The Cutaneous Microcirculation

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The cutaneous microcirculation is organized as two horizontal plexuses. One is situated 1–1.5 mm below the skin surface and the other is at the dermal–subcutaneous junction. Ascending arterioles and descending venules are paired as they connect the two plexuses. From the upper layer, arterial capillaries rise to form the dermal papillary loops that represent the nutritive component of the skin circulation. There are sphincter-like smooth muscle cells at the point where the ascending arterioles divide to form the arteriolar component of the upper horizontal plexus. At the dermal–subcutaneous junction, there are collecting veins with two cusped valves that are oriented to prevent the retrograde flow of blood. Laser Doppler flowmetry has demonstrated vasomotion of red cell flux localized to the sites of ascending arterioles. The simultaneous recording by laser Doppler flowmetry of red cell flux and the concentration of moving red blood cells from individual sites allows one to construct topographic maps of these two values. These two maps, based on initial studies using correlative skin biopsies, can define 1 mm^3 volumes of skin that are predominantly arteriolar in composition, venular in composition, or essentially devoid of all microvascular elements. The electron and light microscopic features that define the microvascular segments, when coupled with that ability of laser Doppler flowmetry to define the predominant microvascular segments under the probe, allow one to study both the mechanisms of normal physiologic states and the pathogenetic mechanisms underlying pathologic skin disorders in which the microvasculature plays a predominant role. ***Key words: 3-dimensional reconstruction/architecture/electron microscopy/laser Doppler flowmetry/microcirculation/organization/skin/topographic mapping/vasomotion. Journal of Investigative Dermatology Symposium Proceedings 5:3–9, 2000***

Our knowledge about the ultrastructure and organization of the cutaneous microvasculature has only been developed in the past 20 y. Prior to that time, the cutaneous vessels, with the exception of those in the human plantar skin, were believed to exist as a randomly anastomosing network without stratification. Physiologists had recognized for some time that the cutaneous blood flow was far greater than that needed for epidermal nutrition and correctly surmised that the excess must be related to regulation of heat loss and temperature control.

The early studies on blood flow using laser Doppler flowmetry (LDF) exhibited significant spatial and temporal variations in its measurements (Tenland *et al*, 1983). This variability implied that LDF was unreliable, because of the assumptions at that time that the skin microvasculature was a uniformly distributed collection of anastomosing vessels. This review summarizes the architecture, organization, and ultrastructure of the cutaneous microcirculation, and how these parameters can be used to develop an understanding of physiologic and pathologic mechanisms that have a significant microvascular component. The presumed unreliability of LDF can be readily explained in terms of the organization and ultrastructure of the cutaneous microvasculature.

The arterioles and venules of the cutaneous microcirculation form two important plexuses in the dermis: an upper horizontal network in the papillary dermis from which the nutritive capillary loops of the dermal papillae arise and a lower horizontal plexus at the dermal–subcutaneous interface (Yen and Braverman, 1976; Braverman and Yen, 1977a; Braverman and Keh-Yen, 1981; Braverman, 1989). The lower plexus, formed by perforating vessels from the underlying muscles and subcutaneous fat, gives rise to arterioles and venules that directly connect with the upper horizontal plexus and also provide lateral tributaries that supply the hair bulbs and sweat glands (**Fig 1**). There are some interconnections among the ascending arterioles and descending venules within the dermis, but these two horizontal plexuses represent the physiologically important areas in the skin. Most of the microvasculature is contained in the papillary dermis 1–2 mm below the epidermal surface. In areas of skin where the dermal papillae are not well developed, arterioles connect with capillaries that course close to the dermal–epidermal junction before the latter move deeper into the dermis to join the postcapillary venules in the upper horizontal plexus. The superficial horizontal plexus is also a thermal radiator, but the exact sites of control for heat regulation remain to be determined. The putative control factors – arteriovenous communications – have not been identified in the skin except in the digits, nose, and ears. Most of the common forms of cutaneous telangiectasias are abnormalities of the horizontal plexus or capillary loops (Braverman and Keh-Yen, 1983b).

Using 1 μm plastic embedded sections, arterioles can be identified with certainty by the presence of an internal elastic lamina; capillaries, on the basis of a thin vascular wall containing pericytes; and venules, on the basis of thicker walls without elastic fibers (Yen and Braverman, 1976; Braverman and Yen, 1977a). Unfortunately, these distinctions cannot be made reliably in 5 μm

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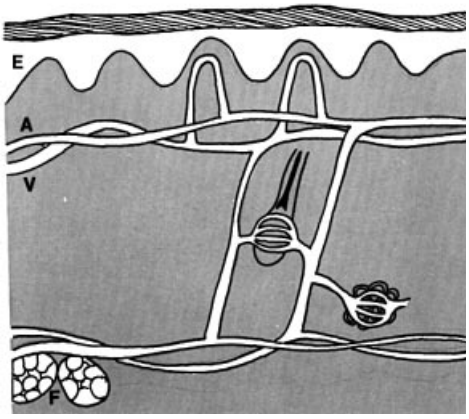

Figure 1. Schematic representation of the microvascular organization in human skin. E, epidermis; A, arteriole; V, venule; F, subcutaneous fat. (From Braverman, 1989; with permission.)

paraffin sections. Electron microscopy (EM) is able to identify definitively the different segments of the microvasculature. EM needs to be used when one is correlating structure with normal or abnormal function in order to confirm the observed light microscopic findings. EM criteria for identifying the three segments of the microvasculature were proposed by Yen and Braverman after reconstructing portions of the horizontal plexus and capillary loops in the dermal papillae of normal human skin as the vessels coursed from the arterial to the venous side (Braverman and Yen, 1977a). Serial 1 μm plastic embedded sections were correlated with EM ultrathin sections. This approach eliminated the inconsistencies and errors of interpretation present in earlier studies of the dermal microvasculature, which had been based on EM observations of individual vessels as they were randomly encountered in human and animal tissues (White and Clawson, 1967; Breathnach, 1971). Conclusions had been drawn without any consideration being given to the location of the vessel within the microvascular bed. The criteria proposed by Braverman and Yen have been confirmed by Higgins and Eady (1981) and parallel the seminal work of Rhodin (1967, 1968) on the subdermal microcirculatory bed in rabbits.

ULTRASTRUCTURE

Microvascular segments The arterioles in the papillary dermis vary from 17 to 26 μm in diameter and represent terminal arterioles (Yen and Braverman, 1976). They most likely function as part of the resistance vessels in the skin. In these arterioles, the endothelial cells are surrounded by two layers of smooth muscle cells. The cells in the inner layer are longitudinally oriented and those in the outer layer form a spiral. The vascular wall is composed of basement membrane material that has a relatively homogeneous appearance by EM and completely surrounds and encompasses the elastic fibers and smooth muscle cells. The inner smooth muscle cells and endothelial cells send cytoplasmic processes toward each other to make frequent tight junctional contacts through breaks in the basement membrane. The subendothelial elastic lamina appears as an interrupted layer between the endothelial and smooth muscle cells in cross-section. In longitudinal sections and in three-dimensional reconstructions, the elastic lamina can be seen to be made up of interwoven longitudinal fibers resembling a wire mesh fence and presumably performing a function analogous to steel rods embedded in concrete. Each bundle of longitudinal elastic fibers lies in a space formed by the cytoplasmic processes of the inner layer of smooth muscle cells and endothelial cells that have made contact with one another.

As the diameter of the arteriole decreases from 26 to 15 μm, the elastic fibers assume a progressively more peripheral position in the vascular wall. At the 15 μm level, the elastic fibers have disappeared from the vascular wall and are present only as an incomplete sheath between the wall and the surrounding adventitial (veil cell) layer. This external elastin sheath disappears at the 10–12 μm level, which corresponds to the beginning of the capillary bed. The basement membrane retains its homogeneous appearance during this transition. Smooth muscle cells – identified by numerous dense bodies and myofilaments – are not found below the 15 μm level. Their place is taken by a single cell with less well-developed dense bodies and many fewer filaments (Braverman and Sibley, 1990). This cell has many circumferential arms, some of which almost completely encircle the endothelial cell tube. The arms overlap each other slightly. The configuration of the cell and the localization of vasomotor activity to this terminal arteriolar segment by LDF suggest that this cell may function both as a precapillary sphincter and as the pacemaker for vasomotor activity, as has been demonstrated for the precapillary sphincters in cat mesentery (Johnson and Wayland, 1967). (See later in paper.)

The arterial capillary, the next contiguous microvascular segment, has an outside diameter of 10–12 μm and an internal endothelial tube diameter of 4–6 μm (Yen and Braverman, 1976). The basement membrane material retains its homogeneous appearance (**Fig 2**). Pericytes form tight junctions with endothelial cells through breaks in the basement membrane. Pericytes differ from smooth muscle cells by being thinner, lacking dense bodies, and having fewer myofilaments; however, pericytes do have the contractile proteins necessary for cellular contraction (Joyce *et al*, 1985). The walls of these capillaries vary from 2 to 3 μm in thickness, but in a few instances the endothelial tube is surrounded by a wall only 0.5–1 μm wide, in contrast to capillaries elsewhere in the body, where the vascular wall is usually only 0.1 μm wide.

As the arterial capillary is traced, the basement membrane material progressively begins to develop lamellae within its homogeneous framework until a segment is reached in which the entire vascular wall is multilaminated (**Fig 2**) (Yen and Braverman, 1976). Dense layers of basement membrane material, 25–100 nm thick, alternate with less-dense zones. As many as 10 lamellae may be present. The outside diameters of the vessels remain at 10–12 μm and their endothelial tubes at 4–6 μm. Pericytes and veil cells remain the important cellular elements in the vascular wall and in the immediately surrounding dermis, respectively.

The venous capillary connects with the postcapillary venule, a vessel whose external diameter increases from 12 to 35 μm and whose endothelial tube diameter enlarges from 8 to 26 μm. Most of the postcapillary venules seen in the papillary dermis measure 18–23 μm in external diameter and 10–15 μm in endothelial tube diameter. Pericytes form 2–3 layers around the vascular wall, in contrast to only one layer in the venous capillary, and cover approximately 80% of the endothelial cell tube surface. The basement membrane of the vascular wall is multilaminated. The wall is usually 3.5–5.0 μm wide. Collagen fibrils may be present between the lamellae or may form a thin sheath in the outer layer of the vascular wall.

The vessels in the papillary dermis are composed entirely of terminal arterioles, arterial and venous capillaries, and postcapillary venules. The majority of vessels, however, are postcapillary venules, the physiologically most reactive segment of the microcirculation. Here the inflammatory cells migrate from the vascular space into the tissues, and endothelial cells often develop intercellular gaps that result in increased vascular permeability in response to acute inflammation. Some of the vessels in the lower third of the dermis are twice as large as the upper dermal vessels and represent arterioles and collecting venules (40–50 μm).

The homogeneous and multilaminated appearance of the vascular basement membrane are much more distinct in tissues fixed in Karnovsky's fixative than in those fixed in buffered osmium tetroxide. Osmium fixation may produce a pseudolaminated appearance. The ultrastructural differences in basement membrane material between the arterial and venous components of the microcirculation are present in all areas of the skin.

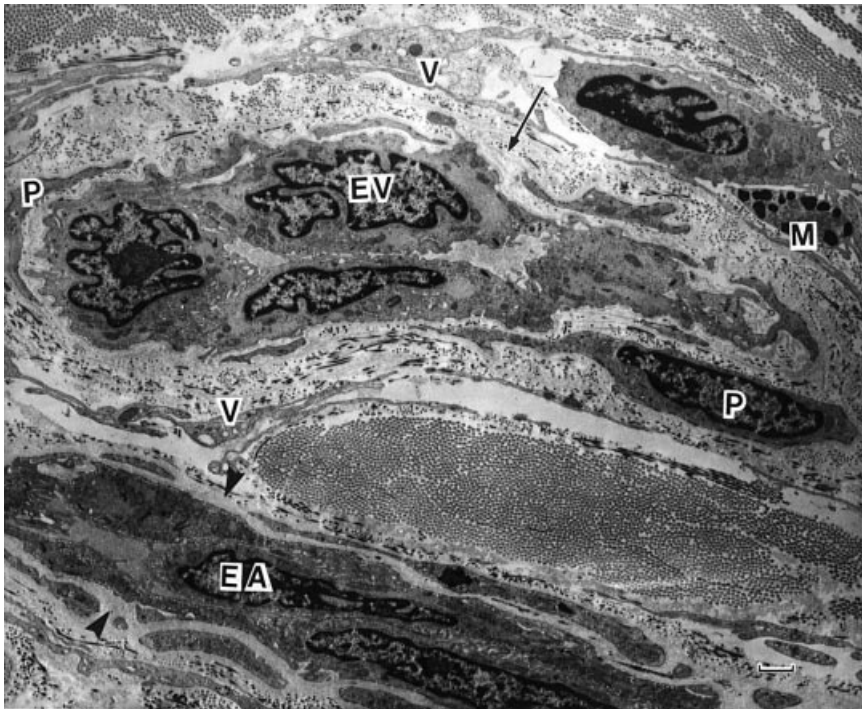

Figure 2. Electron micrograph of postcapillary venule and adjacent terminal arteriole. E, venular endothelial cell; EA, arteriolar endothelial cell; P, pericyte; V, veil cell with thin cytoplasmic processes (*arrows*); M, mast cell. The *small arrowhead* indicates multilayered basement membrane material in venule; the *large arrowhead* points to homogeneous appearing basement membrane material in arterioles. *Scale bar*: 1 μm.

The capillary loop The capillary loop arises from a terminal arteriole in the horizontal papillary plexus and is composed of an ascending limb, an intrapapillary loop having a hairpin turn, and a descending limb that connects with a postcapillary venule in the horizontal plexus (Braverman and Yen, 1977a). Each dermal papilla is supplied by a single capillary loop, which has an intrapapillary and extrapapillary portion defined by an imaginary line drawn between the deepest points of adjacent rete ridges. The ascending limb of the loop arises from the horizontal plexus and passes either directly to a papilla or subdivides once, twice, or three times before giving rise to the final ascending limbs that enter individual papillae. The ascending limb of the capillary loop in its extra- and intrapapillary portion has the characteristic of an arterial capillary, homogeneous appearing basement membrane material in the vascular wall. The endothelial tube at the crest and in the intrapapillary descending portion is 1–1.5 μm wider than that of the ascending limb. The mural basement membrane material of the capillary loop retains its homogeneous appearance throughout its intrapapillary course. At the apex of the loop, the endothelial cells become attenuated, and in some areas cell wall thickness measures 11–30 nm, but true bridged fenestrations are rarely seen.

The character of the descending limb changes abruptly either at the border or 10–30 μm distal to the border, where the loop becomes extrapapillary. The endothelial tube becomes wider and the basement membrane material in the wall loses its homogeneous appearance and develops multilayers characteristic of a venous vessel before it connects with the horizontal plexus. There are no ultrastructural differences between the capillary loops at different sites in the skin, including the lower leg; only their density varies. Bridged fenestrations are normally found in capillaries where there is a need for rapid exchange of molecules between the vascular system and the tissues (renal glomeruli, endocrine glands, intestinal lamina propria, choroid plexus of the brain, and the ciliary body of the eye). In healthy skin, bridged fenestrations are limited to the venous capillaries adjacent to the eccrine sweat gland and dermal papilla of the hair. The capillary loops in lesions of psoriasis, however, are characterized by bridged fenestrations and a multilayered basement membrane. The normal skin of psoriatic patients and their healed lesions do not show these changes. The capillary loops exhibit normal arterial capillary characteristics.

Deep dermal and subcutaneous vessels The ultrastructure of the arterioles and venules in the mid- and lower dermis and in the lower horizontal plexus differ from the ultrastructure of comparable vessels in the superficial horizontal plexus in several ways (Braverman and Yen, 1981, 1983a). The diameters are wider, 50 μm *versus* 25 μm; the walls are thicker, 10–16 μm *versus* 4–5 μm; smooth muscle cells or pericytes are present as 4–5 layers in contrast to 1–2 layers in similar vessels of the superficial plexus; and bundles of collagen fibrils in arterioles are present in subendothelial positions rather than in the periphery of the vascular wall.

The arterioles and venules in the fat lobules are identical in structure and size to those of the lower horizontal plexus. Rarely, an arteriole 100 μm in diameter is seen.

The arteriolar endothelial cells in the mid- and lower dermis and fat contain bundles of actin filaments 4–7 nm wide that tend to course along the abluminal border of the cells (Braverman and Keh-Yen, 1981). These bundles exhibit transverse linear wavy bands of increased density resembling the Z bands of skeletal muscle. In longitudinal sections, these intracellular bundles appear to be associated with extracellular filaments measuring 10–20 nm in diameter. Because these cytoskeletal filaments occur only in the high pressure arterial vessels, they probably serve to maintain cellular shape and to increase the adhesion between adjacent endothelial cells and between the endothelial cells and the basement membrane material of the vascular wall.

Collecting venules with valves Valve-containing vessels pass from the deep dermis into the superficial layer of the fat (Braverman and Keh-Yen, 1983a). These vessels range from 70 to 120 μm in diameter and can be identified easily with a dissecting microscope. Postcapillary venules, 25–50 μm, join these large vessels from all directions. Valves are found at most places where the small vessels join the larger ones, but valves are also present within the large vessels not associated with branch points. All of the valves have two cusps with associated sinuses (pockets formed

between each valve leaflet and the adjacent vessel wall). The free edges of the valves are always directed away from the smaller vessel and toward the larger one at the branch points.

Valves imply a mechanism involved in the forward propulsion of blood, as suggested by the work of Landis (1930) and Eichna and Bordley (1939) and confirmed by the recent work of Mahler *et al* (1979) using the more sophisticated instrumentation of the time. Direct cannulation of human finger nailfold capillaries has demonstrated that the blood pressure is pulsatile in both the arteriolar and the venular limbs and systolic pressures fluctuate from 11 to 75 mmHg. Valves in collecting veins at the dermal–fat interface would seem to be appropriately placed to insure the forward motion of the blood.

Veil cells Veil cells are flat adventitial cells that surround all dermal microvessels (**Fig 2**). The exact nature and function of these cells are still undetermined (Braverman *et al*, 1986). They do not have cells markers for T, B, or Langerhans cells, nor do they stain for HLA-DR. Their ultrastructure most closely resembles a fibroblast. Unlike pericytes that are an integral component of the vascular wall and are enmeshed in the mural basement membrane material, veil cells are totally external to the wall, demarcating the vessel from the surrounding dermis. Perivascular mast cells are usually situated in a space between the vascular wall and the surrounding veil cells. The veil cells are seen infrequently around the microvessels in the subcutaneous fat and it is not known whether they are present in significant numbers around microcirculatory vessels in other organs.

Contractile cells of the microvascular wall Smooth muscle cells and pericytes comprise the contractile elements of the microvascular wall (Braverman and Sibley, 1990). In the upper horizontal plexus, they form unique configurations in each functional segment of the microvasculature.

In the arteriolar segment just before the capillary bed, the contractile cells resemble pericytes more than smooth muscle cells. A single cell with multiple circumferential arms completely or almost completely encircles the endothelial tube. The cell has multiple arms that overlap each other slightly, and exhibits multiple junctional contacts with the endothelial cell. This cell that appears to be at least 6.5 μm long, based on our three-dimensional computer reconstructions (Braverman and Braverman, 1986; Braverman and Sibley, 1990), may function as a precapillary sphincter.

In the postcapillary venule, pericytes and endothelial cells make innumerable contracts over their entire surfaces. They range from cytoplasmic ''fingers'' and ''ridges'' that insert into deep endothelial cell invaginations to ''fingers'' that merely touch the endothelial cell surface. The pericytes grip the endothelial cell tube much like a vise-grip tool. Three-dimensional reconstructions show that a single pericyte makes contact with the 2–4 underlying endothelial cells. This intimate interdigitation has been shown to be rich in fibronectin at the contact points (Courtoy and Boyles, 1983).

MICROANATOMICAL ORGANIZATION

LDF Using LDF studies, Braverman *et al* (1990) have correlated wave patterns with the underlying microvascular segments described above. Because the LDF instuments only gather signals from a depth of 1–1.5 mm below the epidermis, the capillary blood supply to the sweat glands and hair follicles does not contribute to the LDF signal because these structures are 3–5 mm below the skin surface. High amplitude red cell flux waves exhibiting vasomotion corresponded to an underlying ascending arteriole, and low amplitude waves without vasomotor activity corresponded to venular predominance under the 1 mm diameter LDF detecting probe. Using such data one can construct computer-generated topographic maps of the arteriolar supply in the skin (**Fig 3**). By obtaining flux data simultaneously with the concentration of moving red blood cells (CMBC) data a topographic map can be constructed for each parameter (Braverman *et al*, 1992). This

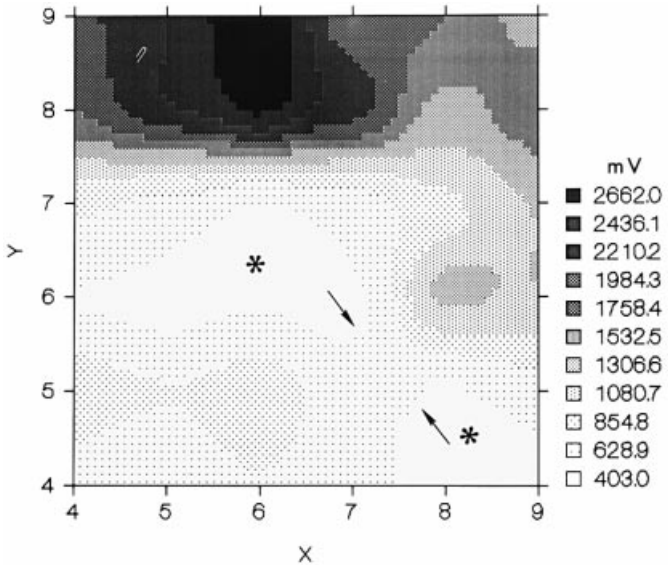

Figure 3. Topographic flux map of flexor forearm (6 × 6 grid, 6 mm × 6 mm area). A Medpacific LD5000 instrument was used at a gain of 10×. Highest flux readings indicate site of ascending arteriole. The asterisks indicate the areas of minimally detectable flux and relative avascularity. Scale of flux is shown in arbitrary millivolt units on *Y*-axis. *Arrows* indicate bridge between two vascular islands. (From Braverman *et al*, 1992; with permission.)

combination of maps correctly predicts the relative proportions of microvascular segments beneath the 1 mm diameter probe (**Fig 4**). Sites of high flux and high CMBC indicate the presence of an underlying ascending arteriole and its paired descending venule. High flux, low CMBC sites indicate arteriolar predominance; low flux, high CMBC sites indicate venular predominance; and low flux, low CMBC sites denote relatively avascular areas.

These LDF studies have demonstrated that the ascending arterioles are randomly spaced at intervals of 1.5–7 mm (Braverman and Schechner, 1991), thus explaining the basis for the spatial heterogeneity of LDF measurements (Tenland *et al*, 1983). Each ascending arteriole divides into 4–5 branches to form a portion of the upper horizontal plexus (**Figs 5**, **6**). The ascending arteriole is accompanied by a post-capillary venule that is formed from the confluence of 8–10 branches within the upper plexus. Each vascular unit is attached to its neighbors along a portion of their respective peripheries. In three dimensions, the vascular unit resembles an umbrella: the handle is represented by the paired ascending arteriole and descending venule (Braverman *et al*, 1990). The umbrella proper is formed by the arteriolar and venular branches, with the arterioles being predominant in the center and the venules predominant in the periphery. The phasic (vasomotor) variation in red cell flux amplitude is greatest over the ascending arteriole and its immediate branches, suggesting that these vessels are the morphologic site for the generation of vasomotor activity detected by LDF in the skin. The putative sphincter, formed by a single encircling smooth muscle cell at the site where the elastic containing arteriole branches to form the arterioles of the upper plexus, may be the origin of the vasomotion detected by LDF. When a single arteriolar site is continuously monitored for 1–2 h, the red cell flux changes from minimum to maximum and vice versa at intervals of 12–20 min at some sites and 70–90 min at other sites, thus explaining in part the temporal heterogeneity of LDF measurements.

This strategy based on LDF-morphologic correlation provides a way to determine the approximate mixture of microvascular elements beneath the 1 mm diameter probe. One can determine the density of ascending arterioles in a defined area of skin, as well as defining the areas of capillary and postcapillary predominance between the arterioles, and the areas of relative avascularity in both

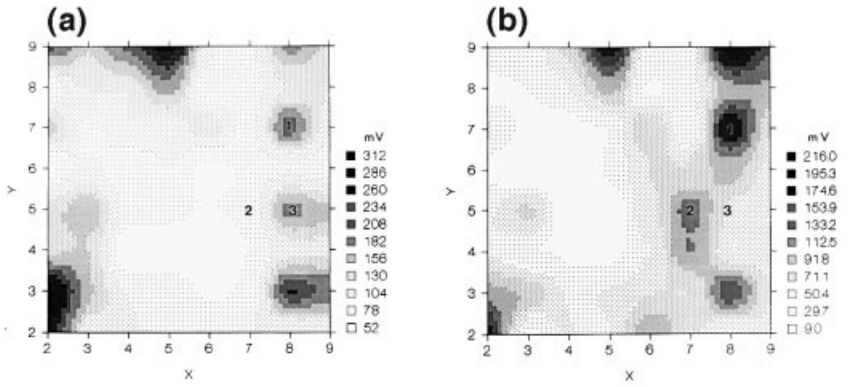

Figure 4. Topographic contour maps of flexor forearm skin. Coordinates of needle probe placement shown on *X*, *Y* axes. Each point is 1.24 mm away from an adjacent coordinate. Scale of LDF values is in arbitrary units of millivolts. (*a*) Map based on CMBC values; (*b*) map based on flux values. Biopsy sites are labeled 1, 2, and 3. Areas that appear clear or have the least dense stipples are relatively avascular. (From Braverman *et al*, 1992; with permission.)

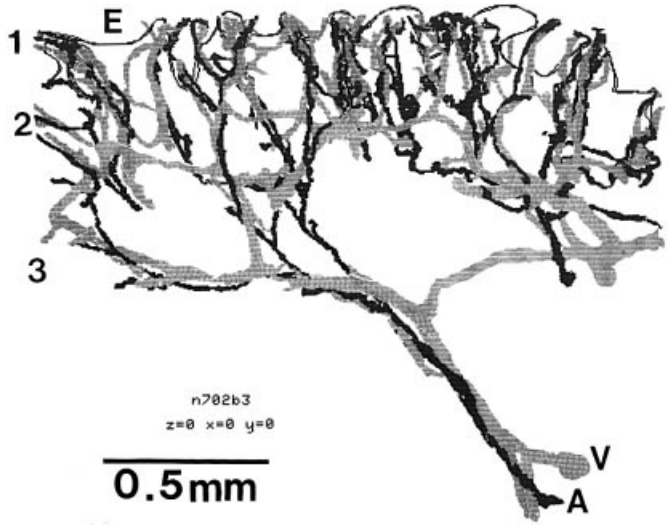

Figure 5. Computer reconstruction of vessels present in papillary dermis. E, epidermis. Zone 1–2 contains dermal capillary loops and zone 2–3 contains vessels of horizontal plexus. A, ascending arteriole with five immediate branches; V, accompanying descending venule formed by nine converging postcapillary venules. (From Braverman *et al*, 1990; with permission.)

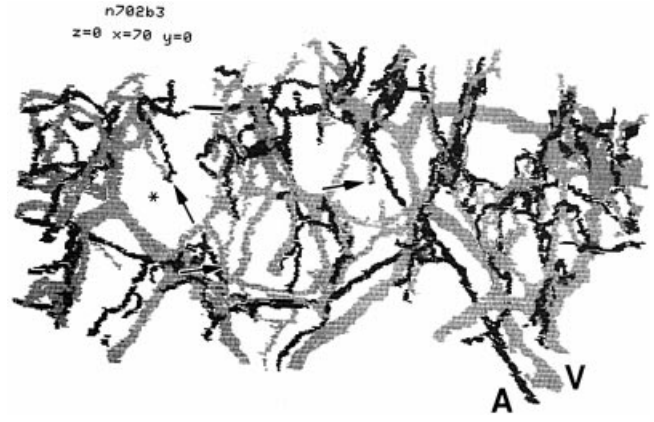

Figure 6. Computer reconstruction in Fig 5 rotated 70° on *X*-axis. Asterisk indicates space empty of arterioles and postcapillary vessels. *Arrows* indicate capillary loops in dermal papillae. A, ascending arteriole; V, descending arteriole. Width of reconstruction = 2 mm. (From Braverman *et al*, 1990; with permission.)

experimental designs and pathologic states. The vascular islands with attachments at their peripheries coupled with intervening avascular spaces explains the livedo pattern in skin following heat stress and impaired blood flow for other reasons (Braverman *et al*, 1992). Although the above studies were performed with a template probe holder that was manually moved precisely 1 mm for each data measurement, the same results were obtained in later experiments with a scanning laser Doppler instrument (Wårdell *et al*, 1994).

CLINICAL APPLICATIONS

Cutaneous disorders with a microvascular component

Psoriasis In psoriasis, the dermal capillary loops are venous capillaries as defined by multilaminated mural basement membrane and endothelium with bridged fenestrations (Braverman, 1972; Braverman and Yen, 1974, 1977b). In the normal skin of psoriatics, the loops are arterial capillaries. When photochemotherapy with oral psoralen and UVA irradiation (PUVA) and UVB therapies induce remissions in psoriatic lesions, the first ultrastructural morphologic event is a shortening of the capillary loop with reversion from a venous capillary to an arterial capillary. This reversion occurs 3–7 d before the abnormally high labeling index of the basal cell layer begins to return toward normal (Braverman and Sibley, 1982). Remissions induced by methotrexate or topical steroids produce clinical and histologic normalization of the epidermis, including the labeling index of the basal cell layer, but without any return of the capillary loops to normal (Braverman and Sibley, 1985).

The postcapillary venules in psoriatic lesions, and to a lesser extent in the normal-appearing skin of psoriatics, also exhibit endothelial cell gaps that are indistinguishable from those produced by intradermal histamine. Three-dimensional computer reconstructions of both psoriatic and histamine-induced gaps show that they occur both at endothelial cell junctions and through the endothelial cells themselves (Braverman and Keh-Yen, 1986). Neal and Michel have shown similar changes in endothelial cell gaps in frog microvessels induced by ionophore A23187 (Neal and Michel, 1995).

The increased vascular dilatation in psoriasis represents increased vascular mass (Barton *et al*, 1992), and the flow has been shown to be 10 times greater in psoriatic lesions than in normal skin when measured by ^{133}Xenon washout (Klemp and Staberg, 1983). Although the latter technique measures the entire cutaneous microcirculation and subcutaneous fat, the bulk of the increased flow does occur in the upper horizontal plexus as evidenced by increased temperature and redness of the lesion. When measured by LDF, the flux is 2.5–4.5 times greater than normal in localized areas around a psoriatic plaque. In these zones, there are no histologic abnormalities observed by light microscopy, yet these are the sites

from which the psoriatic plaque grows in an asymmetrical manner (Hull *et al*, 1989). The increased flow in these sites precedes any morphologically detectable inflammatory or epidermal change (Goodfield *et al*, 1994). Similarly, intravital microscopy has demonstrated that there is an expansion of existing vessels rather than new vessel formation in the upper plexus and its derived vessels (Bull *et al*, 1992). These LDF studies also showed that the red cell flux decreased significantly 4–8 d before there was evidence of clinical improvement in the psoriatic lesion (Khan *et al*, 1987), an observation identical to those of Braverman and Sibley (1982) who used only morphologic endpoints.

In the past decade, studies by Detmar, Dvorak and others have implicated vascular endothelial growth factor/vascular proliferation factor (VEGF/VPF), a multifunctional cytokine found in tumors and in a variety of tissues including keratinocytes, as being the major angiogenic factor in psoriasis (Detmar *et al*, 1994, 1995; Dvorak *et al*, 1995). VEGF/VPF is mitogenic for microvascular endothelial cells and is also 50 000 times more potent than histamine as an inducer of hyperpermeability to macromolecules such as albumin in postcapillary venules. The mitogenic activity would produce the elongation of the capillary loops in psoriasis through the proliferation of the endothelial cells in the venous limb of the capillary loop. VEGF/VPF is believed to enhance the functional activity of the vesicular/vacuolar organelle (VVO) system in the postcapillary venules. The VVO system forms a continuous channel from luminal to abluminal surfaces through poral interconnections of membrane bound vesicles and vacuoles. These channels are believed to be the transport mechanism for macromolecules and are probably the basis for the transendothelial cell gaps observed by routine EM in psoriatic vessels and in normal vessels following the intradermal injection of histamine (Feng *et al*, 1997). The VVO system is most likely a more highly developed version of the caveolae in capillaries (Frokjaer-Jensen, 1980, 1991), much as the smooth muscle cells are a more highly developed version of pericytes. VEGF/VPF is most likely the unidentified angiogenic factor that our laboratory had found in psoriatic and normal epidermis, but not in dermis, a decade ago (Malhotra *et al*, 1989).

Endothelial cell gaps may occur between cells, through cells, and in combination with one another. The mechanism(s) of formation in relation to the type of associated inflammation needs to be restudied. Because VEGF/VPF is induced by TGFα in keratinocytes, a fruitful avenue of inquiry would be the relationship between UV light exposure and the improvement of psoriasis via capillary loop normalization based on a mechanism that included UVB, UVA, TGFα, and VEGF/VPF.

Vasomotion Maximum vasomotion of 6–10 cycles per min occurs over sites of ascending arterioles. When vasomotor activity was measured at paired arteriolar sites separated by 5.7–20 mm on ipsilateral limbs or at comparable sites on contralateral limbs, vasomotor waves were synchronous approximately 60% of the time (Schechner and Braverman, 1992). Previous work had shown it to be asynchronous, but the probes had been randomly placed 1.5–2.0 cm apart. Studies by Bernardi *et al* (1996, 1997) have confirmed this synchronicity and provided additional evidence to support the existence of a central sympathetic autonomic modulator of vasomotion.

Vasomotor waves can be quantitated by amplitude and frequency or by spectral analysis. The latter technique, which is more useful, demonstrates that the beat-to-beat rate of the cardiac cycle varies around two means: 0.1 Hz and 0.25 Hz. The former is believed to be related to sympathetic tone and the latter to parasympathetic tone and the respiratory rhythm. When a patient is quickly tilted from the supine to the erect position, the 0.1 Hz frequency becomes accentuated at the expense of the 0.25 Hz frequency, presumably because of baroreceptor generated increased sympathetic tone. Using laser Doppler methodology, Bernardi *et al* (1989) demonstrated that these spectra are present in cutaneous vasomotion in the human forearm, and that their response to tilting is identical. These responses can be reduced significantly by sympathetic blockade of the limb. In diabetics, this response is impaired in both the skin and the heart in the presence of autonomic neuropathy (Spallone *et al*, 1996). The study of cutaneous vasomotor responses to a variety of stimuli by spectral analysis is a new and promising approach to investigating the effect of the autonomic nervous system on the cutaneous microcirculation.

Based upon the correlative studies of LDF vasomotor and erythrocyte flux wave patterns with the morphology of the underlying vasculature described above (Braverman *et al*, 1990, 1992; Braverman and Schechner, 1991), it is now possible to identify from the LDF patterns 1 mm^3 areas of skin in which there is a predominantly arteriolar composition or a predominantly capillary/venular composition. This should allow experiments to be designed that will test pathogenetic mechanisms involving dermal perfusion in the vast spectrum of microvascular disorders that are encountered in clinical medicine. These techniques coupled with advances in the culture and biochemistry of endothelial cells, and the significant roles of adhesion molecules and cytokines in endothelial cell biology, especially as they relate to the transendothelial migration of inflammatory cells, should provide a comprehensive approach to understanding the role of the microvasculature in cutaneous disorders. The combination of EM to define the nature of the microvascular segment and measurements of flow by LDF in the study of psoriasis can serve as a paradigm for the study of other disorders in which the microvasculature plays a significant role.

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REFERENCES

Barton SP, Abdullah MS, Marks R: Quantification of microvascular changes in the skin of patients with psoriasis. *Br J Dermatol* 126:569–574, 1992

Bernardi L, Hayoz D, Wenzel R, Passino C, Calciati A, Weber R, Noll G: Synchronous and baroreceptor-sensitive oscillations in skin microcirculation: evidence for central autonomic control. *Am J Physiol* 273:H1867–H1878, 1997

Bernardi L, Radaelli A, Solda PL, *et al*: Autonomic control of skin microvessels: assessment by power spectrum of photoplethysmographic waves. *Clin Sci* 90:345–355, 1996

Bernardi L, Rossi M, Fratino P, Finardi G, Mevio E, Orlandi C: Relationship between phasic changes in human skin blood flow and autonomic tone. *Microvasc Res* 37:16–27, 1989

Braverman IM: Electron microscopic studies of the microcirculation in psoriasis. *J Invest Dermatol* 59:91–98, 1972

Braverman IM: Ultrastructure and organization of the cutaneous microvasculature in normal and pathologic states. *J Invest Dermatol* 93:2S–9S, 1989

Braverman M, Braverman IM: Three dimensional reconstruction of serial sections using a microcomputer graphics system. *J Invest Dermatol* 86:290–294, 1986

Braverman IM, Keh-Yen A: Ultrastructure of the human dermal microcirculation. III. The vessels in the mid- and lower dermis and subcutaneous fat. *J Invest Dermatol* 77:297–304, 1981

Braverman IM, Keh-Yen A: Ultrastructure of the human dermal microcirculation. IV. Valve-containing collecting veins at the dermal-subcutaneous junction. *J Invest Dermatol* 81:438–442, 1983a

Braverman IM, Keh-Yen A: Ultrastructure and three-dimensional reconstruction of several macular and papular telangiectases. *J Invest Dermatol* 81:489–497, 1983b

Braverman IM, Keh-Yen A: Three-dimensional reconstruction of endothelial cell gaps in psoriatic vessels and their morphologic identify with gaps produced by the intradermal injection of histamine. *J Invest Dermatol* 86:577–581, 1986

Braverman IM, Schechner J: Contour mapping of the cutaneous microvasculature by computerized laser Doppler velocimetry. *J Invest Dermatol* 97:1013–1018, 1991

Braverman IM, Sibley J: Role of the microcirculation in the treatment and pathogenesis of psoriasis. *J Invest Dermatol* 78:12–17, 1982

Braverman IM, Sibley J: The response of psoriatic epidermis and microvessels to treatment with topical steroids and oral methotrexate. *J Invest Dermatol* 85:584–586, 1985

Braverman IM, Sibley J: Ultrastructural and 3-dimensional analysis of the contractile cells of the cutaneous microvasculature. *J Invest Dermatol* 95:90–96, 1990

Braverman IM, Yen A: Microcirculation in psoriatic skin. *J Invest Dermatol* 62:493–502, 1974

Braverman IM, Yen A: Ultrastructure of the human dermal microcirculation II. The capillary loops of the dermal papillae. *J Invest Dermatol* 68:44–52, 1977a

Braverman IM, Yen A: Ultrastructure of the capillary loops in the dermal papillae of psoriasis. *J Invest Dermatol* 68:53–60, 1977b

Braverman IM, Sibley J, Keh-Yen A: A study of the veil cells around normal, diabetic, and aged cutaneous microvessels. *J Invest Dermatol* 86:57–62, 1986

Braverman IM, Keh A, Goldminz D: Correlation of laser Doppler wave patterns with underlying microvascular anatomy. *J Invest Dermatol* 95:283–286, 1990

Braverman IM, Schechner JS, Silverman DG, Keh-Yen A: Topographic mapping of the cutaneous microcirculation using two outputs of laser-Doppler flowmetry: Flux and the concentration of moving blood cells. *Microvasc Res* 44:33–48, 1992

Breathnach AS: *An Atlas of the Ultrastructure of Human Skin*. London: Churchill, 1971, pp. 214–226

Bull RH, Bates DO, Mortimer PS: Intravital video capillaroscopy for the study of the microcirculation in psoriasis. *Br J Dermatol* 126:436–445, 1992

Courtoy P, Boyles J: Fibronectin in the microvasculature; localization in the pericyte-endothelial interstitium. *J Ultrastruct Res* 83:258–273, 1983

Detmar M, Brown LF, Claffey KP, *et al*: Overexpression of vascular permeability factor/vascular endothelial growth factor and its receptors in psoriasis. *J Exp Med* 180:1141–1146, 1994

Detmar M, Yeo K-T, Nagy JA, *et al*: Keratinocyte-derived vascular permeability factor (vascular endothelial growth factor) is a potent mutagen for dermal microvascular endothelial cells. *J Invest Dermatol* 105:44–50, 1995

Dvorak HF, Brown LF, Detmar M, Dvorak AM: Vacular permeability factor/vascular endothelial growth factor, microvascular permeability, and angiogenesis. *Am J Pathol* 146:1029–1039, 1995

Eichna LW, Bordley J III: Capillary blood pressure in man. Comparison of direct and indirect methods of measurement. *J Clin Invest* 18:695–704, 1939

Feng D, Nagy JA, Hipp J, Pyne K, Dvorak HF, Dvorak AM: Reinterpretation of endothelial cell gaps induced by vasoactive mediators in guinea pig, mouse and rat; many are transcellular pores. *J Physiol* 504:747–761, 1997

Frokjaer-Jensen J: Three dimensional organization of plasmalemmal vesicles in endothelial cells. An analysis by serial sectioning of frog mesenteric capillaries. *J Ultrastruct Res* 73:9–20, 1980

Frokjaer-Jensen J: The endothelial vesicle system in cryofixed frog mesenteric capillaries analyzed by ultrathin serial sectioning. *Nj Electron Microscop Technique* 19:291–304, 1991

Goodfield M, Hull SM, Holland D, Roberts G, Wood E, Reid S, Cunliffe W: Investigation of the "active" edge of plaque psoriasis: vascular proliferation precedes changes in epidermal keratin. *Br J Dermatol* 131:808–813, 1994

Higgins JC, Eady RAJ: Human dermal microvasculature: a morphological and enzyme histochemical investigation at the light and electron microscope levels. *Br J Dermatol* 104:117–129, 1981

Hull SM, Goodfield M, Wood EJ, Cunliffe WJ: Active and inactive edges of psoriatic plaques: identification by tracing and investigation by laser-Doppler flowmetry and immunocytochemical techniques. *J Invest Dermatol* 92:782–785, 1989

Johnson OPC, Wayland H: Regulation of blood flow in single capillaries. *Am J Physiol* 212:1405–1415, 1967

Joyce NC, Haire MF, Palade GE: Contractile proteins in pericytes. II. Immunocytochemical evidence for the presence of two isomyosins in graded concentrations. *J Cell Biol* 100:1387–1395, 1985

Khan A, Schall LM, Tur E, Maibach HI, Guy RH: Blood flow in psoriatic skin lesions: the effect of treatment. *Br J Dermatol* 117:193–201, 1987

Klemp P, Staberg B: Cutaneous blood flow in psoriasis. *J Invest Dermatol* 81:503–506, 1983

Landis EM: Micro-injection studies of capillary blood pressure in human skin. *Heart* 15:20–228, 1930

Mahler F, Muheim MH, Intaglietta M, Bollinger A, Anliker M: Blood pressure fluctuations in human nailfold capillaries. *Am J Physiol* 236:H888–H893, 1979

Malhotra R, Stenn KS, Fernandez LA, Braverman IM: The angiogenic properties of normal and psoriatic skin associate with the epidermis – not the dermis. *Lab Invest* 61:162–165, 1989

Neal CR, Michel CC: Transcellular gaps in microvascular walls of frog and rat when permeability is increased by perfusion with the ionophore A23187. *J Physiol* 488:427–437, 1995

Rhodin JAG: The ultrastructure of mammalian arterioles and precapillary sphincters. *J Ultrastruct Res* 18:181–223, 1967

Rhodin JAG: Ultrastructure of mammalian venous capillaries, venules, and small collecting veins. *J Ultrastruct Res* 25:452–500, 1968

Schechner JS, Braverman IM: Synchronous vasomotion in the human cutaneous microvasculature provides evidence for central modulation. *Microvasc Res* 44:27–32, 1992

Spallone V, Bernardi L, Maiello MR, Cicconetti E, Ricordi L, Fratini P, Menzinger G: Twenty-four hour pattern of blood pressure and spectral analysis of heart rate variability in diabetic patients with various degrees of autonomic neuropathy. Comparison to standard cardiovascular tests. *Clin Sci* 91 (Suppl.):105–107, 1996

Tenland T, Salerud EG, Nilsson EG, Oberg PA: Spatial and temporal variations in human skin blood flow. *Int J Microcirc Clin Exp* 2:81–90, 1983

Wårdell K, Braverman IM, Silverman DG, Nilsson GE: Spatial heterogeneity in normal skin perfusion recorded with laser Doppler imaging and flowmetry. *Microvasc Res* 48:26–38, 1994

White JG, Clawson CC: Blood cells and blood vessels. In: AS Zelickson, ed. *Ultrastructure of Normal and Abnormal Skin*. London: Henry Kimpton, 1967, pp 261–303

Yen A, Braverman IM: Ultrastructure of the human dermal microcirculation: the horizontal plexus of the papillary dermis. *J Invest Dermatol* 66:131–142, 1976