Microcirculation

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Physiology and pathophysiology of the microcirculation

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The microcirculation consists of a highly dynamic system of vessels that not only subserves the local metabolic needs of individual organs, but also functions as a site for integration of a variety of physiological processes, including regulation of arterial pressure (in arterioles), plasma volume (capillaries), and inflammation (venules). This integrative function of the microvasculature can be largely attributed to the responses of a single cellular component of the vessel wall, ie, the endothelial lining. There is growing recognition that dysfunctional endothelial cells contribute to the pathogenesis of a number of cardiovascular diseases, including atherosclerosis, hypertension, and shock. This paper reviews: (i) the morphology and ultrastructure of the microcirculation, and (ii) the functions and responses of three key elements of the microcirculation, ie, arterioles, capillaries, and venules, in health and disease.

Keywords: ischemia/reperfusion; endothelium-dependent vasodilation; capillary growth; inflammation; atherosclerosis; leuckocyte–endothelial cell adhesion

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The ultimate function of the cardiovascular system, ie, the delivery of oxygen, nutrients, and water to tissues, occurs in the microcirculation. These critical exchange processes are finely controlled to meet the needs of tissues. Readjustments in blood flow and O_2 (and nutrient) extraction that allow for this fine control are attributed to the responses of two vascular elements—arterioles and capillaries. Of equal importance are the capacitance, immune surveillance, and plasma protein exchange functions that occur in the postcapillary segment of the microcirculation (ie, venules).

Over the past decade, much has been learned about the chemical and physical factors that influence microvascular function. A focal point of this attention has been the endothelial cell, which is known to produce a variety of substances that can affect underlying smooth muscle cells and circulating blood cells, and exert an autocrine influence on adjacent endothelial cells. The novel information gained in this field of research has resulted in profound changes in the prevailing concepts on the regulation of microvascular function and led to a greater appreciation of the role of endothelial cells and the microcirculation in different disease processes.

In this article, consideration is given to some of the new prevailing concepts concerning the factors that regulate three structurally and functionally important elements of the microcirculation—arterioles, capillaries, and venules. Attention is also given to the potential role of these elements of the microvasculature in different disease states.

ANATOMICAL AND FUNCTIONAL ORGANIZATION OF THE MICROCIRCULATION

The general architecture of the microcirculation varies among organs, but the fundamental elements of the

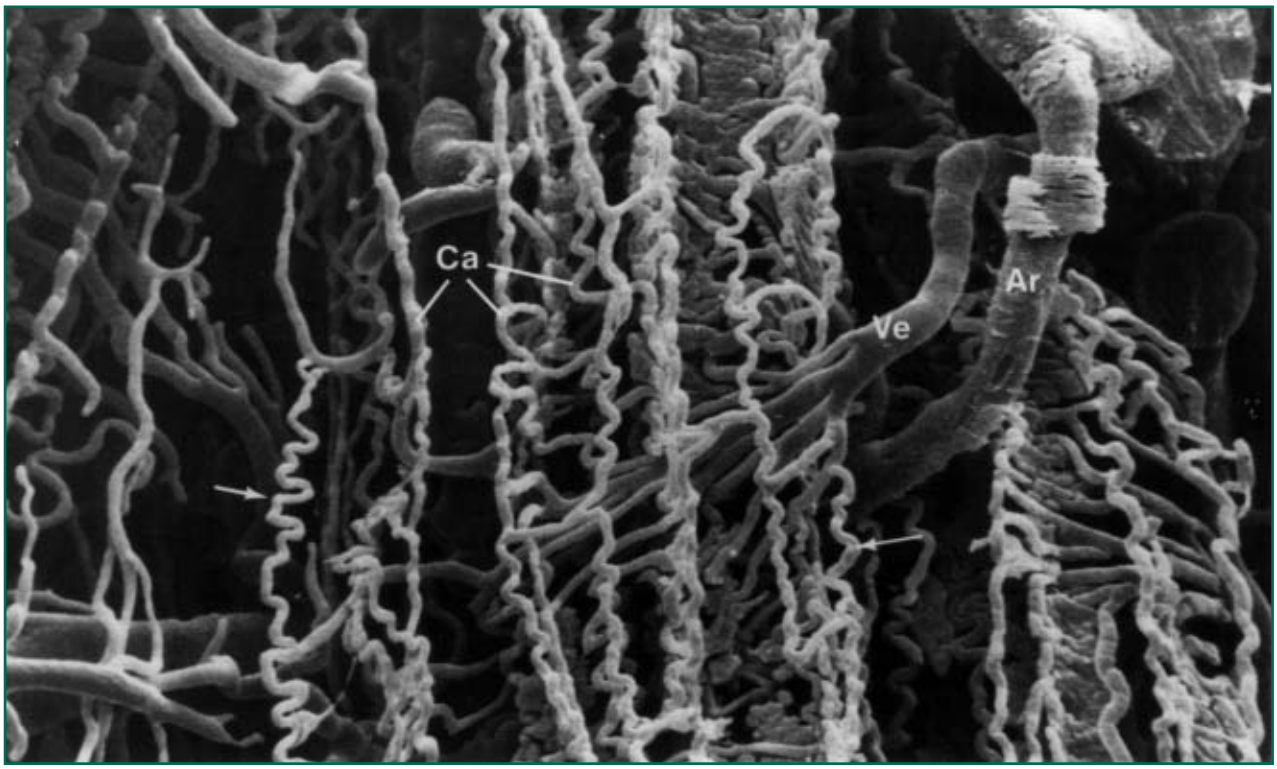

Figure 1. *Cast of the microvasculature of skeletal muscle. Capillaries (Ca) are oriented longitudinally in the same direction as the muscle fibers they supply. The capillary network is supplied by an arteriole (Ar) and drained by a venule (Ve). Reproduced from: Kessel RG, Kardon RH, eds.* Tissues and Organs: A Text-Atlas of Scanning Electron Microscopy. *San Francisco, Calif: W.H. Freeman & Co. Copyright © 1979, W.H. Freeman & Co. With permission.*

microvasculature are common to all vascular beds. In general, the microcirculation consists of arterioles, capillaries, and venules, which form a branching, tapered network of approximately circular tubes *(Figure 1)*. The fundamental interorgan variations in microvascular anatomy relate primarily to branching patterns, vessel densities, and the fine structure of capillaries.[1-4]

Arterioles

Each small artery can give rise to several (1-4) arterioles as its diameter decreases toward the tissue core (or periphery). Arterioles are generally less than 500 µm in diameter, with an external muscular coat that consists of 2 to 4 circumferentially arranged smooth muscle cells. As arteriolar diameter decreases, so does the number of smooth muscle layers. The terminal (precapillary) arterioles have an internal diameter of l5 to 20 µm and are surrounded by only one layer of smooth muscle cells. Arterioles, like their parent vessels (large and small arteries), have an inner lining of endothelial cells, and the periphery of the vessel may be invested by fibroblasts and a network of nonmyelinated nerves. Arterioles can undergo active changes in diameter, about two- to threefold in the smallest vessels and 20% to 40% in the larger arterioles, depending on the initial state of vascular tone.

Capillaries

Arterioles give rise to capillaries when the internal arteriolar diameter falls below 50 µm, but in the majority of cases capillaries are derived from terminal arterioles. Capillaries largely consist of a tube (of 4 to l0 µm internal diameter) lined by a single layer of endothelial cells and a thin basement membrane. The ultrastructure and endothelial thickness of capillaries vary considerably between and within organs.[2] Based on the fine structure of their endothelium, capillaries are generally divided into the following categories: (i) fenestrated; (ii) continuous; and (iii) discontinuous. In many tissues, only a fraction (eg, 20% to 30%) of the capillaries are open to perfusion under resting conditions.[5] The ability of tissues to recruit additional perfused capillaries during periods of stress (eg, hypoxia) has been attributed to the existence of "precapillary sphincters," which may represent one or two layers of smooth muscle that surround the entrance of a capillary. The capillary network, with its large surface area and an endothelial

barrier that is highly permeable to lipid-soluble and small water-soluble molecules, appears well suited for the exchange of gases, nutrients, and water between the blood stream and tissues.

Venules

Capillaries drain into larger vessels that are also devoid of a smooth muscle coat. These postcapillary venules represent the segment of the microvasculature that is most reactive to inflammation and contain intercellular endothelial junctions that can open to allow plasma proteins and circulating cells (leukocytes) to escape from the blood stream.[4] Smooth muscle appears on the media of larger venules (muscular venules) that drain the postcapillary venules. The organization of the venular network is similar to that of the arterioles except that venules are two to three times wider and are somewhat more numerous (eg, 2 to 4 times in heart) than the arterioles. Furthermore, smooth muscle is more abundant in arterioles than in muscular venules. The passive, distensible nature of the postcapillary and muscular venules accounts for the ability of these microvessels to store and mobilize significant quantities of blood in certain organs.

Endothelial cells and smooth muscle cells

These cells represent the major functional elements of the blood vessel wall that allow arterioles, capillaries, and venules to carry out their functions. While the two cell types are clearly capable of functioning independently, there are processes that enable one cell type to influence the other. The phenomenon of endothelium-dependent vasodilation (discussed below) perhaps best exemplifies how endothelial cells can exert control over the tone of adjacent vascular smooth muscle cells through the production and liberation of vasoactive substances. There are, however, other cells that are either in contact with, or adjacent to, the blood vessel wall that can exert an influence on the activity of endothelial cells and/or vascular smooth muscle (VSM) cells. Pericytes and mast cells are examples of such auxiliary cells that can exert a profound influence on the function of arterioles, capillaries, and/or venules.

SELECTED ABBREVIATIONS AND ACRONYMS

bFGF	basic fibroblast growth factor
EDRF	endothelium-derived relaxing factor
I/R	ischemia and reperfusion
ICAM-1	intercellular adhesion molecule–1
mAbs	monoclonal antibodies
NO	nitric oxide
PAF	platelet-activating factor
PDGF	platelet-derived growth factor
PGI_2	prostacyclin
SOD	superoxide dismutase
TxA_2	thromboxane A_2
VCAM-1	vascular cell adhesion molecule–1
VEGF	vascular endothelial growth factor
VSM	vascular smooth muscle

Pericytes

Pericytes are ameba-shaped, actin-containing cells that are associated abluminally with capillaries and postcapillary venules.[6] These cells extend long, slender processes that are embedded within the basement membrane to directly contact the underlying endothelium. The density of pericytes in a vascular bed varies among tissues and between different-sized vessels. Pericytes are more numerous and have more extensive processes (more contact with endothelium) on venous capillaries and postcapillary venules. Studies on individually cultured and cocultured (with endothelial cells) pericytes reveal an ability of these cells to contract and to produce substances that modulate the development and function of endothelial cells (and vice versa). Accordingly, pericytes have been invoked as playing a role in the regulation of capillary blood flow, capillary growth, and vascular permeability, as well as being precursors to VSM cells.[5]

Mast cells

Mast cells are usually found closely apposed to the microvasculature, particularly the postcapillary venules. These cells are exquisitely sensitive to activation by a variety of stimuli, including neuropeptides (eg, substance P), oxygen radicals (eg, superoxide), lipid mediators (platelet-activating factor, leukotrienes), and bacterial peptides. Upon activation, mast cells release a number of substances that can influence the function of endothelial cells and vascular smooth muscle in all segments of the microvasculature. Mast cell–derived modulators of microvascular function include histamine, adenosine, nitric oxide, cytokines (eg, tumor necrosis factor, interleukin-1), proteases (eg, cathepsin G), and oxidants.[6]

Electrical communication

There is also evidence that propagation of electrical impulses along the endothelial cell monolayer as well as between endothelial cells and VSM cells may contribute to the coordination of microvessel responses to physiological stimuli.[7] Vasoactive agents applied directly onto capillaries have been shown to elicit dilation of upstream arterioles via a process that appears to involve electrical communication between the endothelial cells of capillaries and arterioles. Similar evidence has been presented to support the possibility of upstream propagation of an endothelial response from venules to capillaries. Measurements of intracellular potentials in endothelial cells and VSM cells of arterioles suggest that these cells also function as an electrical syncytium. Gap junctional tracers appear to move bidirectionally between adjacent smooth muscle or adjacent endothelial cells, as well as between endothelial cells and VSM cells, but not in the reverse direction. These observations indicate that the anatomical configuration of gap junctions in microvessels allows ready communication of electrical and perhaps chemical signals among the different control elements of the microcirculation, ie, arterioles, capillaries, and venules.

ARTERIOLES

Contribution of different-sized vessels to total vascular resistance

The profile of pressures within different segments of the microcirculation provides valuable insight into the distribution of resistances within a vascular bed as well as into the impact of a change in the caliber of specific vessels on total vascular resistance. In most tissues studied to date, it appears that vascular resistance primarily lies in arterioles with diameters less than 200 µm. As illustrated in *Figure 2*, 30% of basal vascular resistance in the coronary circulation can be attributed to 150 to 250 µm diameter arterioles, with an additional 40% of the total resistance to flow generated by arterioles of smaller caliber (<150 µm).[7,8] Capillaries and veins account for only 25% of the total resistance. These observations support the view that a major portion of the resistance in the coronary circulation, as in other vascular beds, is controlled by the arterioles. This characteristic imparts a dominant role to the smallest arterioles in the regulation of blood flow under resting conditions. However, this role can be shifted towards larger arterioles as well large conducting

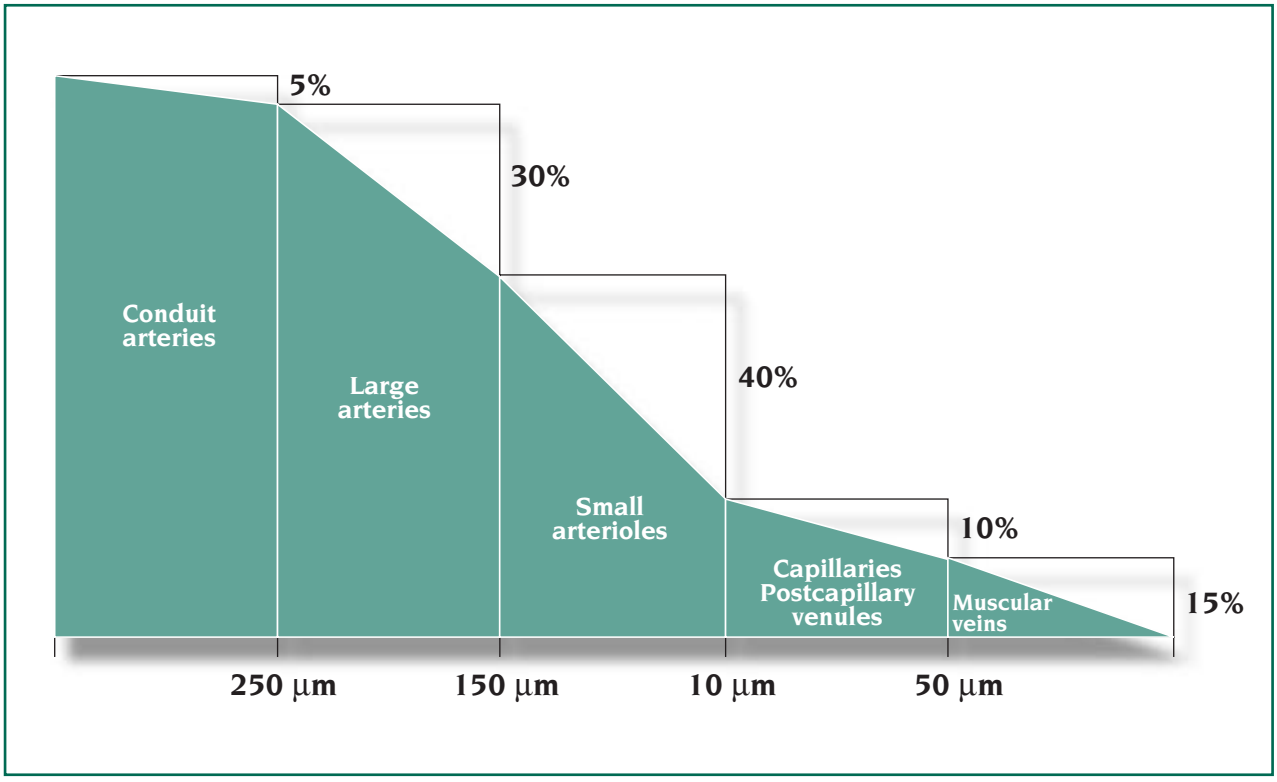

Figure 2. *The distribution of resistances along the different segments of the microvasculature. The resistances were deduced from the drop in pressure between vascular segments. Modified from ref 8.*

arteries under conditions of intense vasodilation and in coronary artery disease.[7,8] There is also mounting evidence that the various factors that act to regulate blood flow in tissues exert their actions at discrete sites along the arterial tree. This site-specificity of microvessels' responsiveness to physiological stimuli suggests that regulatory mechanisms are not uniform throughout the vascular tree.[9]

Factors governing the tone of resistance microvessels

Role of endothelial cells

The resting tone of arterioles appears to result from a complex interaction of metabolic, myogenic, neurohumoral, and physical (eg, stretch or shear) signals received by the blood vessel wall. Until recently, the responses of arterioles to physiological stimuli were believed to be initiated almost exclusively by signals sensed by VSM cells. There is now clear evidence that endothelial cells play an important role in maintaining vascular tone by releasing substances that modulate the delicate balance between vasodilation and vasoconstriction. An appreciation for the contribution of endothelial cells to vascular tone comes from studies demonstrating that acetylcholine dilates arterial smooth muscle only if the endothelium is intact and viable.[10] Indeed, the loss of endothelial cell integrity can result in the transformation of potent dilators (eg, acetylcholine, substance P) to vasoconstrictors. This mechanism may also account for the asymmetrical responses of human coronary arteries to acetylcholine: it is a dilator when applied intraluminally, but a constrictor when applied abluminally.

Further investigations of the phenomenon of endothelium-dependent vasodilation have implicated a short-lived (biological half-life = 30 s) substance that resembles nitrosovasodilators in that it activates VSM cell soluble guanylate cyclase, producing a rise in cGMP.[11,12] Furthermore, this substance, termed endothelial-derived relaxing factor (EDRF) is inactivated by superoxide anions and protected by superoxide dismutase (SOD). Nitric oxide (NO), a potent VSM relaxant that is produced in endothelial cells through the oxidation of L-arginine by the enzyme NO synthase, is now generally recognized to be EDRF. A role for NO in modulating arteriolar tone is supported by studies employing L-arginine analogs that inhibit NO synthase, as well as L-arginine supplementation to enhance NO production. For example, the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) has been shown to constrict coronary microvessels and to inhibit the vasodilation normally elicited by cardiac pacing or adenosine administration.[7]

Endothelial cells produce a variety of substances, in addition to nitric oxide, that can contribute to basal vascular tone.[11,12] Prostacyclin (PGI_2) and adenosine are both potent VSM relaxants that are produced by endothelial cells. However, endothelial cells are equally capable of producing vasoconstrictor agents, the most powerful of which is endothelin (ET). The endothelin family of vasoactive peptides (ET-1, ET-2, ET-3) can interact with receptors found on VSM cells (ET_A) or endothelial cells (ET_B), with the former receptors accounting for ET-induced constriction of arterioles. Angiotensin-converting enzyme (ACE) is produced by, and expressed on, the lumenal surface of endothelial cells, thereby allowing these cells to make a local contribution to the powerful renin-angiotensin system. Other vasoconstrictor agents generated by endothelial cells include thromboxane A_2 (TxA_2) and superoxide. Indeed, it has been suggested that under normal conditions a balance exists between the production of PGI_2 and TxA_2, and between NO and superoxide, which allows the maintenance of normal arteriolar tone. Conditions that upset this balance between endothelial cell–derived vasodilators and vasoconstrictors can result in profound alterations in tissue perfusion.[9]

Metabolic control

It is widely held that factors (metabolites and tissue pO_2) that are linked to oxidative metabolism may represent the most important mechanism for blood flow regulation in metabolically active tissues.[3] When metabolic activity increases, tissue pO_2 falls and metabolites accumulate in the tissue. Arterioles dilate in response to either a reduction in tissue pO_2 or upon exposure to metabolites such as adenosine. Hence, it has been proposed that the changes in tissue milieu that are associated with an increased oxygen consumption result in vasodilation and a consequent increase in blood flow, which serves to deliver more oxygen to the tissue.[3] The increase in oxidative metabolism associated with cardiac pacing results in dilation of coronary arterioles of different caliber, however, the magnitude of the vasodilation is inversely related to arteriolar diameter.[10]

Myogenic control

Arteriolar smooth muscle intrinsically responds to stretch by contracting, and it relaxes when smooth muscle tension is reduced.[3] An extension of this phenomenon is the myogenic mechanism that enables arterioles to contract when transmural pressure is

increased and relax when transmural pressure falls. This mechanism invokes a role for arteriolar wall tension, rather than blood flow, as the controlled variable in the vasculature. According to the Laplace relationship (T=P×r), when intravascular pressure (P) is doubled, arteriolar radius (r) must decrease to half its initial value in order to restore tension (T) to its original value. By its nature, the myogenic mechanisrn tends to maintain a constant intravascular pressure at the microvascular level. Myogenic responses have been demonstrated in arterioles of the coronary, renal, intestinal, and other vascular beds.

Flow-dependent dilation

Increase in blood flow through various vascular beds is accompanied by dilation of small arteries and arterioles.[10] This response is endothelium-dependent and may be explained by the action of shear stress on endothelial cells. Increased shear stress has been shown to elicit a calcium-dependent activation of endothelial cell NO synthase and to activate phospholipase A_2. These changes likely result in an increased endothelial production of NO and PGI_2, and consequently lead to relaxation of the underlying VSM cells. A role for NO in flow-dependent dilation of isolated coronary arterioles is supported by the observation that NO synthase inhibitors abolish the response.

Spatial heterogeneity of vasoregulatory mechanisms

The technique of intravital videomicroscopy has enabled investigators to assess the importance of each of the aforementioned regulatory mechanisms in arterial microvessels of varying sizes. These studies have revealed that different segments of the arterial tree can respond in a profoundly different manner to various physiological and pharmacological stimuli. *Figure 3* illustrates this heterogeneity of responsiveness along an arterial circuit, ie, from small arteries to terminal arterioles.[8,10] The first panel under the illustration of the branching pattern of the vascular bed depicts a simplified hypothetical relationship between vessel diameter and location along the coronary vascular circuit. The next three panels illustrate the relative responsiveness of the different-sized vessels to changes in pressure (myogenic responses), flow (flow- or shear-induced vasodilation), or metabolic stimuli (adenosine). These patterns of vascular responsiveness indicate the following: (i) while small arteries do not respond to a myogenic stimulus, arterioles respond with powerful contractions; (ii) flow-induced

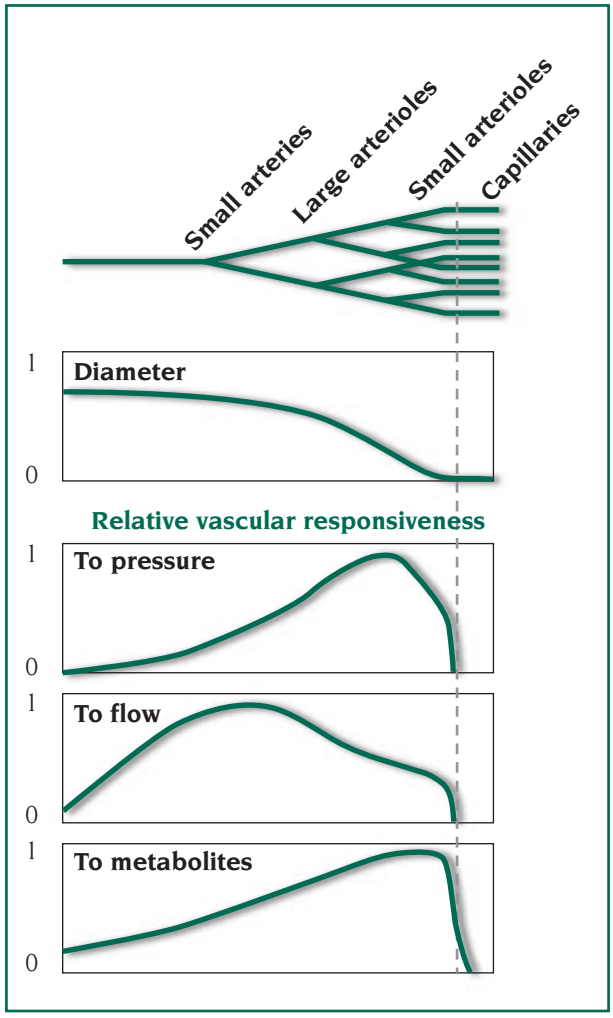

Figure 3. *Relative responsiveness of different segments of the arterial tree to pressure (myogenic responses), flow (shear- or flow-induced dilation), and metabolic stimuli. Modified from ref 10.*

(endothelium-dependent) dilation is observed throughout most of the arterial tree with large arterioles exhibiting the most intense response to flow changes; (iii) the responsiveness to metabolic factors increases as arterial diameter decreases, such that metabolic control is a dominant influence on the tone of terminal arterioles; and (iv) there is considerable redundancy of blood flow control mechanisms within those segments of the arterial tree (arterioles) that normally contribute most to total vascular resistance.

The differential sensitivity of different-sized microvessels to vasoactive stimuli can also be demonstrated using various pharmacologic agents.[8] For example, norepinephrine infusion into the coronary circulation (which activates α-adrenergic receptors) leads to constriction of arterioles larger than 100 µm in diameter

and simultaneously dilates arterioles less than 100 µm in diameter. The dilation of smaller arterioles likely results from myogenic VSM relaxation occurring secondary to a reduction in intravascular pressure (caused by the upstream vasoconstriction). Nitroglycerin elicits a dose-dependent dilation of coronary microvessels larger than 200 µm, with little or no effect on smaller arterioles. The calcium blocker nifedipine, on the other hand, elicits strong dilation of different-sized coronary microvessels.

Arteriolar dysfunction and circulatory disorders

Recent advancements in our understanding of the multiple factors that regulate the tone of arterial microvessels have had a profound influence on the evolution of concepts proposed to explain the pathogenesis of several circulatory disorders.[11,12] The abnormal tissue perfusion associated with certain diseases has long been attributed to dysfunction of specific components of the vessel wall. Well-documented examples of this include the hypertrophy and altered function of arterial VSM cells in arterial hypertension as well as the loss of pericytes and basement membrane thickening that accompanies the development of diabetes mellitus. However, in recent years, the focus of attention has shifted almost entirely to endothelial cells as the primary dysfunctional component of the vessel wall in different disease states.[9,11,12] *Table I* lists some of the cardiovascular disorders for which there is evidence of dysfunctional endothelium-dependent regulation of vascular tone. It is noteworthy that the circulatory disorders listed in *Table I* include conditions characterized by either a reduced (eg, ischemia/reperfusion), elevated (sepsis), or normal (hypertension) level of tissue perfusion (blood flow). An element common to most of these conditions is an abnormality in basal and/or stimulated nitric oxide bioavailability in the affected tissues. Some disorders (eg, sepsis, inflammation) appear to be associated with an overproduction of NO, while other conditions (ischemia/reperfusion, atherosclerosis) are characterized by a diminished production and/or reduced bioavailability (inactivated by superoxide) of the endothelial cell–derived vasodilator.

This new appreciation of the potential role of endothelial cells in disease progression has also led to an improved understanding of how circulating factors (eg, cholesterol) and blood cells (eg, leukocytes) that normally interact with vascular endothelium can contribute to microvessel dysfunction. Hypercholesterolemia, a well-established risk factor for coronary artery disease, results in a profoundly attenuated endothelium-dependent relaxation to vasodilator stimuli such as acetylcholine and bradykinin.[12] This phenomenon is manifested in both large arteries and arterioles, and has been demonstrated in the forearm and coronary microcirculations of patients with hypercholesterolemia.[13] Studies employing NO synthase inhibitors in these subjects have revealed that stimulated, but not basal, production of NO is impaired.[13] Some investigators have proposed that an enhanced superoxide production by endothelial cells leads to a reduced bioavailability of NO in microvessels subjected to hypercholesterolemia. There is also evidence suggesting that L-arginine (the substrate for NO production) administration restores endothelium-dependent vasodilation in hypercholesterolemic subjects. While the clinical relevance of these observations has not been firmly established, the recognition that hypercholesteromia affects the function of arterioles as well as conduit arteries may have important therapeutic implications, particularly in view of recent reports describing an association between endothelial dysfunction and cholesterol levels in the high normal range in humans.[14]

Leukocytes can also exert a modulating influence on endothelium-dependent vascular reactivity. Various tissues exposed to ischemia/reperfusion (I/R) exhibit an impaired reactivity of small arteries and arterioles to acetylcholine and other endothelium-dependent vasodilators. This response is accompanied by a diminished capacity of endothelial cells in postischemic tissues to generate NO. Two experimental strategies have proven effective in reversing the I/R-induced deficit in endothelium-dependent vasodilation, ie, scavenging oxygen-

CARDIOVASCULAR DISORDERS	
Arterial hypertension	Diabetes mellitus
Hypercholesterolemia	Smoking
Septic shock	Graft atherosclerosis
Vasospasm	Ischemia/reperfusion
Chronic heart failure	Syndrome X

Table I. *Cardiovascular disorders with dysfunctional endothelium-dependent regulation of vascular tone (from refs 9 and 12).*

derived free radicals and preventing leukocyte–endothelial cell adhesion. The protective action of scavenging enzymes such as SOD is consistent with evidence that I/R is associated with an excessive production of superoxide, which can inactivate NO. The preservation of endothelium-dependent vascular reactivity observed in animals receiving monoclonal antibodies against leukocyte adhesion molecules or that are genetically deficient in the same adhesion glycoproteins implicates adherent leukocytes in the I/R-induced vascular dysfunction. These observations, coupled with the protective actions of SOD treatment, suggest that adherent leukocytes, rather than endothelial cells, may be the major source of superoxide that inactivates NO after I/R. Furthermore, the results obtained from these studies raise the possibility that leukocytes may account for the impaired endothelium-dependent vasodilation associated with other circulatory diseases. Such a mechanism has been invoked to explain some of the altered vascular reactivity associated with hypertension.[15]

CAPILLARIES

Role of capillaries as exchange vessels

There are several anatomical and physiological properties of a typical capillary bed that enable this segment of the microcirculation to serve as the major site of exchange of gases (O_2, CO_2), nutrients (glucose), and water between blood and parenchymal cells. The numbers of capillaries, capillary lumenal volume (internal diameter), as well as the dimensions and frequency of pores that pierce the capillary endothelium determine the effective surface area available for exchange across capillaries. The low red blood cell velocity and low intravascular pressure in capillaries also serve to optimize the rates of O_2 transport and fluid filtration, respectively. The capacity for pore-bound diffusion of small water-soluble nutrients (eg, glucose) across capillaries varies according to the ultrastructural classification of capillaries, with the smaller pores of continuous-type capillaries allowing lower exchange rates than capillaries of the fenestrated and discontinuous type. Nonetheless, under physiological conditions, the capacity for transcapillary nutrient diffusion is several times the rate of delivery of these nutrients to the tissue by blood flow.

The substantial organ-to-organ variability in capillary exchange parameters is believed to reflect the specific functional and metabolic demands placed on the microvasculature by different tissues. This point is illustrated by the profound differences in capillary surface area estimated for various tissues *(Table II)*.[16] The pulmonary microcirculation, with its unique role as a gas exchange membrane, exhibits the largest capillary surface area of all tissues. The myocardium is also well-endowed with capillaries, having a capillary surface area per gram of tissue that is nearly 10 times higher than that noted for the less metabolically active skeletal muscle. The potential impact of such a large capillary surface area on tissue oxygenation can be appreciated when one considers that the number of capillaries per cardiac muscle fiber ranges between 0.9 and 1.1, with an average diffusion distance between capillary and cardiac muscle fiber of 8.0 µm, which compares to a diffusion distance of 15 to 20 µm in skeletal muscle.[17]

REGIONAL DIFFERENCES IN CAPILLARY DENSITY

Organ	Capillary density (cm^2/g)
Lung	3000
Heart	500
Brain	240
Small intestine	125
Skeletal muscle	70

Table II. *Capillary density in various organs. Data from ref 15.*

Modulation of functional and anatomical capillary density

Because of the demands normally placed on the coronary circulation by the working heart, nearly all capillaries are always open to perfusion. However, in most other vascular beds, only a small fraction (eg, 20% to 30%) of the total capillary population is normally patent for blood perfusion. Physiologic stresses such as an increased metabolic demand and/or reduced blood flow in these tissues are generally associated with the recruitment of addi-tional perfused capillaries. Consequently, the classic concept has been that alterations in functional capillary density allow local modulation of O_2 exchange area and capillary-to-cell diffusion distances.[3] The structural element that governs the patency of exchange vessels (so-called "precapillary sphincters") has not been clearly defined in many vascular beds. This has led to the proposal that terminal arterioles (10 to 15 µm diameter) regulate functional capillary density because these microvessels exhibit vasomotion (entrained fluctuations in microvessel diameter and flow) and account for only a

fraction of total microvascular resistance.[3] Alternatively, local alterations in capillary lumen diameter mediated by contractile elements in or around capillaries could provide extremely fine spatial control of the number of perfused capillaries. Pericytes may represent the contractile element that mediates subtle narrowing of the capillary lumen that is sufficient to hinder the passage of red blood cells.[5] Although the structural equivalent of the "precapillary sphincter" remains undefined, it is clear that this element exerts its influence on capillary patency by responding to sudden changes in the tissue environment (eg, decreased oxygen tension) that necessitate an appropriate change in perfused capillary surface area.

Capillary proliferation represents another mechanism whereby tissues can compensate for chronic alterations in oxygen delivery and/or metabolic demand, and restore organ function after injury.[17] The endothelium that lines normal capillaries is an extremely stable population of cells with very low mitotic activity; only 0.01% of endothelial cells in the body are dividing at any given time.[18] Hence, capillary growth and proliferation are rarely observed in normal adult tissues except during wound healing and cyclical events in the female reproductive cycle (ovulation, menstruation).[18] In the presence of appropriate stimuli, the process of angiogenesis (development of new blood vessels from an existing vascular network) can be initiated. Endothelial cells exposed to such stimuli first release proteases that degrade the underlying basement membrane and surrounding structural elements. The cells then migrate toward the angiogenic (chemotactic) stimulus within the extravascular space, with a concomitant proliferation of the endothelial cells lining the vessel wall to replace the previously migrated cells. The migrating and proliferating endothelial cells form cord-like structures in target tissues that later canalize to form functional vessels, which are further stabilized by surrounding pericytes. The initiation of angiogenesis is often associated with an increased capillary permeability that serves to enrich the adjacent interstitial compartment with plasma components.[19,20]

A variety of physiologic factors have been implicated in the modulation of angiogenesis *(Table III).*[18-20] These factors have been shown to exert an influence on the angiogenic process through actions on the migration and/or proliferation of endothelial cells and/or smooth muscle cells and/or smooth muscle cells. Vascular endothelial growth factor (VEGF), one of the best studied angiogenic factors, is produced by normal as well as by many tumor cells, and can be found at elevated levels in the serum and urine of cancer patients.[19] This angiogenic peptide increases capillary permeability and stimulates both the migration and proliferation of capillary endothelium in vivo. Transgenic animals with a null mutation for the VEGF gene exhibit embryonic lethality, characterized by absent or delayed endothelial cell differentiation and impaired angiogenesis. In vitro, VEGF is a potent and selective mitogen for endothelial cells, and this effect appears to be mediated by nitric oxide since NO synthase inhibitors block VEGF-induced endothelial cell proliferation.

Less is known about endogenous inhibitors of angiogenesis *(Table III).* Thrombospondin, which is secreted by normal cells, is believed to contribute to the capillary quiescence observed in healthy tissue. There is also a growing body of evidence that implicates proteolytic fragments of the extracellular matrix as inhibitors of angiogenesis. These factors may play an important role in the resolution of transitory angiogenic responses, such as wound healing. Part of the intense interest in defining the factors that either inhibit or induce angiogenesis relates to the desire to identify therapeutic strategies that will enhance capillary proliferation in ischemic tissues or restore a functional endothelial monolayer after angioplasty.[20]

ANGIOGENIC FACTORS

Inducers	Inhibitors
Basic fibroblast growth factor (bFGF)	Thrombospondin
Vascular endothelial growth factor (VEGF)	Angiostatin*
Platelet-derived growth factor (PDGF)	Proteolytic fragments of laminin, fibronectin, and collagen
Transforming growth factor β(TGFβ)	Interleukin-12

***Table III.** Modulators of capillary proliferation. *Circulating proteolytic fragment of plasminogen.*

Pathological alterations in functional capillary density

Alterations in perfused capillary density have been implicated in the adaptive responses of tissues to prolonged hypoxia (eg, exercise) and as a critical step in the pathogenesis of certain disease states (diabetes mellitus, cancer).

Such alterations in capillary perfusion can result from intravascular events that lead to the acute obstruction of microvessels or from long-term changes in total capillary density that reflect either the dissolution or proliferation of capillaries. Hemorrhagic shock and ischemia are conditions that exemplify the potential complications that can arise from acute capillary obstruction. In both instances, circulating blood cells (leukocytes, platelets) and/or aggregates of these cells with fibrin (microthrombi) can lodge within the capillary lumen, thereby preventing the flow of blood through these vessels.[21] Furthermore, some of the leukocytes that become lodged in ischemic capillaries remain stuck upon restoration of perfusion pressure, causing "capillary no-reflow." A potential consequence of this capillary no-reflow is an increased resistance to blood flow in postischemic tissues and a diminished capacity to recover from the ischemic insult. For example, "no-reflow" can contribute to myocardial cell necrosis following I/R.

The propensity for capillary plugging with leukocytes in ischemic tissues has been attributed to a number of events, including increased stiffness of activated leukocytes, endothelial cell swelling, leukocyte–endothelial cell adhesion, and low driving pressures for leukocyte movement along the capillaries. It likely that the relative contribution of each of these factors to capillary no-reflow varies between organs. Thus, organs (eg, heart) that are perfused by capillaries with small internal diameters will be more sensitive to leukocyte plugging during and following periods of hypotension. Other tissues (eg, skin) that have large arterial-venous anastomoses, which shunt leukocytes past the capillary bed, will be less prone to leukocyte capillary plugging. The physical restriction or trapping of leukocytes within capillaries has been implicated as a major contributor to the leukocyte accumulation observed in postischemic myocardium, liver, brain, kidney, and skeletal muscle.[21] The strong correlation between the percentage of capillaries exhibiting no-reflow and the percentage of capillaries that contain granulocytes in postischemic tissues suggests that leukocyte trapping likely accounts for capillary no-reflow. Additional supportive evidence is provided by reports that demonstrate virtual elimination of capillary no-reflow in animals that are either rendered neutropenic or receive antibodies that interfere with leukocyte–endothelial cell adhesion in postcapillary venules.[21] Since the level of adhesion molecule expression on capillary endothelium is quite low (compared to venular endothelium), the ability of adhesion molecule–directed antibodies to attenuate capillary no-reflow has been attributed to an action on the downstream postcapillary venules. Adherent leukocytes in postcapillary venules appear to promote leukostasis in upstream capillaries by enhancing fluid and protein filtration across venular endothelium. The resulting interstitial edema raises interstitial fluid pressure to a level sufficient to occlude the capillary lumen and thereby facilitate the entrapment of leukocytes. *Figure 4* illustrates some of the potential mechanisms for the development of the no-reflow phenomenon.

Capillary proliferation provides a mechanism for tissues to compensate for the inadequate delivery of oxygen that is associated with chronic hypoxia, ischemia, and/or an increased metabolic demand. Capillary growth has been demonstrated in heart, brain, and skeletal muscle during exposure to high-altitude hypoxia, and in the heart and skeletal muscles during some types of exercice.[17] Humans exposed to a 24-week endurance training program exhibit an increased capillary density surrounding slow oxidative fibers. Furthermore, a 70% increase in myocardial capillary density has been demonstrated in rabbits after 3 weeks of bradycardial pacing (heart rate was half normal).[17] The mechanisms involved in these conditions of enhanced capillary proliferation remain poorly defined, but it seems likely that the hypoxic stimulus and/or chronically elevated blood flow (via flow-dependent production of nitric oxide) contribute to the angiogenic response.

The adaptive responses to a reduced capillary density in ischemic tissue appear rather slow and inefficient, since capillary surface area in a healed myocardial infarct (at 40 days after infarction) is not significantly higher than the value noted 3 days after the infarct. Dramatic increases in the expression of VEGF and its receptors on endothelial cells have been noted in a rat model of myocardial infarction.[19] Exogenously administered VEGF as well as other angiogenic factors (platelet-derived growth factor [PDGF] and basic fibroblast growth factor [bFGF]) have been shown to increase capillary density and induce vessel regrowth in different animal models of regional ischemia.[20] These findings have led to the concept of therapeutic angiogenesis whereby growth factor production in ischemic tissues is stimulated using drugs or gene therapy.[20]

Inhibition of capillary proliferation is also a logical therapeutic strategy for shrinking primary tumors and diminishing metastatic spread. Tumors grow without vessel proliferation up to a diameter of 1 to 2 mm (10^5) cells. Because the O_2 consumption of tumor cells is high, further tumor growth is not possible without

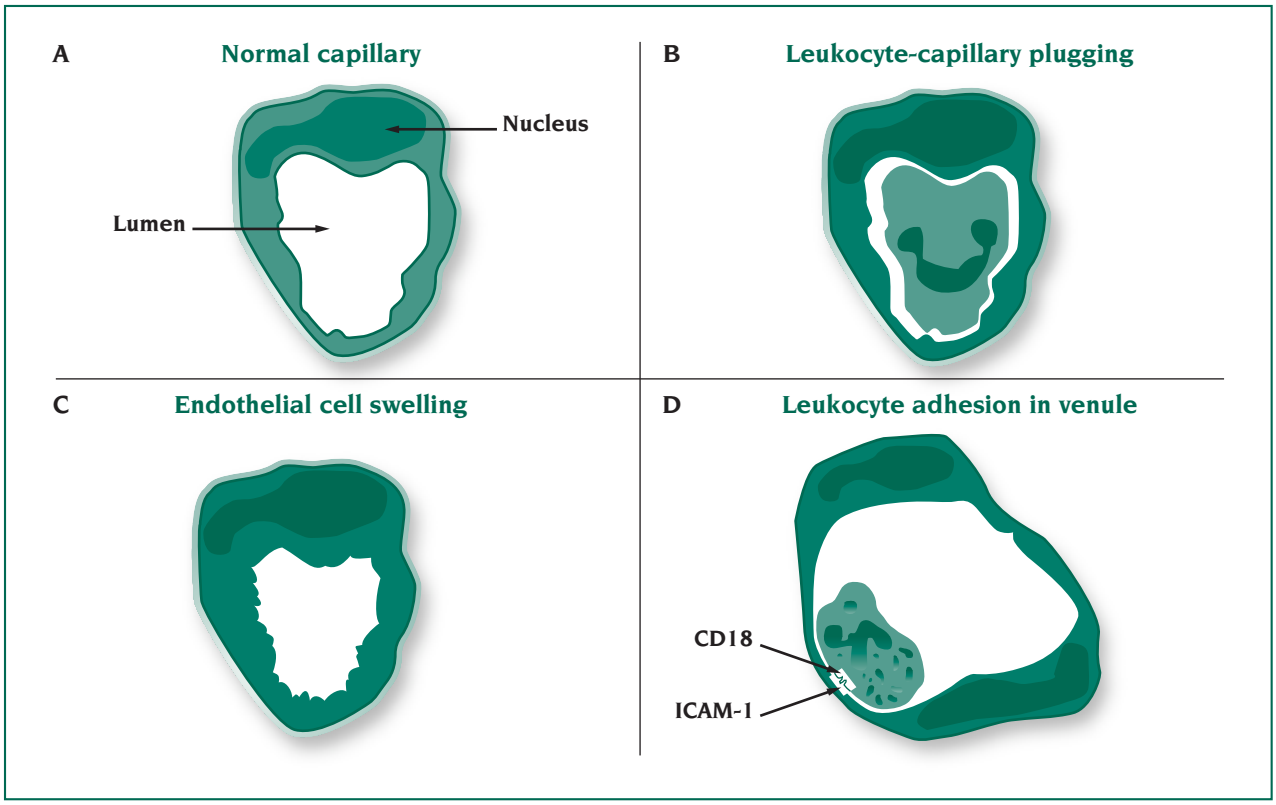

Figure 4. *Mechanisms that underlie the development of the "no-reflow" phenomenon. A. Normal capillary. B. Leukocyte-capillary plugging may be related to an increased stiffness of granulocytes and/or endothelial cell swelling. C. Increased endothelial cell swelling also decreases the diameter of the capillary lumen, which increases microvascular resistance. D. Leukocyte adherence in postcapillary venules can also increase the resistance to flow, either as a consequence of lumenal obstruction or by causing interstitial edema, which collapses upstream capillaries. ICAM-1, intercellular adhesion molecule–1.*

angiogenesis. In some tumors, the capillaries grow at such an incredible rate that 40% of the tumor mass is endothelial cells. Hence, reports describing inhibition of tumor growth in animals receiving monoclonal antibodies to VEGF provide hope for the eventual use of comparable therapeutic strategies in humans. Antiangiogenic agents are also being tested in trials for treatment other conditions, such as diabetic ocular neovascularization, childhood hemangiomas, psoriasis, and arthritis.[19]

VENULES

Postcapillary venules and small venules have long been known for their contribution to the capacitance function of the cardiovascular system. These distensible microvessels serve as the major storage site for blood in many tissues, particularly in splanchnic organs. To certain physiological stimuli, the venules respond with intense constriction, which expels the stored blood into the central circulation. This mechanism accounts in part for the ability of sympathetic activation to maintain adequate levels of cardiac output during periods of exercise or following hemorrhage.

The application of intravital videomicroscopic techniques to the field of microvascular research has led to the recognition that venules represent the major site of transvascular protein exchange (vascular permeability to plasma proteins) and leukocyte trafficking (leukocyte–endothelial cell adhesion).[2] The localization of these inflammatory functions in venules is believed to reflect the unique characteristics of endothelial cells in this segment of the microcirculation.[22] Consequently, the literature is replete with reports that describe the responses of cultured venous endothelial cells (usually derived from human umbilical vein) to various inflammatory stimuli. These in vitro studies, coupled with data derived from experiments utilizing intravital microscopy, have improved our understanding of the potential contribution of venules to the pathogenesis of certain cardiovascular diseases and vascular disorders in which there is a significant inflammatory component.[23]

Leukocyte–endothelial cell adhesion

Circulating leukocytes are recruited to sites of inflammation and tissue injury by a highly coordinated process that occurs primarily in postcapillary venules *(Figure 5)*. As leukocytes exit capillaries, hemodynamic forces give rise to an outward radial movement of leukocytes toward the venular endothelium. This margination process is generally attributed to red blood cells (which normally pile up behind the larger leukocytes in capillaries) that overtake the leukocytes and tend to push them toward the venular wall. The initial adhesive interaction between the leukocytes and venular endothelium is rolling. This low-affinity (weak) interaction is subsequently strengthened, such that the leukocytes attach to the endothelium and remain stationary. The leukocytes are then able to migrate into the interstitium through spaces between adjacent endothelial cells. These interactions are regulated by sequential activation of different families of adhesion molecules expressed on the surface of neutrophils and endothelial cells.[23]

Table IV summarizes some of the adhesion molecules expressed on the surface of endothelial cells and their respective counterreceptors on circulating leukocytes. Lectin-like adhesion glycoproteins, called the selectins, mediate leukocyte rolling. Both P- and E-selectin expression are increased on endothelial cells when the appropriate stimuli are present in inflamed tissue. P-selectin, which is stored in endothelial cells as a preformed pool, can be rapidly mobilized to the cell surface by stimuli such as histamine, hydrogen peroxide, and leukotrienes. A slower, more prolonged (transcription-dependent) expression of P-selectin can also be demonstrated (peaking at 4 h) after cytokine or endotoxin stimulation. E-selectin, which does not exist in a preformed pool, is entirely under transcriptional regulation and requires about 3 h to reach peak expression on intestinal endothelial cells.
Endothelial cell adhesion molecules that mediate firm adhesion of leukocytes include intercellular adhesion molecule–1 (ICAM-l) and vascular cell adhesion molecule–1 (VCAM-1). Both of these glycoproteins are constitutively expressed on venular endothelial cells, but the density of expression can be profoundly increased following challenge with cytokines or bacterial endotoxin. The expressions of P-selectin, E-selectin, ICAM-1, and VCAM-1 on vascular endothelial cell are temporally coordinated to ensure that the processes of leukocyte rolling and firm adhesion/emigration can occur for several hours after the initiation of an inflammatory response *(Figure 6).* The importance of these endothelial cell adhesion molecules and their counterreceptors on leukocytes in different animal models of

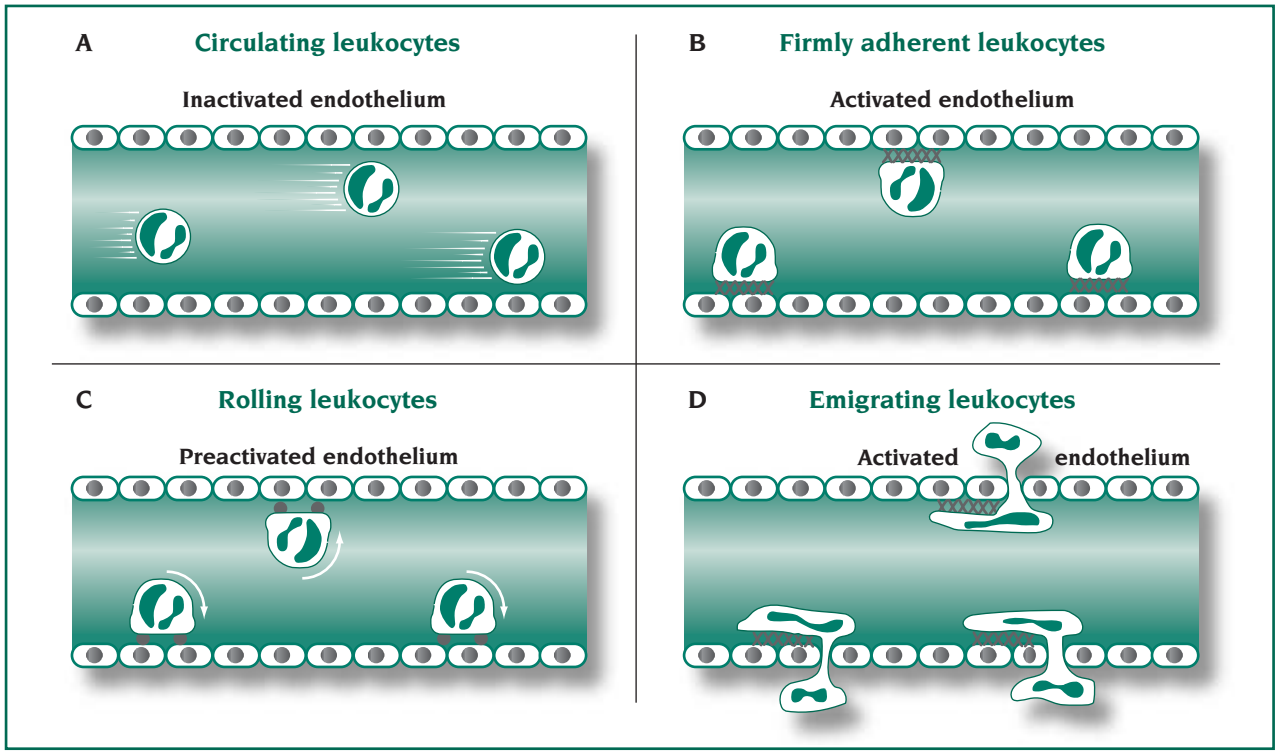

Figure 5. *Sequence of events that lead to the binding of leukocytes to activated endothelial cells and extravasation into inflamed tissue.*

ENDOTHELIAL CELL ADHESION MOLECULES: LIGANDS AND FUNCTIONS

Endothelial cell adhesion molecule	Leukocyte receptors	Adhesion response
P-selectin	L-selectin PSGL-1	Rolling
E-selectin	L-selectin	Rolling
ICAM-1	CD11/CD18	Adherence Emigration
VCAM-1	VLA_4	Adherence Emigration

Table IV. *ICAM-1, intercellular adhesion molecule–1; PSGL = P-selectin glycoprotein ligand; VCAM-1, vascular cell adhesion molecule–1; VLA_4 = very late antigen–4.*

inflammation has been demonstrated using either adhesion molecule–specific blocking monoclonal antibodies (mAbs) or mice that are genetically deficient in one or more adhesion molecules.[23]

There are several other factors that influence leukocyte–endothelial cell adhesion in postcapillary venules. Nitric oxide, adenosine, and prostacyclin produced by endothelial cells tend to prevent adhesion, while the oxygen radicals (superoxide, hydrogen peroxide) generated by activated leukocytes, and endothelial cells promote leukocyte adhesion.[23] These agents appear to exert their actions by interfering either with the production of inflammatory mediators (eg, platelet-activating factor [PAF]) that induce leukocyte adhesion or with the induction of adhesion molecule expression on endothelial cells and/or leukocytes. Auxiliary cells, such as mast cells (histamine), macrophages (cytokines), and platelets (leukotrienes), also produce substances that can promote leukocyte–endothelial cell adhesion.[6] Agents that promote the accumulation of endogenous antiadhesion molecules (eg, nitric oxide) as well as agents that either neutralize adhesion glycoproteins (mAbs) or prevent the accumulation of proinflammatory factors (eg, cytokines) provide therapeutic avenues for inhibition of leukocyte recruitment in postcapillary venules.

The physical forces generated by the movement (flow) of blood in the microcirculation also play an important role in the modulation of leukocyte–endothelial cell adhesion.[23] The prevailing shear rate exerted on the walls of postcapillary venules determines the level of leukocyte rolling and firm adherence, and dictates the contact area between leukocytes and the endothelial cell surface. Even in the absence of an inflammatory stimulus, graded reductions in venular shear rate for brief periods (<2 min) elicit progressive recruitment of

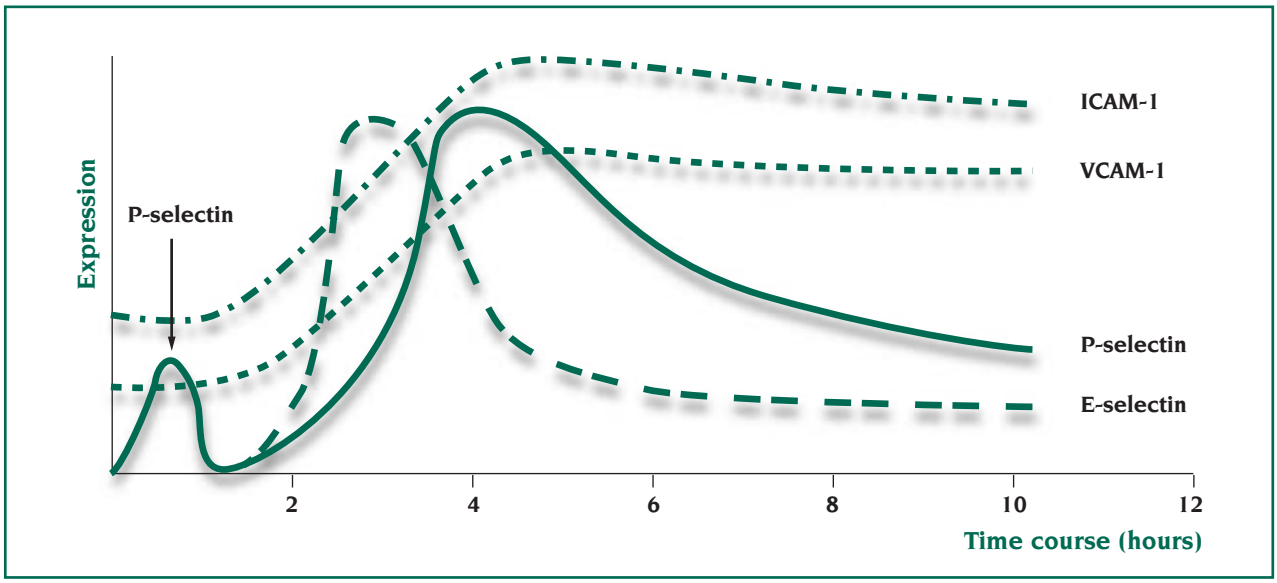

Figure 6. *Time course of expression of adhesion glycoproteins on the surface of histamine- (early rise in P-selectin expression) or cytokine-activated endothelial cells in vivo. ICAM-1, intercellular adhesion molecule–1; VCAM-1, vascular cell adhesion molecule–1.*

both rolling and firmly adherent leukocytes. Similarly, it has been noted that the number of adherent leukocytes recruited into venules by an inflammatory stimulus is inversely proportional to the wall shear rate, suggesting that it is easier for leukocytes to create strong adhesive bonds with endothelial cells at low shear rates and that high shear rates may prevent the creation of such bonds. While the higher wall shear rates experienced by endothelial cells in arterioles (compared to venules) have been invoked to explain the rarity of leukocyte adhesion in arterioles, recent evidence suggests that the leukocyte's preference for the venular endothelium reflects the higher density of adhesion molecules expressed by these cells.[22]

The firm adhesion of neutrophils to vascular endothelial cells is usually associated with the enhanced production and release of highly reactive and potentially toxic substances from neutrophils. Activated neutrophils utilize the plasma membrane–associated enzyme reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase to produce superoxide, which subsequently reacts with itself to generate hydrogen peroxide.[24] The potent oxidizing and chlorinating agent hypochlorous acid (HOCl) is also produced when hydrogen peroxide and extracellular chloride ions react with myeloperoxidase, an enzyme released from neutrophil granules. Activated neutrophils also secrete a variety of proteases, which can lead to uncontrolled proteolysis of vascular wall elements (eg, basement membrane) and of the interstitial matrix. Many of these proteases are secreted in an inactive (latent) form that is dependent on oxidative mechanisms (HOCl) for activation.[24] Extracellular fluid is well-endowed with antioxidants and antiproteases that limit the cytotoxic potential of circulating neutrophils. However, when neutrophils adhere to venular endothelial cells, a sequestered microenvironment is created (at the leukocyte–endothelial cell interface) that allows neutrophil-derived oxidants and proteases to overwhelm plasma antioxidants and antiproteases, thereby enabling the neutrophil to exert its full cytotoxic potential at the vessel wall. The accompanying vascular injury is often manifested as increased vascular permeability, increased fluid and protein filtration, and interstitial edema.[4]

Vascular permeability to plasma proteins

Vascular endothelial cells normally serve as a barrier that minimizes the movement of fluid and proteins from blood to interstitium. When this barrier function is lost, either as a consequence of endothelial cell damage or contraction of adjacent endothelial cells, plasma proteins gain greater access to the interstitial compartment, which results in an elevated oncotic pressure and excess fluid accumulation, ie, interstitial edema. While capillaries are the major source of fluid that is filtered into the interstitial spaces, postcapillary venules represent the major site of vascular protein leakage (extravasation).[4] The role of venules in protein extravasation is particularly evident in inflamed tissue because the accumulated inflammatory mediators and immune cells can act on venules to diminish endothelial barrier function.[4]

There are several characteristics of postcapillary venules that allow this segment of the microvasculature to regulate vascular permeability to plasma proteins. Ultrastructural analyses of the pathways for transvascular exchange have revealed that both the size and frequency of interendothelial junctions and endothelial fenestrae are higher in postcapillary venules than in either arterioles or capillaries.[2] These pathways are normally large enough to allow a low basal amount of plasma protein leakage that is driven by both diffusive and convective (coupled to fluid filtration) mechanisms. Venular endothelium also appears to possess a higher density of cell surface receptors for inflammatory mediators than their counterparts in arterioles and capillaries.[4] Engagement of certain inflammatory mediators (eg, histamine, PAF) with their receptors on venular endothelial cells elicits cell contraction, which results in a widening of the junctions (gaps) between adjacent endothelial cells and a consequent increase in protein extravasation. Furthermore, since postcapillary venules are the preferred site for leukocyte trafficking (due to the high density of leukocyte adhesion receptors), these endothelial cells are more frequently exposed to neutrophil products (proteases, oxidants), which can diminish barrier function, than their counterparts in arterioles and capillaries. The process of leukocyte emigration also appears to render venular endothelial cells more vulnerable to barrier dysfunction. In some models of inflammation, a strong positive correlation has been shown between venular albumin leakage and the rate of transendothelial migration of leukocytes.[23]

Pathological alterations in venular function

Leukocyte–endothelial cell adhesion and increased vascular permeability have been implicated in a number of pathological conditions, including a variety of cardiovascular diseases and regional circulatory disorders *(Table V)*.[23] In many instances, the organ

PATHOLOGIC CONDITIONS	
Diabetes mellitus	Arterial hypertension
Septic shock	Ischemia/reperfusion
Stroke	Hemorrhagic shock
Frostbite	Thermal trauma
Meningitis	Hypersensitivity reactions

***Table V.** Pathologic conditions associated with increases in leukocyte–endothelial cell adhesion and vascular permeability (from ref 23).*

dysfunction and/or parenchymal cell necrosis associated with these conditions is preceded by alterations in venular structure and/or function. These observations have often led to the assertion that venules are more sensitive to the deleterious effects of these disease processes and that venular dysfunction may play a role in the initiation and/or progression of the disease.

A condition that exemplifies the changes in venular function that can occur during disease progression and shows how vascular permeability changes can be linked to enhanced leukocyte trafficking through the generation of oxygen radicals by endothelial cells, is ischemia/reperfusion (anoxia/reoxygenation).[25] Over two decades ago, it was first demonstrated that the reintroduction of molecular oxygen (or reperfusion) into previously hypoxic (or ischemic) tissue can result in irreversible cellular damage (necrosis). Subsequent studies have revealed that increased microvascular permeability is an early pathological change that occurs after reperfusion of ischemic tissues (I/R) and that the mechanisms underlying the microvascular dysfunction also account for the parenchymal cell injury and organ dysfunction that is elicited by I/R.[21,26]

While ischemia per se can elicit an increased vascular permeability, the same duration and intensity of ischemia followed by a brief period of reperfusion results in a substantially greater (eg, fourfold) rise in permeability.[25] This additional increment in permeability caused by reperfusion apppears to be linked to the generation of oxygen free radicals, such as superoxide, hydrogen peroxide, and hydroxyl radicals.[26,27] *Table VI* summarizes the lines of evidence that implicate oxygen radicals in the pathogenesis of reperfusion-induced microvascular dysfunction.[25-27] The most compelling evidence that favors a role of oxygen radicals in this injury process is the blunted reperfusion-induced vascular responses observed in experimental animals that are either treated with oxygen radical scavenging enzymes (eg, catalase, superoxide dismutase) or that are genetically-engineered to overexpress these same enzymes.

Two enzymatic sources of oxygen radicals in postischemic tissues that have received considerable attention are xanthine oxidase and NADPH oxidase.[25,26] Xanthine oxidase, which can generate both superoxide and hydrogen peroxide, is localized primarily in vascular endothelial cells of humans and many animal species.[28] A role for xanthine oxidase in I/R-induced microvascular dysfunction is supported by studies that show blunted injury responses in animal models treated with allopurinol or other agents that inhibit or inactivate the enzyme. Although the enzyme exists as a dehydrogenase (which cannot generate oxygen radicals) in normal tissues, conditions (such as ischemia) associated with hypoxia, acidosis, and/or limited proteolysis can initiate its conversion to the radical-generating oxidase form.[28] Since ischemia also results in the accumulation of purines from ATP hydrolysis, both substrates for xanthine oxidase are provided upon reperfusion, ie,

EVIDENCE IMPLICATING OXYGEN RADICALS IN ISCHEMIA/REPERFUSION-INDUCED INCREASE IN VASCULAR PERMEABILITY
• Reintroduction of molecular oxygen at reperfusion required for permeability response
• Endothelial cells produce oxygen radicals after I/R (or anoxia/reoxygenation)
• Oxygen radical scavengers (SOD and catalase) attenuate I/R-induced increases in vascular permeability
• Inhibitors of oxygen radical generation by endothelial cells (allopurinol) attenuate I/R-induced increases in vascular permeability
• Exogenous oxygen radical–generating systems (xanthine oxidase–hypoxanthine) mimic the vascular responses to I/R

***Table VI.** Oxygen radials mediate ischemia/reperfusion injury. I/R, ischemia/reperfusion; SOD, superoxide dismutase.*

molecular oxygen and hypoxanthine, which leads to the generation of superoxide and hydrogen peroxide.[25] The preferential localization of xanthine oxidase to vascular endothelial cells is often invoked to explain the vulnerability of the microvasculature to I/R injury.

Another source of oxygen radicals and potential mediator of I/R injury is the activated neutrophil. In several organs, the evidence supporting a role for leukocytes (primarily neutrophils) in reperfusion-induced microvascular dysfunction is as compelling as that described for xanthine oxidase.[21,26] Neutrophils adhere in, and emigrate from, postcapillary venules of postischemic tissues, with the magnitude of the neutrophil recruitment dependent on the severity of the ischemic insult.[29,30] Furthemore, animals that are either rendered neutropenic or which receive monoclonal antibodies that neutralize leukocyte or endothelial cell adhesion molecules show markedly diminished microvascular dysfunction after I/R. Recent studies have revealed that mice that are genetically deficient in these leukocyte adhesion receptors also exhibit blunted reperfusion injury responses.

There is evidence in some vascular beds (eg, skeletal muscle, brain, and intestine) that links the generation of oxygen radicals by endothelial cell–associated xanthine oxidase with the recruitment and activation of leukocytes in postcapillary venules subjected to I/R *(Figure 7)*.[25] Agents that either prevent the generation of oxygen radicals from xanthine oxidase (allopurinol) or scavenge these radicals have been shown to inhibit the recruitment of adherent and emigrating leukocytes in the microcirculation. These observations have led to the proposal that xanthine oxidase–derived oxygen radicals produced at the time of reperfusion elicit the upregulation of endothelial cell adhesion molecules and lead to the formation of substances (eg, PAF, complement, leukotriene B_4) that activate and attract neutrophils. The activated neutrophils release oxygen radicals and proteases as they adhere to and emigrate across venules, thereby impairing the endothelial barrier, and move toward parenchymal cells, where additional damage may be inflicted.

The mechanisms that underlie the microvascular dysfunction that is associated with I/R and other vascular disorders with an inflammatory component suggest that there are several potential avenues for therapeutic intervention. For I/R injury, beneficial therapeutic effects might be expected for agents that either: (i) prevent the production of (or scavenge) oxygen radicals; (ii) inhibit the formation of (or antagonize) inflammatory mediators (PAF) generated by endothelial

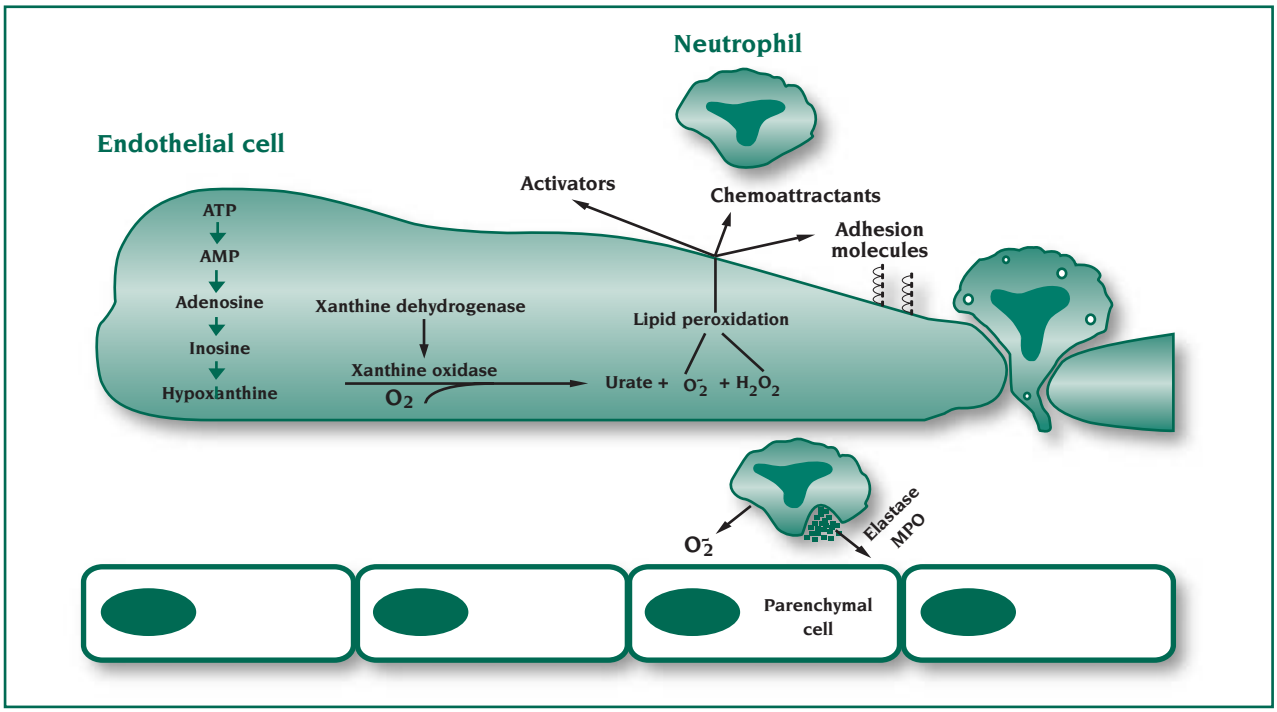

Figure 7. *Mechanism proposed to explain the role of xanthine-derived oxidants in the recruitment and activation of circulating leukocytes. The adherent and emigrated leukocytes mediate injury to endothelial cells and parenchymal cells by releasing oxygen radicals and proteases. ATP, adenosine triphosphate; AMP, adenosine monophosphate; MPO, myeloperoxidase.*

cells in response to oxygen radicals; (iii) interfere with leukocyte–endothelial cell adhesion; or (iv) antagonize the effects of neutrophil-derived proteases. While these animal experiment–based strategies have not been rigorously tested or gained acceptance in the clinical setting, it might be expected that agents that act at multiple sites in this inflammatory cascade hold more promise for eventual clinical use. NO-donating compounds, for example, reduce reperfusion injury in different animal models via mechanisms that appear to involve scavenging superoxide radicals, inhibiting the release of inflammatory mediators from activated mast cells, and preventing leukocyte–endothelial cell adhesion.[6,30]

FUTURE DIRECTIONS AND UNRESOLVED ISSUES

In recent years, we have witnessed a resurgence of interest in, and information on, the role of the microcirculation in the initiation and maintenance of different disease states. Rapid progress in this field of investigation has resulted from technological advancements that allow visualization and quantification of events occurring within the vessel wall and at the blood-endothelial interface. Improved methods for culturing microvascular endothelial cells as well as the availability of specific reagents (eg, NO synthase inhibitors, monoclonal antibodies against adhesion molecules) or gene-targeted mice that are directed towards the products of endothelial cell activation have also contributed to this progress.

Whether or not the progress made in the understanding of the contribution of the microvasculature to different pathological processes during the next decade matches the remarkable insights gained in the past few years will depend on the focus of future research efforts. The implication of microvascular dysfunction in several disease processes has often relied on data derived from a variety of animal models of unknown relevance to human disease as well as indices of vascular dysfunction that are not clearly related to a specific segment of the microvasculature, ie, arterioles, capillaries, or venules. Until additional work is performed to address these issues more directly and systematically, a critical and detailed analysis of the available evidence in favor of dysfunctional microcirculation in specific cardiovascular diseases is needed. In this issue, three articles are devoted to defining the role of the microcirculation in the pathogenesis of **hypertension** (Struijker Boudier), **heart failure** (William Chilian et al), and **reperfusion injury in man** (Allan and David Lefer).

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Microcirculation

Expert Answers to Three Key Questions

1

The microcirculation: is it a key player in hypertension?

H.A.J. Struijker Boudier

2

Does the coronary microcirculation play a role in heart failure?

W.M. Chilian, D. Weihrauch, D. Stepp, C. Wright, Y. Nishikawa

3

Is the microcirculation important in reperfusion injury in man?

A.M. Lefer, D.J. Lefer

The microcirculation: is it a key player in hypertension?

H.A.J. Struijker Boudier, PhD

Professor and Chairman - Department of Pharmacology - Cardiovascular Research Institute Maastricht (CARIM) - Universiteit Maastricht, Maastricht - THE NETHERLANDS

The major hemodynamic abnormality underlying the elevated blood pressure in hypertension is an increase in vascular resistance.
The microcirculation is a key site of increased vascular resistance in hypertension. A lesser density of arterioles and capillaries is an important common characteristic of various microvascular beds in many forms of hypertension.
This microvascular rarefaction has been observed even in very early stages of hypertension development. A hypothesis is discussed to explain early microvascular rarefaction as the result of genetic and fetal mechanisms of decreased small blood vessel growth.

Keywords: hypertension; microcirculation; vascular resistance; angiogenesis; blood vessel

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The major hemodynamic abnormality in hypertension, in addition to the elevated blood pressure, is a rise in vascular resistance.[1,2] Total peripheral vascular resistance is increased in almost all forms of experimental and clinical hypertension. A rise in cardiac output is the major determinant of the increased blood pressure only in the early stages of certain forms of hypertension.[3]
The rise in vascular resistance does not occur simultaneously in all tissues. In both human essential hypertension and spontaneous hypertension in rats, the kidneys are among the first organs in which resistance increases.[3,4] However, eventually, resistance increases in nearly all tissues.

RESISTANCE CONTROL

The localization and mechanisms of the vascular resistance increase have been a central theme in hypertension research of the last decades. Direct measurements of blood pressure have shown only a small reduction in pressure along the larger conduit arteries or the smaller muscular arteries. Both in hypertensives and in normotensives, a pronounced drop only occurs further downstream at the level of the branchings within the tissue proper. This is the level of the microcirculation. The fact that the major pressure dissipation occurs at the level of the microcirculation implies that this section of the cardiovascular system is a key player in resistance control.
The degree to which the microcirculation contributes to resistance control differs according to the type of tissue. For instance, in the rat mesentery or cheek pouch of the hamster, a reduction in systemic blood pressure of 35% to 40% may have taken place already before the level of the largest arterioles (100 to 150 µm in diameter).
On the other hand, in skeletal muscle or the coronary microcirculation, only a 10% to 20% pressure drop has been measured at that level in both hypertensive and normotensive conditions.[5] The pattern of pressure drop and resistance control also differs within various tissues, depending on the specific microvascular architecture.

MICROVASCULAR ARCHITECTURE

Microvascular networks are organized in different ways.
Certain organs have very specific arrangements, such as the kidney with its double—glomerular and peritubular—microvascular networks. The simplest and most general architecture is that of a tree-like, branching network.
One major arteriole feeds such a network, which then branches off into ever smaller (A1, A2, A3, etc) arterioles to reach the capillary level (internal diameters 5 to 8 µm).

Blood is drained into increasingly bigger venules to leave the tissue via a limited number of relatively large venules. The cremaster muscle is an example of a tissue with this type of architecture.

Another common form of microvascular architecture is that of arcading networks. The arrangement of these networks is such that large arterioles feed blood into a network of smaller arterioles from which the branching is largely in the form of side-branches in parallel circuits. These circuits make up a series of arcades that serve to ensure a more uniform spatial apportionment of blood within the tissue. Arcade-like arrangements are found in embryonic tissues[6] and in various skeletal muscles in experimental animals.[7] Unfortunately, technologies for intravital microscopy have not yet been developed well enough at the human level to allow detailed microvascular architectural analyses in hypertensive and normotensive subjects. Stanton et al[8] have recently introduced a promising methodology for microvascular network analysis in the human retina.

NETWORK CHARACTERISTICS AND RESISTANCE

The determinants of vascular resistance of a single blood vessel can be relatively simply described by Poiseuille's law. Blood viscosity, vessel length, and—most powerfully—vessel diameter are the determinants, according to this law. The situation is considerably more complex in a (micro)vascular network consisting of many vessels. The amount of vessels, their branching pattern, and their geometry have a large impact on the resistance of the network. Relatively simple extensions of Poiseuille's law can be used for the tree-like branching networks.[9] However, the arcade-like networks require a much more complex approach to determine resistance.[6,7] Computer simulations, rather than analytical mathematics, are required to calculate resistance characteristics of more complex microvascular networks. In spite of these methodological complexities, a few general statements can be made concerning network characteristics that affect resistance: (i) the number of terminal arterioles is inversely related to the resistance of a branched network[9,10]; (ii) branching angles of the arterioles affect resistance[8]; and (iii) the length and diameters of individual arterioles contribute to the overall resistance and its distribution throughout a network.[9,11]

MICROCIRCULATION IN HYPERTENSION

Intravital microscopy has enabled the detailed study of microvascular network characteristics in a range of tissues in experimental animal models of hypertension (for review, see 11,12). Similar observations in human hypertension are less abundant, but intravital microscopy and related methods are now rapidly becoming standard techniques in vascular medicine.[12] Thus, some of the major hypotheses derived from work in animal models will be tested at the clinical level in the next decade.

The most consistent change of the microcirculation in primary forms of hypertension in animals and humans is the decreased number of small arterioles and capillaries. This phenomenon is usually referred to as microvascular rarefaction. Arteriolar and capillary rarefaction are early phenomena in the development of primary forms of hypertension. In spontaneously hypertensive rats, they are already observed before pressure rises significantly.[13] A similar observation was made in young adult humans with an enhanced risk of developing hypertension[14] and in persons with borderline hypertension.[15] It is of interest that rarefaction is a more systematically observed early phenomenon in primary hypertension than arteriolar diameter decrease. In the early stages of secondary forms of hypertension, arteriolar constriction, rather than rarefaction, is the primary mechanism of resistance increase.[3,11] However, in the later stages of both primary and secondary forms of hypertension, microvascular rarefaction is a generalized phenomenon. This has led us to propose two mechanisms of rarefaction: an early form related to primary defects in blood vessel growth, and a later form, which is part of the adaptive remodeling of the vascular system due to altered mechanical stresses in hypertension.[3,11]

Other changes in microvascular network characteristics in hypertension are still less well established. Retinal arteriolar branching angles are narrower in both established hypertension[8] and in men with low birth weight, a recently much debated risk factor for hypertension development.[16] Finally, in experimental animal models of hypertension, distinct differences have been described in arcade structure of skeletal muscle microvascular networks.[7]

MECHANISMS OF MICROVASCULAR RAREFACTION

Hutchins and Darnell[17] were the first to suggest that microvascular rarefaction represents a pathogenic mechanism in primary hypertension.

They proposed that arteriolar and capillary rarefaction are mechanisms for long-term control of blood flow. Skalak and Price[10] have recently delineated the role of mechanical stresses in microvascular remodeling. Both shear stress (flow-related) and circumferential wall stress (pressure-related) affect microvascular network characteristics. Microvessels can actually disappear through a process of endothelial cell apoptosis in hypertensive conditions.[18]

A second hypothesis on the mechanism of microvascular rarefaction comes from Prewitt and coworkers.[19] These authors distinguish phases of functional and structural (or anatomical) rarefaction. Functional rarefaction is the result of microvascular constriction to the point of nonperfusion of the vessel, whereas structural rarefaction represents the actual disappearance of the vessel. During functional rarefaction the vessel can be reopened by powerful vasodilator stimuli. Prewitt and coworkers[19] proposed that functional rarefaction is the consequence of increased sensitivity of small arterioles to vasoconstrictor stimuli, with subsequent chronic vasoconstriction.
The "ghost arterioles" thus created would gradually disappear anatomically. Although this hypothesis provides an attractive explanation for microvascular rarefaction in models of secondary hypertension, it is not compatible with several observations in primary forms of hypertension. In primary hypertension, structural rarefaction is observed even in very early stages of hypertension. Furthermore, enhanced vasoconstrictor sensitivity is generally not observed in small arterioles in early primary hypertension.

We favor an alternative explanation for early microvascular rarefaction in primary forms of hypertension. This explanation is based on a decreased angiogenic potential of the microvasculature in subjects prone to develop primary hypertension.

HYPERTENSION AND ANGIOGENESIS

Early microvascular rarefaction in hypertension may be caused by a decreased formation rather than active disappearance of small arterioles and capillaries. Two different mechanisms are involved in the formation of blood vessels: vasculogenesis, which is the new formation of blood vessels from in situ differentiating endothelial cells, and angiogenesis, the sprouting of capillaries from preexisting vessels. A third form of vascular growth is the remodeling of existing small vessels to larger ones, by the addition of vascular smooth muscle cells and extracellular matrix material around a larger diameter. This form of growth is of particular importance in collateral formation. The first two forms of vascular growth are of particular importance during embryonic development and in situations of mechanical or ischemia-related stresses exerted on tissues.[20]

The formation of new blood vessels is controlled by a number of factors: (i) mechanical stresses, such as shear stress and circumferential wall stress; (ii) cell-cell interactions near and in the vessel wall; (iii) extracellular matrix components and their breakdown products; (iv) metabolic factors; and (v) growth factors. The activity of these control mechanisms is determined by genetic and early environmental stimuli. Recent research has pointed to the intrauterine, fetal, period as a particularly important one in setting the stage for later vascular development.[20-22] We may thus envisage that the growth potential of the microvascular system is determined by the combined action of genetic and fetal influences. In hypertension-prone individuals, this potential is reset towards a lesser potential for growth. This leads to a gradual rarefaction of capillaries and arterioles, which—in turn—is the basis for the gradual resistance increase in hypertension. A number of aspects of this hypothesis remain to be proven. However, it is an attractive hypothesis since it integrates some of the recent insights on the possible genetic and fetal origins of hypertension and explains the gradual rise in vascular resistance during the development of hypertension.

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Does the coronary microcirculation play a role in heart failure?

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Heart failure is conventionally attributed to direct alterations in cardiac myocyte contractile function. However, therapeutic attempts to improve pump function appear to have little effect on the long-term outcome of the disease. Attention is now focusing on a possible role of the coronary microcirculation, impairment of which would result in alterations in extravascular compressive forces that could limit dilation of intramural coronary resistance vessels. This hypothesis is borne out by findings of marked reduction of endocardial-epicardial distribution of myocardial perfusion in the failing heart, and, in various models of heart failure, of blunted endothelium-dependent vasodilator responses and augmented constriction in coronary arterioles. This could result in impaired or even insufficient vasodilation at the microvascular level and, in turn, in myocyte death and patchy fibrosis, both of which are frequently observed in heart failure.

Keywords: coronary circulation; endothelium-dependent dilation; nitric oxide; endothelin; coronary insufficiency

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Despite significant advances over the last several years in the understanding of the mechanisms, consequences, and treatment of congestive heart failure (CHF), this pathology continues to cause enormous cardiovascular morbidity and mortality. Although early therapeutic trials focused on improvement of depressed left ventricular systolic function, these interventions (positive inotropes) are associated with either no change or increased mortality.[1-3] While most investigations into the etiology of heart failure focus on cardiac problems, there are other features of this disorder, such as neuroendocrine problems and abnormalities in the distribution of flow in peripheral circulation, which undoubtedly contribute to the progression of the disease. Recently, several trials have clearly demonstrated the benefit of angiotensin-converting enzyme (ACE) inhibition in patients with CHF.[4] Considering that ACE inhibition has potent effects on the coronary microvasculature, we speculate that the salutary action of this therapy may be multifactorial, in that ACE inhibitors target both cardiac muscle and the coronary vasculature. In the subsequent discussion, we will summarize results from clinical and experimental studies addressing whether alterations in the coronary circulation may contribute to the pathological sequelae of heart failure. Our goal in this short review is to answer the question, does the coronary microcirculation play a role in heart failure?

Before we elaborate on the hypothesis that inadequate coronary vasodilation and excessive constriction may contribute to the evolution of failure, it is worth considering the cogent question, is there evidence for coronary insufficiency and myocardial ischemia in the failing heart? This question has important implications, because if the answer is affirmative, then it would be obvious how coronary abnormalities could contribute to poor cardiac pump function. There are numerous clinical studies that do not show overt evidence of myocardial ischemia, but an important and overlooked factor is that generally most measurements were made under basal resting conditions with relatively low metabolic demands. If vasodilator reserve is limited, or hemodynamic factors such as end-diastolic pressure increase during a stress, the result may be myocardial ischemia.
In animal models of heart failure, increases in metabolic demands can produce coronary insufficiency.[5-9] In patients with varying types of heart failure, evidence for ischemia is observed as alterations in the high energy content of the myocardium,[10] increased incidence of sudden death,[7] angina,[6,11] and echocardiographic evidence during

stress testing.[6] Taken together, there is evidence supporting the hypothesis that myocardial ischemia may contribute to ventricular dysfunction during heart failure.

CORONARY VASCULAR MORPHOMETRY IN HEART FAILURE

An important consideration of establishing a causal relationship between heart failure and inadequate vasodilatory responses of the coronary circulation pertains to coronary vascular morphometric characteristics. Although there is a relative paucity of literature in this area (in contrast to many reports of coronary morphometrics in the hypertrophied, but not failing, heart), there are a number of key observations that buttress the hypothesis that abnormalities in the coronary microcirculation causally contribute to the pathophysiological sequelae of heart failure.

Anversa and Capasso reported that in rats with hypertension-induced failure there was a decrease in the density of arterioles, but not capillaries.[12] Associated with the areas devoid of arterioles were grossly enlarged cardiac myocytes. Sabbah et al[13] found that capillary density was decreased in areas of the myocardium that also had profound interstitial fibrosis. The fibrosis, which was mainly due to collagen deposition, appeared to replace degenerated cardiac myocytes. These investigators speculated that the combination of collagen deposition around viable myocytes and the decrease in capillary density together would increase the diffusion distance for oxygen. The net result would render the viable myocytes more susceptible to further reductions in oxygen delivery. In the hypertrophied heart, there is evidence that the increase in oxygen diffusion distance alters normal cellular organization. Normally, mitochondria are distributed randomly in cardiac myocytes, but in the hypertrophied heart their distribution becomes spatially polarized toward the nearest capillary.[14] This implies that in cardiac hypertrophy the increased diffusion distance resulting from increased myocyte diameters hampers oxygen delivery sufficiently to provoke compensatory cellular adaptations. None of the studies, however, established if the decrease in vascular density is causally related to the evolution of the pathology. In fact, we would be remiss to not point out that in many types or stages of cardiac hypertrophy, with no evidence for failure, there are decreases in arteriolar or capillary density.[15-21] Although this does not eliminate the possibility that alterations in coronary morphometric parameters can contribute to cardiac failure, we must admit that a definitive link has not yet been established.

CORONARY BLOOD FLOW IN THE FAILING HEART

Prior to any discussion of abnormalities in the regulation of coronary blood flow, it is important to first highlight normal physiological control mechanisms. *Figure 1* illustrates a microvascular smooth muscle (MVSM) cell that is receiving input from physical forces (stretch due to transmural pressure changes) and compression due to active contraction, ventricular dilation, and/or elevations in end-diastolic pressure. There are many vasoactive factors released from cardiac myocytes, endothelial cells, and autonomic nerves that can produce either vasodilation or vasoconstriction. Local intrinsic factors, eg, metabolites, myogenic (stretch-dependent) mechanisms, and flow-dependent production of nitric oxide, exert dominant control over the tone of resistance vessels.[22-24] Certain paracrine factors, eg, endothelin, bradykinin, and angiotensin II, also seem to exhibit significant vasoactive influences.[25,26] In addition, neurohumoral factors, eg, norepinephrine or acetylcholine, produce constriction or dilation, respectively.[27,28] The aggregate effect of these, and likely several more unknown or unmentioned regulatory factors, is tight coupling of flow to metabolism and ample vasodilator reserve. Moreover, at rest and even during substantial dilation, the endocardial-epicardial distribution of flow is about 1.0 to 1.5, indicating sufficient perfusion to the subendocardium. The above elementary description of coronary control and hemodynamics pertains to the normal heart and coronary circulation. Many pathophysiological disturbances affect the transmural distribution of flow, coronary vasodilator reserve, and the influences of regulatory mechanisms.[29,30] Abnormalities in the coronary circulation in heart failure could potentially contribute to the development of the disease either through excessive vasoconstriction or insufficient vasodilation. Increases in extravascular compressive forces could also increase total coronary resistance. Before we commence with discussion of the previously mentioned specific issues, we will first provide a general description of coronary vascular hemodynamics in the failing heart.

CHF can be caused by left ventricular hypertrophy (LVH) secondary to systemic pressure overload. Many investigators reported that coronary reserve is progressively compromised during the hypertrophic process due to inadequate

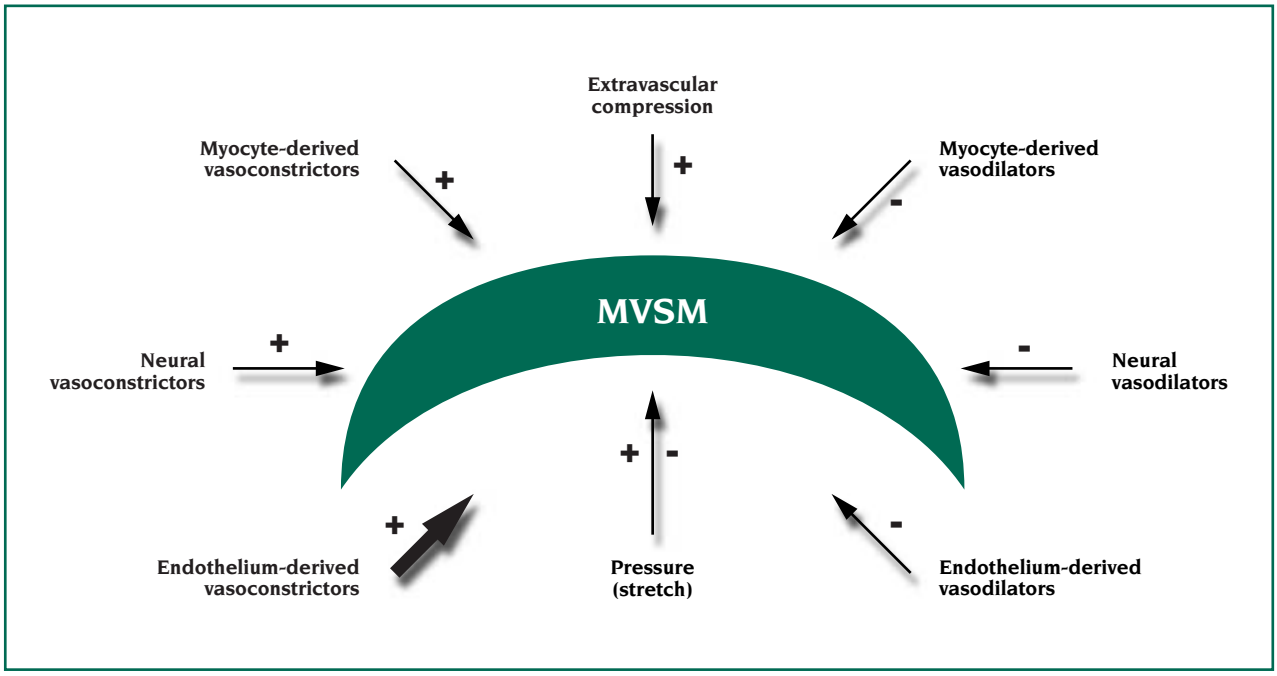

***Figure 1.** Regulatory factors involved in the control of coronary microvascular smooth muscle (MVSM) tone. Factors that increase tone (produce constriction) are designated by a plus (+), and those that lessen tone are marked with a minus (–). Extravascular compression can increase resistance during either systole or diastole (associated with ventricular stretch or high ventricular end-diastolic pressures). Intraluminal pressure can influence tone via the myogenic mechanism, whereby tone and pressure are directly related. Contractile factors can be released from cardiac myocytes and endothelial cells, and vasodilators can be released from these two vascular cells. Autonomic nerves, parasympathetic and sympathetic, can also release vasodilators and vasoconstrictors. The final tone of a resistance vessel is critically dependent on the magnitude of stimulus and the concentration of the vasodilators and vasoconstrictors.*

growth of the coronary vasculature.[15-21] This suggests the hypothesis that coronary reserve is exhausted and causes myocardial hypoperfusion and cardiac decompensation. Previous studies have shown that myocardial perfusion is compromised in the subendocardium in experimental models of CHF, implying that inadequate perfusion to the subendocardium may lead to failure from compensated cardiac hypertrophy.[5] Moreover, in severe left ventricular hypertrophy secondary to aortic stenosis, coronary vasodilator reserve can be absent, and many of these patients have angina.[11,31] Taken together, these studies indicate that cardiac hypertrophy arising from pressure overload or from other pathological conditions compromises coronary function, which may be manifested as suboptimal coronary dilation and focal areas of ischemia.

Considerable data have accumulated from human and animal models of CHF indicating that myocardial blood flow reserve is impaired in response to pacing, exercise, and pharmacological vasodilation.[5,8,32-34] Although the maximal increase in coronary blood flow is less in cardiomyopathic patients than in normal heart, angina is not a common manifestation of patients with heart failure in the absence of significant coronary artery disease and marked left ventricular hypertrophy.[35] A notable exception to this is the angina that occurs during severe aortic stenosis and the accompanying failure.[11] Even though coronary flow reserve is impaired during exercise under most conditions of heart failure, it is generally accepted that the impairment is not sufficiently severe to cause overt symptoms of myocardial ischemia. A caveat about these remarks is that at the microvascular level regional inadequacies in perfusion may produce patchy areas of fibrosis, which are suggestive of ischemia, in the absence of the accepted hallmarks, eg, ST-segment changes, lactate production, and angina.

Although congestive heart failure is associated with disturbances in global myocardial perfusion, an even more striking consequence of this pathology pertains to the distribution of transmural myocardial blood flow. In experimental models of heart failure, a reduced myocardial endocardial/epicardial blood flow ratio suggests a compromised subendocardial reserve.[8,9] The endocardial/epicardial ratio decreases further with vasodilation, eg, adenosine or reactive hyperemia. These data suggest that one mechanism of failure of the severely

hypertrophied heart may involve inadequate coronary perfusion to the subendocardium. In addition, a marked increase in left ventricular end-diastolic pressure in CHF contributes to a decreased endocardial flow.[36,37] Dilation of the ventricle, which occurs during heart failure, may also increase the extravascular resistive element of the coronary microcirculation. Within this context, one laboratory has reported that passive stretch of the myocardium dramatically increased vascular resistance.[38,39] Taken together, these results suggest that ventricular dilation, cardiac hypertrophy, and increased end-diastolic pressure—consequences of the failing heart—may together, or alone, impair dilation of subendocardial resistance vessels, thereby accelerating the progression of the disease.

MICROVASCULAR REGULATION IN THE FAILING HEART

It is conceivable that alterations in vascular control may also impair the distribution and amount of coronary flow in CHF. Within this context, abnormalities in endothelial, neurohumoral, and local mechanisms may all contribute to abnormal microvascular vasoactive reactions.

Endothelium-dependent regulation

Endothelium-dependent vasodilation is an important microvascular regulatory scheme and depends on many factors, including the stimulus (particular agonist or shear stress) and the location within the vascular tree where the stimulus is acting. It is well accepted that nitric oxide (NO), endothelium-derived hyperpolarizing factor, and prostacyclin are three of the most important endothelium-dependent vasodilators. Our discussion will concentrate on interferences with NO production in heart failure by cytokines and oxygen-derived free radicals, and how decreases in the production of NO may magnify the actions of vasoconstrictor(s).

Increased levels of cytokines, especially tumor necrosis factor (TNF), may be involved in the development of peripheral endothelial dysfunction in CHF.[40,41] TNF can impair the stimulated release of NO[42] and thus blunt normal endothelium-dependent vasodilatory mechanisms. In addition, TNF is associated with induction of inducible nitric oxide synthase (iNOS),[42,43] which may explain the observations of increased NO levels in CHF.[44-46] Increased levels of NO can potentially decrease the production of endothelium-dependent hyperpolarizing factor (EDHF), another vasodilating factor produced in the vascular endothelium.[47,48] Furthermore, if basal release of NO is increased, there may be a downregulation of endothelial nitric oxide synthase (eNOS)[49] leading to impaired agonist- or shear-induced production of NO. Perhaps this is a reason for relatively normal resting flows, with evidence for ample production of NO, but depressed endothelium-dependent vasodilation. Further studies are required to define the source of the basal release of NO (iNOS or eNOS), and its effects on vascular reactivity in the failing heart.

Oxygen-derived free radicals may lead to endothelial dysfunction via a number of mechanisms. These chemical species quench NO—the reaction of superoxide with NO yields peroxynitrate, a powerful oxidant that can damage vascular cells.[50] Free radicals alter the redox state, shifting the balance between NAD or NADP and NADH or NADPH—cofactors for NO synthase. These species also damage endothelial cell membranes by peroxidation, thus altering signaling, membrane permeability, and ionic homeostasis.[51] There is evidence of increased production of free radicals in patients with heart failure of many etiologies.[52,53]
Antioxidant therapy has favorable effects on the progression of heart failure. Specifically, probucol, a lipid-lowering agent and potent antioxidant, provides complete protection against adriamycin-induced cardiomyopathy in rats.[54,55] Carvedilol, a newly developed β-blocker/antioxidant, improved the mortality of patients with heart failure more than other β-blockers without antioxidant actions.[56-58] These data support the hypothesis that free radicals contribute to the pathogenesis of heart failure.
A caveat about the above discussion is that it remains unknown whether the beneficial actions of antioxidant therapy in heart failure are mediated by its actions, even in part, on the coronary microcirculation.

The levels and actions of vasoconstrictors that produce increases in coronary resistance, including catecholamines,[59-63] angiotensin II[64] and neuropeptide Y,[65] are augmented in CHF. In addition to these substances, endothelin (ET) is frequently implicated as playing an important pathophysiological role in CHF and is among the most potent constrictors of the coronary microcirculation.[66,67] Under normal physiological conditions, the release of endothelin is attenuated by NO.[68,69]
Thus, we advance the following hypothesis: the impairment in the production of NO in heart failure allows the release of endothelin and the consequential microvascular constriction to be augmented.

There is also the potential for substantial interactions between many

vasoconstrictors and NO.
For example, norepinephrine-induced activation of the endothelial α_2-adrenoceptor results in NO production.[70-72] If this vasodilatory mechanism is impaired, then the vasoconstrictor action should be augmented. The actions of virtually any vasoconstrictor can also be augmented if endothelium-dependent production of NO is impaired. We make this argument because, as the caliber of a microvessel is decreased during vasoconstriction, the vascular shear stress is likely to increase. Normally, shear stress produces potent NO-mediated vasodilation of the coronary microcirculation,[24,73] and this mechanism can act to "brake" the actions of virtually any vasoconstrictor.[74] However, if the shear stress–induced production of NO is compromised, then the actions of constrictors in the coronary microcirculation would be unopposed and would cause increases in the tone of certain microvessels. This constriction could potentially impede oxygen delivery and compromise coronary vasomotor adjustments to a variety of stresses. One point that deserves mention is that heart failure is generally associated with a depression of endothelium-dependent vasodilation.[75] Yet it is unresolved whether the failing heart leads to the depression of endothelium-dependent dilation or if depressed vasodilation can promote the evolution of the disease. However, we would like to suggest that if microvascular vasodilatory mechanisms, such as shear stress–induced dilation, are blunted or nonexistent in the microcirculation of the failing heart, the result may lead to a loss of vascular control and an imbalance of oxygen delivery and demand.

Additional literature supports the hypothesis that insufficient coronary dilation, primarily mediated by diminished production of NO, contributes to the etiology of heart failure. Specifically, inhibition of ACE appears to offer significant increases in survival and lessens morbidity in patients with heart failure.[4,76,77] The mechanism of ACE inhibition can be explained on the basis of inhibited formation of angiotensin II or increased levels of bradykinin resulting in direct effects on the heart and/or salutary effects mediated by the decrease in peripheral resistance and excretion of salt.[77] Our discussion will concentrate on the direct myocardial effects of ACE inhibition in the context of inhibiting the breakdown of bradykinin or preventing angiotensin II formation.

Inhibition of ACE or kininase II may potentially increase tissue levels of bradykinin. Bradykinin is a potent coronary vasodilator and its vasodilation is mediated by NO, EDHF, and/or prostacyclin.[78,79] In an experimental model of heart failure, ACE inhibition dramatically increased NO production by increasing the amount of available kinins.[80] Inhibition of ACE augments coronary microvascular vasodilation by bradykinin[79,81] and appears to restore endothelium-dependent dilation to many agonists in the microcirculation of the failing heart.[82,83] If the production of NO constitutes a serious deficiency in the failing heart and contributes to hypoperfusion, then the potentiation of the levels and actions of bradykinin by ACE inhibition may underlie the beneficial action.

In the aggregate, diminished endothelial production of NO in heart failure may lessen the vasodilation to physiological stresses and potentiate the actions of constrictors. These two consequences may not allow proper vasomotor adjustments in the failing heart and contribute to dysfunction.

Endothelium-independent regulation

Heart failure is associated with significant alterations in the production of pressors, their receptor profile, and the sensitivity of smooth muscle to the actions of many vasoconstrictors.[84-87] The following discussion will focus on how the unfettered actions of certain vasoconstrictors, endothelin-1 (ET-1), angiotensin II, and catecholamines, may compete with metabolic coronary vasodilation leading to hypoperfusion of the myocardium.

ET-1 is a paracrine factor produced by many cells in the heart, including cardiac myocytes and vascular smooth muscle cells,[88,89] and is a potent constrictor of coronary resistance vessels.[67,90] In CHF, the circulating levels of ET-1 are increased[86] and its release from cardiac myocytes from failing hearts is increased substantially over myocytes from normal hearts.[91] In conjunction with these observations, the coronary vascular response to ET-1 is reported to be altered in heart failure. Specifically, in a model of pacing-induced canine heart failure, intracoronary infusion of sarafotoxin, an ET_B receptor agonist, caused a significant decrease in coronary blood flow; whereas, in normal animals, this agonist produces vasodilation.[92] The observation that sarafotoxin produced constriction in the coronary circulation of the failing heart suggests that the normal balance of ET_A-induced constriction and ET_B-induced dilation is shifted in coronary microcirculation in the failing heart. Finally, administration of bosentan, a nonselective endothelin receptor antagonist, reduced systemic vascular resistance and improved overall left ventricular performance in dogs with CHF.[93,94] This suggests

that mitigation of the vasoconstrictive effects of endothelin may translate into improved myocardial function in heart failure. It is imperative to point out that the mechanism of action—systemic or coronary vasodilation, or both—is not yet resolved. We would like to summarize this discussion about endothelin by reiterating that it is among the most potent constrictors in the coronary microcirculation, and if its release and the sensitivity of coronary resistance vessels are augmented in the failing heart, the consequence may lead to coronary insufficiency and further impairment of cardiac function.

Angiotensin II, produced by the action of ACE on angiotensin I, is another potent constrictor of coronary resistance vessels.[95,96] ACE activity in cardiac myocytes is increased in the failing heart[97] and may, therefore, induce local (myocardial) increases in the level of angiotensin II, as well as decreases in tissue concentrations of bradykinin. As discussed above, ACE inhibitors are efficacious in the treatment of CHF,[4,76,77,98] and the therapeutic action may be mediated in part via the inhibition of angiotensin II formation. ACE inhibitors increase coronary blood flow in patients suffering from heart failure,[97,99-101] indicating that there is dilation of coronary resistance vessels.

CHF is also associated with elevations in cardiac sympathetic nerve activity and circulating levels of catecholamines.[102-105] Either of these influences could potentially contribute to an acute worsening of failure because α-adrenergic coronary constriction would limit coronary vasodilation and β-adrenergic stimulation would increase myocardial metabolism. However, it should be mentioned that β-adrenergic sensitivity of the failing heart is greatly reduced.[106,107] In addition, there is evidence for decreased α-adrenergic coronary constriction of large arteries,[108] but the total number of α-adrenergic receptors increases during heart failure.[109] The situation of α-adrenergic constriction of coronary resistance vessels is complicated by some unique aspects of this transduction system. Specifically, administration of an α-adrenergic agonist or stimulation of sympathetic nerves elicits coronary constriction in vivo,[110,111] but in vitro the arteriolar microvessels do not respond.[112] We have found in a preliminary report that α_1-adrenergic activation of cardiac myocytes causes the release of a factor(s) that produce(s) constriction of coronary resistance vessels.[113,114] Although we have not unequivocally identified this factor, we do know it produces sustained constriction of isolated arterioles. If the number of α-adrenergic receptors is increased in heart failure, then we speculate that the release and actions of myocyte-derived contractile factors is augmented, and, in the setting of compromised reserve or blunted metabolic mechanisms, this could provoke ischemia.

SUMMARY

Our goal in this review was to address the possibility that structural and functional alterations in the coronary microcirculation contribute to the sequelae of developing heart failure. We presented evidence that suggests such a link, which is summarized in *Figure 2*. This figure illustrates the abrogated vasodilator mechanisms and augmented vasoconstrictor influences imposed on coronary resistance vessels in the failing heart. Despite these myriad pathophysiological effects, one could argue that coronary microvascular function should be "normal" because of the frequent use of positive inotropes in the treatment of the disease.
A positive inotrope may increase myocardial oxygen demands, which would seem contraindicated if there are existing abnormalities in coronary vascular reserve.
The situation is far too complex for such simplistic reasoning: a positive inotrope would decrease chamber size and lessen wall tension, thus it may reduce the oxygen requirements of a failing heart, despite the increase in contractility.
Importantly, a decrease in end-diastolic pressure would lessen the diastolic compression on the endocardial microcirculation and facilitate subendocardial perfusion.
Finally, the use of inotropes is not associated with a reduction in the mortality and morbidity of heart failure, whereas vasodilator therapies are—again suggesting the possibility that altered coronary microvascular regulation is causally involved in heart failure.

Another point of contention is the small number of patients suffering from heart failure with concomitant myocardial ischemia. Compared to the total number of patients with heart failure, the occurrence seems rare. However, it is worth noting that virtually all patients with heart failure have some type of diffuse fibrosis and microscopic areas devoid of myocytes. These patchy areas of necrosis suggest problems with tissue oxygenation.
Because coronary vasodilator reserve is spatially variable,[115-118] it is conceivable that areas with less than normal intrinsic reserve could be those most vulnerable to insults during heart failure—augmented activity of constrictors, decreased activity of vasodilators, increased extravascular compression—culminating in microareas of cell death, which would take on

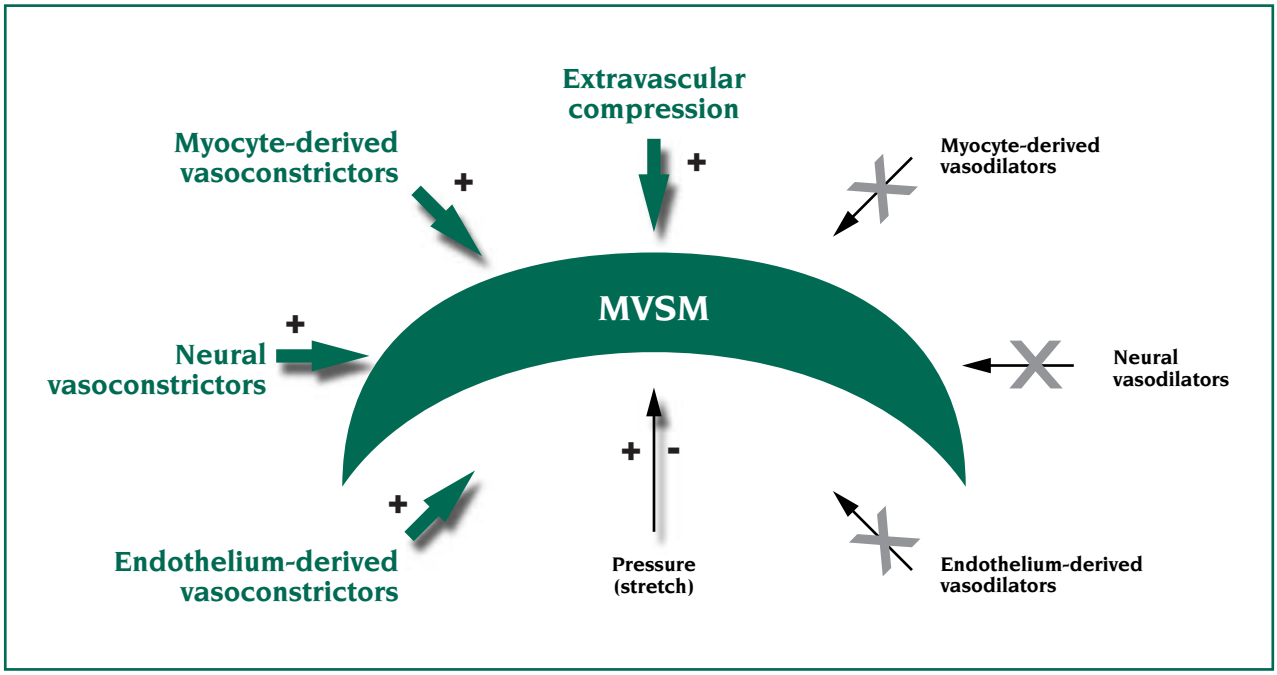

Figure 2. *Pathophysiological disturbances in the coronary microcirculation produced by heart failure: increased compressive forces, increased production of vasoconstrictors (norepinephrine, angiotensin II, endothelin), decreased production of vasodilators by the endothelium, and decreased efficacy of vasodilators, eg, acetylcholine, to induce the release of endothelium-derived vasodilators. The arrows* ***in bold*** *and pluses indicate the augmented effects, whereas the* ***X****s on the arrows indicate an abrogated effect. The decreased production of nitric oxide by the endothelium would increase endothelin release. Increases in angiotensin-converting enzyme activity would inactivate bradykinin and increase production of angiotensin II. The net effect of these influences would be inappropriate dilation, excessive constriction, and blunted vasodilator reserve. MVSM, microvascular smooth muscle.*

the appearance of diffuse fibrosis. Microareas of ischemia would be difficult, perhaps impossible, to diagnose clinically, because there would not be the usual overt symptoms of ischemia.

Taken together, we believe there is strong evidence in the literature that links problems with control and morphometry of the coronary microcirculation with the development of heart failure.

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Is the microcirculation important in reperfusion injury in man?

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The microcirculation is the key site involved in the pathophysiology of reperfusion injury. The microvasculature is an important site of nitric oxide (NO) production as well as of superoxide formation. Moreover, it is a primary location for leukocyte–endothelial cell interaction, which is the hallmark of reperfusion injury.
Reperfusion injury is initiated within minutes of reperfusion by the generation of superoxide radicals that inactivate NO. The reduced bioavailability of NO triggers an endothelial dysfunction that promotes neutrophil adherence and concomitant injury to the ischemic-reperfused tissue.
This neutrophil amplification stage is the primary mechanism of reperfusion injury to the heart or other organs subjected to ischemia and reperfusion.

Keywords: endothelium; nitric oxide; neutrophil; leukocyte adherence; P-selectin; cardiomyocyte

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Over recent years the microcirculation has gradually emerged as the most critical site in the cardiovascular system as the technology and tools for its study have been developed and attention has been focused on it as a potential target for disease. Physiologists and physicians have always considered the microcirculation to be very important since it is the "business end" of the circulatory system; indeed, to paraphrase Elizabeth Barrett Browning, "Let us count the ways."

Firstly, the microcirculation comprises by far the greatest portion of the peripheral vasculature in terms of length and surface area of blood vessels, so it is the bulk of the peripheral circulation. Second, the microcirculation is the site of gaseous exchange (eg, O_2 and CO_2), as well as the major locus for fluid permeability, which are the primary functions of the circulatory system. Third, the microcirculation has the greatest concentration of endothelial cell adhesion molecules, which become important in pathophysiologic conditions like reperfusion injury. Fourth, the microvascular endothelium synthesizes and releases many important humoral agents, which modulate vascular function. Thus, nitric oxide (NO) is produced very extensively by the microvascular endothelium. Therefore, it is vital that we focus our attention on the microcirculation in order to understand the pathophysiology of reperfusion injury. What role does the microcirculation play in reperfusion injury?

The next major question that needs to be answered is: why are we interested in reperfusion injury? Reperfusion injury is an inappropriate response to a sudden and full reestablishment of blood flow after a period of cessation of flow. Such an event occurs clinically after thrombolysis or balloon angioplasty of a blocked coronary artery, during cardiopulmonary bypass surgery, or following surgical correction of an occluded mesenteric or celiac artery. In the case of coronary thrombolysis or angioplasty, reperfusion injury can occur as cardiac arrhythmias, an uneven distribution of blood flow, cardiac stunning, or sudden cardiac death. In the case of mesenteric ischemia/reperfusion, reperfusion injury can result in a severe form of circulatory shock (ie, bowel ischemia/reperfusion) with resultant injury to the heart caused by the formation and release of a myocardial depressant factor (MDF), a small peptide produced by the ischemic pancreas.[1]

Reperfusion injury is an acute inflammatory response of the organism to a rapid reoxygenation of a previously hypoxic vascular bed. Although there are a variety of theories promulgated to explain the initial event in reperfusion injury,

the emerging consensus is that a large surge of superoxide radicals is released from the vascular endothelium, which overwhelms the NO formed by the vascular endothelium. This combination of events at the endothelium results in a phenomenon known as "endothelial dysfunction" and occurs within 2.5 to 5 minutes after coronary artery reperfusion.[2] Endothelial dysfunction is now recognized as the trigger mechanism for reperfusion injury.[3] In a sense, the endothelium poisons itself by eradicating the important humoral agent NO, which is nature's means of protecting the endothelium.

As endothelial dysfunction progresses, the endothelium becomes sticky[4] (ie, increased expression of endothelial cell adhesion molecules, particularly P-selectin,[5] occurs). This leads to a marked increase in neutrophil adherence 20 minutes following reperfusion.[4] Many of the adhered neutrophils undergo transendothelial migration and infiltrate the ischemic/reperfused myocardium by 180 minutes after reperfusion. These infiltrated neutrophils release a variety of humoral mediators (eg, oxygen-derived free radicals, proteases, cytokines, etc) all of which are cytotoxic to cardiac myocytes.[6] Therefore, the second major component of reperfusion injury is known as the "neutrophil amplification step."[7] The above description is a brief outline of the major steps in the chain of events generally accepted in the pathophysiology of reperfusion injury.

This paradigm of reperfusion injury has been carefully elucidated in myocardial ischemia/reperfusion, but applies to mesenteric ischemia/reperfusion injury[8,9] and renal ischemia/reperfusion as well.[10] In fact, since the mesenteric microcirculation is much easier to observe in vivo than the coronary microvasculature, many of the cell-to-cell interactions so vital for our understanding of the mechanisms of reperfusion injury have been elucidated in the mesenteric microvasculature.[8,9]

The next question is: how does endothelial dysfunction relate to myocardial reperfusion damage? The first clear demonstrations that ischemia/reperfusion of the heart results in an impaired vasodilator response to an endothelium-dependent dilator were by Van Benthuysen et al[11] and Mehta et al.[12] They showed a significantly attenuated vasodilator response to acetylcholine several hours after reperfusion of dog hearts. Since endothelium-dependent dilators (eg, acetylcholine) achieve dilation by releasing NO, their degree of vasodilation is a test of the ability of the endothelium to synthesize and release NO. The functional importance of endothelium-derived NO will become apparent later. The second major finding was made in 1990 by Tsao et al[4] who showed that the coronary endothelial dysfunction occurred as early as 2.5 min post-reperfusion and became progressively more severe over the next 4.5 hours. These studies were conducted in the cat heart, thus extending the concept to a second species. Subsequently, the phenomenon has been reported in the rat[2] and mouse[13] coronary vasculature. In general, endothelial dysfunction occurs in the absence of overt physical damage to the endothelium.

The next major development was to relate this very early endothelial dysfunction to the generation of superoxide radicals[2,14] by the endothelium itself. This dysfunction can be dramatically attenuated by recombinant human superoxide dismutase (rhSOD), an enzyme that inactivates superoxide radicals. Tsao and Lefer[2] showed that the endothelial dysfunction, which had previously been demonstrated only in large coronary arteries, also occurred in the coronary microcirculation. Furthermore, Sheridan et al[15] elegantly showed that neutrophils accumulate in the coronary microcirculation upon reperfusion. Moreover, in the perfused cat heart, a moderate but significant endothelial dysfunction occurs in the absence of neutrophils

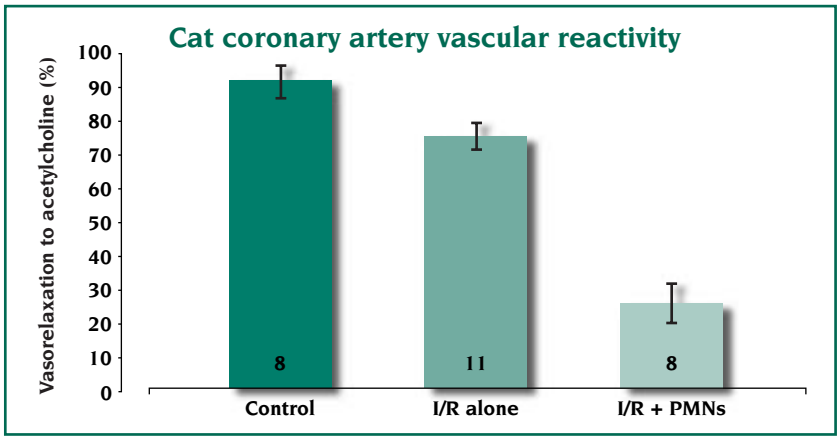

***Figure 1.** Bar graph of isolated cat coronary artery rings following perfusion of isolated cat hearts under conditions of ischemia/reperfusion (I/R). Bar heights are percent vasorelaxation to 100 nM acetylcholine; brackets indicate ± SEM; numbers at bottom of bars indicate number of observations. I/R alone is P<0.05 from control; I/R + PMNs (polymorphonuclear neutrophils) is P<0.01 from control.*

in the perfusate, but there is severe exacerbation of the endothelial dysfunction when reperfusion occurs in the presence of neutrophils. This is illustrated in *Figure 1*.

In terms of treatment of myocardial ischemia, one can combine specific therapeutic agents with a thrombolytic drug (eg, streptokinase, tissue-type plasminogen activator [tPA], etc) or administer a cardioprotective agent, just prior to angioplasty. In a surgical operation such as that carried out in cases involving cardiopulmonary bypass, agents of this type can be given in the fluid used to prime the pump or upon recovery of normal coronary perfusion. These conditions offer attractive clinical opportunities to treat impending reperfusion injury. In these cases, the microcirculation is the primary target since the act of reperfusion washes out the coronary macrocirculation of debris (ie, broken plaque, thrombi, leukocyte plugs, etc), which is transported downstream into the coronary microcirculation.

Most of the leukocyte-endothelium interaction occurs at the level of the microcirculation since this is the major site of high-density cell adhesion molecule (CAM) expression, probably to the greatest extent on the endothelium of postcapillary venules, but also to a significant degree on arteriolar endothelium. Studies have shown both small arteries and venules to be sites of leukocyte adhesion in the ischemic/reperfused dog coronary vasculature.[16] Recently, Banda et al[13] showed that mice deficient in either P-selectin or intercellular adhesion molecule–1(ICAM-1) do not develop a significant degree of endothelial dysfunction following mesenteric ischemia/reperfusion. These so-called "gene knockout" mice offer a very nice model with which to study the role of a specific molecule by its total absence in an animal. They offer a variety of advantages, primarily by allowing one to bypass the use of drugs or antibodies to block the effects of a relevant molecule. As these gene-targeted mice gain wider acceptance, they will be used to answer many specific physiologic and pathophysiologic questions.

At this point, we need to discuss the process of leukocyte–endothelial cell interaction at the level of the microcirculation. This process is much more complex than simply having leukocytes adhere to the endothelium, and comprises at least three important sequential events. By employing the technique of intravital microscopy, one is able to study these processes. As detailed in the lead article by Granger in this issue, intravital microscopy is an important technique for dissecting the steps in leukocyte-endothelium interaction. The leukocytes normally flow in the axial stream of the blood with the erythrocytes and platelets. When an inflammatory event occurs, leukocytes start to roll along the venular endothelium. This slows the leukocytes down to a velocity that allows "capture" by the endothelium if certain CAMs are present. This "rolling" is governed by the selectin family of adhesion glycoproteins, primarily by P-selectin. Many of these rolling leukocytes, which are primarily polymorphonuclear neutrophils, will adhere to the vascular endothelium largely due to the interaction of the β_2 integrin CD11b/CD18 present on the neutrophil surface as well as its counterreceptor ICAM-1 on the endothelial cell surface. Once adhered, many neutrophils flatten out on the endothelial surface and then can undergo transendothelial migration, a process whereby they burrow through the endothelium at the junction of two endothelial cells. This transmigration is largely regulated by platelet–endothelial cell adhesion molecule–1 (PECAM-1), which is constitutively present at high levels at interendothelial cell junctions.

Normally, isolation of the rat mesentery and equilibration for 15 to 20 minutes does not result in any significant degree of rolling or adherence of leukocytes to 40- to 50-µm venules. However, if one superfuses the normal mesentery with 25, 50, or 100 µM N^G-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase (NOS) inhibitor, there is a concentration-dependent increase in leukocyte rolling and adherence within 30 minutes. L-NAME rapidly inhibits synthesis of NO from the vascular endothelium. This reduced NO allows the translocation of P-selectin from granules within endothelial cells to the endothelial surface, thus upregulating P-selectin expression and promoting leukocyte rolling.[17] Many rolling leukocytes subsequently adhere, partly due to the adhesive actions of P-selectin. Addition of L-arginine to the perfusate overrides the L-NAME–induced inhibition of NOS, allowing NO synthesis, which suppresses leukocyte rolling and adherence. Similar inhibitory effects occur if one treats the rat intravenously with a monoclonal antibody directed against P-selectin. Moreover, in P-selectin knockout mice, there is a virtual absence of rolling of leukocytes along the endothelium.[18] The absence of P-selectin in knockout mice can be confirmed by immunohistochemistry in the mesenteric microvasculature. Thus, P-selectin is expressed on the microvascular endothelium in the presence of L-NAME and is markedly attenuated by L-arginine. The final contribution to the under-

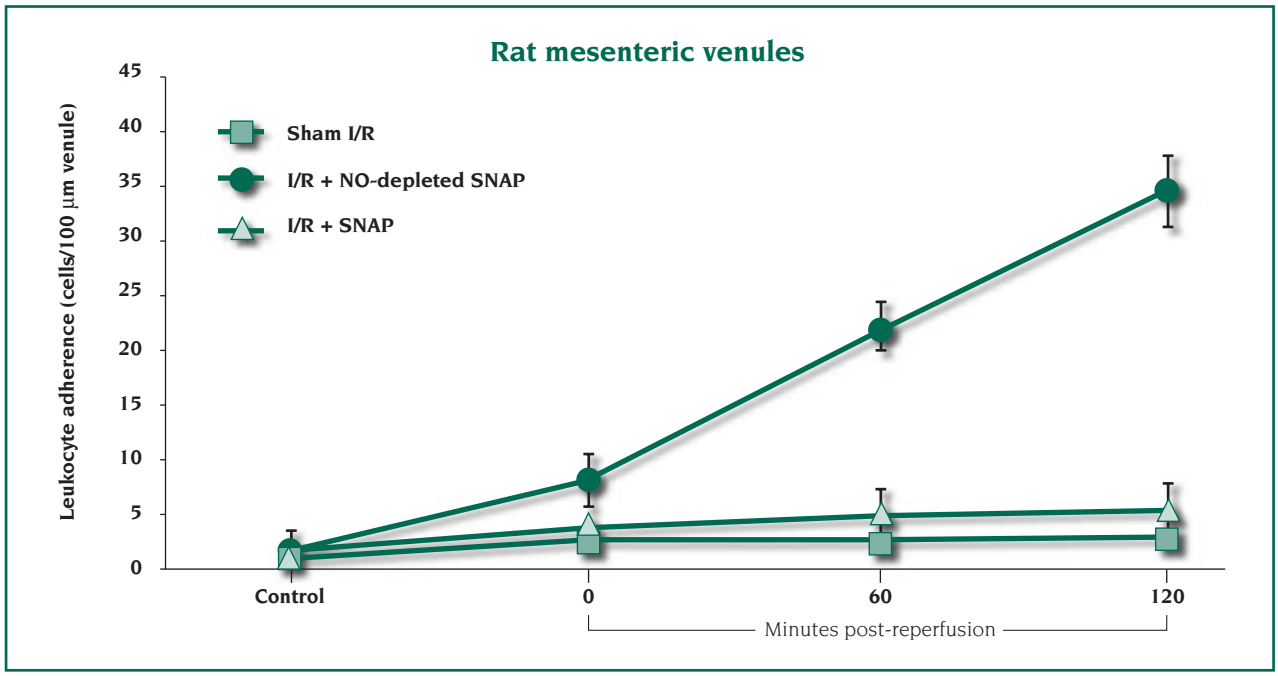

Figure 2. *Leukocyte adherence to rat mesenteric venules by intravital microscopy, over 120 minutes of reperfusion following total ischemia of the superior mesenteric artery for 60 minutes. Control is preischemic value. All values are means ± SEM for six rats in each group. SNAP = S-nitroso-acetylpenicillamine, an NO donor. A significant increase in leukocyte adherence was observed 60 and 120 minutes post-reperfusion (P<0.01 from other two groups). I/R, ischemia/reperfusion.*

standing of this process was made by Gauthier et al,[19] who showed that ischemia of the mesenteric circulation for 60 minutes followed by reperfusion produced a rapid, progressive, and sustained leukocyte rolling and adherence. *Figure 2* illustrates the postreperfusion adherence of leukocytes in mesenteric venules. Within 30 minutes of reperfusion, leukocyte adherence was significantly increased, and continued to increase over the next 90 minutes to a peak (ie, a 6- to 7-fold increase). However, when a NO donor was given at the time of reperfusion, the increased rolling was almost totally inhibited.[20] Thus, the loop is closed: (i) inhibition of endothelium-derived NO increases leukocyte rolling and adherence to the microvascular endothelium; (ii) reduced NO in turn upregulates P-selectin expression on the microvascular endothelium; and (iii) replenishment of NO with an NO donor can attenuate rolling and adherence and blunt P-selectin surface expression on the microvascular endothelium.

Nevertheless, when leukocytes adhere to the microvascular endothelium following ischemia/reperfusion, as in the case of the coronary microcirculation,[15] the stage is set for a marked exacerbation of myocardial necrosis (ie, reperfusion injury). This can be accomplished by at least three separate mechanisms at the microvascular level. The first is by accumulation of leukocytes in the microvascular vessels, forming a type of "plug" that effectively cuts off flow to the region supplied by the affected vessels.[20] The leukocytes may even aggregate to form microvascular plugs by homotypic aggregation. This is a form of the "no-reflow" phenomenon, and clearly causes selected regions of the myocardium to become grossly ischemic even though macrocirculatory reperfusion has occurred.

The second mechanism by which leukocytes may contribute to reperfusion injury is by the release of mediators following adherence of the leukocytes to the coronary microvascular endothelium. In the heart, each myocardial cell is in close apposition to a capillary, and thus the diffusion distance between the leukocyte and cardiac myocyte is virtually the thickness of an endothelial cell, about 1 μm. This distance is short enough to allow some diffusion of leukocyte-released mediators to the nearby cardiomyocytes. Buerke et al[6] showed that isolated adult rat cardiomyocytes rendered hypoxic for 20 minutes and reoxygenated for 20 minutes are almost all normal (ie, 95%±4% viable). However, when incubated in the presence of

supernatants of leukocytes, the viability was only 53%±3%. Thus, leukocytes release humoral mediators of cell injury and death under conditions very similar to ischemia/reperfusion.

The third mechanism of leukocyte-induced reperfusion injury is via leukocyte transendothelial migration out of the microcirculation to the myocytes. These transmigrated leukocytes are like loaded pistols waiting to fire bullets that can injure or kill cardiomyocytes. Their bullets may be small, but as the Bard of Avon would say, "twill do." There is even significant evidence that cardiomyocytes express ICAM-1 on their surface acting to attract leukocytes which subsequently adhere to the ischemic/reperfused myocytes.[21] Of course, leukocytes that are adherent to myocytes can release their cytotoxic mediators at point-blank range, exerting effects at higher concentrations than the same mediator diluted by a longer diffusion distance.

In all three cases, leukocytes and their activation products aggravate an already sensitized tissue that has been ischemic for some time. The resultant combination of events acts to worsen a marked inflammatory state, thus promoting cell injury to the affected region in a manner that compromises organ function.

CONCLUSION

Regional reperfusion injury occurs in the myocardium, kidneys, splanchnic organs (eg, liver, intestine), and in the limb. Hemorrhage and transfusion actually is a form of total body ischemia/reperfusion. In all these cases, the pathophysiology features an early endothelial dysfunction followed by a neutrophil amplification stage sometime later. *Figure 3* shows a schematic representation of the key events occurring in the microcirculation that are responsible for reperfusion injury. These events have been found across several species including the rat, mouse, rabbit, cat, dog, and primate, and are thought to occur to a large extent in man, although the data are less certain in humans due mainly to the limitations on invasive measurements in man. This represents a new frontier to attain in the future, one that is less invasive, as more precise technologies become available. The overwhelming answer to the question posed by the title of this article, in general, is "Yes." However, we lack conclusive data in man. There are no compelling reasons to think that man is very different from other mammals in response to ischemia/reperfusion, but we must strive to obtain carefully controlled, precise measurements in man to finally answer this key question.

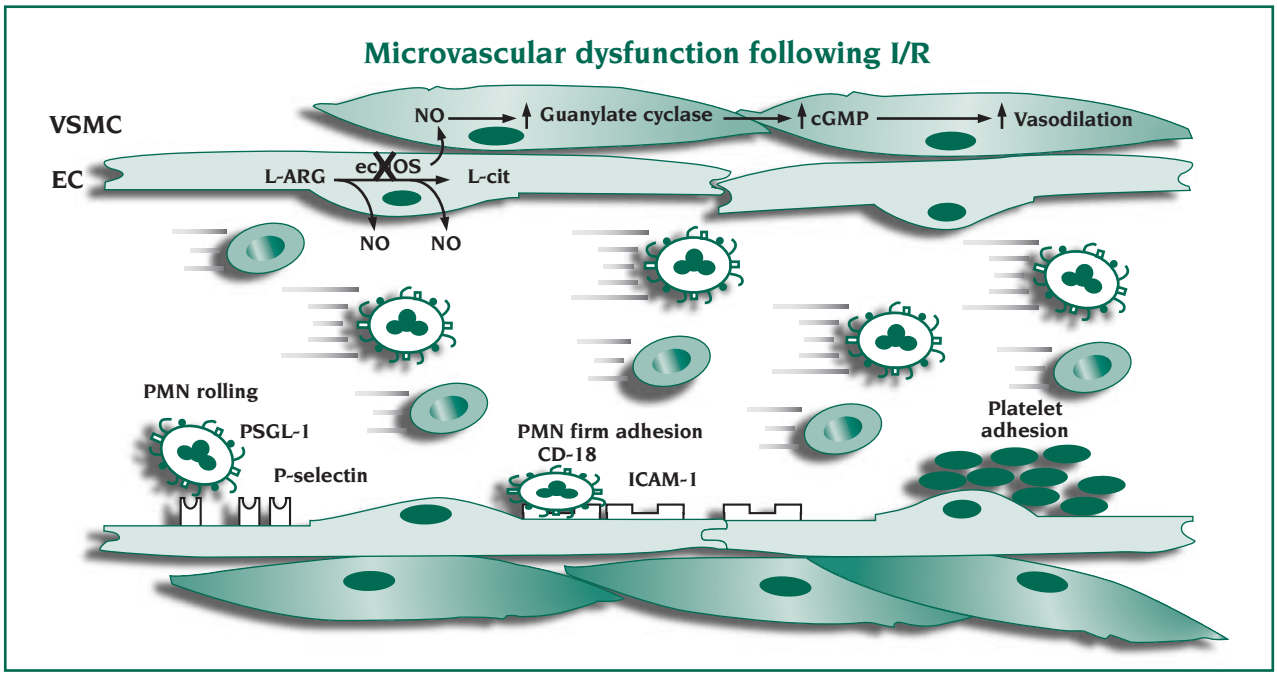

Figure 3. *Schematic diagram of microvascular dysfunction following ischemia/reperfusion (I/R). VSMC, vascular smooth muscle cells; EC, endothelial cells; PMN, polymorphonuclear neutrophils; cGMP, cyclic guanosine monophosphate; L-cit, L-citrulline; PSGL-1, P-selectin glycoprotein ligand–1; NO, nitric oxide; ecNOS, endothelial constitutive nitric oxide synthase; ICAM-1, intercellular adhesion molecule–1. Discoid cells in the axial stream are erythrocytes. PMNs and platelets are shown interacting with the endothelium.*

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Microcirculation

Summaries of Ten Seminal Papers

1

The supply of oxygen to the tissues and the regulation of the capillary circulation

A. Krogh. *J Physiol (Lond).* **1919**

2

Regional differences in capillary permeability

H.S. Mayerson and others. *Am J Physiol.* **1960**

3

Endothelial contraction induced by histamine-type mediators: an electron microscopic study

G. Majno and others. *J Cell Biol.* **1969**

4

Isolation of a tumor factor responsible for angiogenesis

J. Folkman and others. *J Exp Med.* **1971**

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Quantitative investigations of the adhesiveness of circulating polymorphonuclear leukocytes to blood vessel walls

A. Atherton, G.V. Born. *J Physiol (Lond).* **1972**

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The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine

R.F. Furchgott, J.V. Zawadzki. *Nature.* **1980**

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Superoxide radicals in feline intestinal ischemia

D.N. Granger and others. *Gastroenterology.* **1981**

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Leukocyte capillary plugging in myocardial ischemia and reperfusion in the dog

R.L. Engler and others. *Am J Pathol.* **1983**

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A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1

R. Rothlein and others. *J Immunol.* **1986**

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Leukocyte rolling and extravasation are severely compromised in P-selectin–deficient mice

T.N. Mayadas and others. *Cell.* **1993**

Selection of seminal papers by
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Highlights of the years by **Dr P.B. Garlick**
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The supply of oxygen to the tissues and the regulation of the capillary circulation

A. Krogh

J Physiol (Lond). 1919;52:457-474

This seminal paper is the first to describe the regulation of the capillary circulation in relation to the supply of oxygen to tissues. Prior to this publication in 1919, essentially nothing was known regarding vasomotion of the microcirculation, reactive hyperemia, or the regulation of blood flow. Using the best equipment and techniques available at the time, August Krogh very meticulously describes changes that occur in capillary perfusion of a number of animals exposed to a variety of conditions. The results of the investigations are given in great detail, and the style of writing employed by Krogh makes the paper very interesting to read.

August Krogh had previously reported that oxygen pressure in the muscles was equivalent to that in the capillary blood and suggested that capillaries were responsible for the delivery of blood to skeletal muscle. The focus of this study was to understand how blood flow in capillaries was adjusted during exercise to supply adequate amounts of blood to the working muscle. The author believed that at rest only a small number of capillaries were open for blood flow and that during exercise muscular work could cause the opening of additional capillaries. The idea of vessel recruitment had not been previously advanced, and the hypothesis put forth in this classic publication is extremely astute.

Krogh tested his hypothesis using microscopic examination of a number of tissues in the frog and guinea pig under various conditions. He observed that under resting conditions most of the capillaries are in a state of contraction and therefore closed to the passage of blood flow. Stimulation of muscles by gentle massage caused a large number of capillaries to open up. Similarly, Krogh also observed a large number of open capillaries in spontaneously contracting muscles. He also describes for the first time the use of india ink as a method to quantify the number of patent vessels in a tissue sample. Using india ink, Krogh also reports that the diameters of capillaries are increased in working tissues and this may serve to enhance tissue perfusion. Evidence is provided that capillaries are not merely passively autoregulated by blood pressure, but constantly perform active changes in their diameter. Finally, Krogh indicates that hyperemia is a result of changes in capillary diameter and increase in the number of patent capillaries.

This outstanding paper was clearly years ahead of its time. Using very simple technologies and a straightforward approach, Krogh was able to discover the changes that can occur in capillaries to regulate blood flow and oxygen delivery to tissues. The concepts that are described in this paper are still true today and represent a substantial degree of what is still being actively investigated by microcirculatory physiologists. The concepts of regulation of capillary perfusion presented in this paper are vital to our understanding of derangements in the microcirculation that occur in cardiovascular disease. It is clear that this publication represented the culmination of a large number of extremely time-consuming experiments that were very meticulously performed. The paper is brilliantly written and a joy to read. It is also evident from this publication that August Krogh was an outstanding scientist.

1919

British troops massacre 500 people at the Golden Temple at Amritsar; Ernest Rutherford splits the atom; and Felix the Cat is born

Regional differences in capillary permeability

H.S. Mayerson, C.G. Wolfram, H.H. Shirley Jr, K. Wasserman

Am J Physiol. 1960;198:155-160

This highly ingenious publication resulted in the development of the concept that capillary permeability might be regulated by pores of differing sizes. This study was based on earlier work by Mayerson and colleagues in which they infused dextran fractions of different molecular weights into dogs and followed their appearance in thoracic duct lymph. The authors had postulated that capillary beds contained pores of varying sizes that regulate the passage of molecules.

To address this issue, the authors investigated the distribution of dextran fractions ranging from 10 600 to 412 000 in molecular weight in animals. In order to examine regional differences in capillary permeability, the authors investigated the disappearance of dextran in the intestine, liver, and spinal column. This paper laid the groundwork for all future experimental studies in which lymph/plasma ratios were utilized as a measure of tissue-capillary permeability.

One of the most striking findings of this study is the discovery that there are at least two different sets of pores in the same capillary beds. The authors also propose an alternative hypothesis that macromolecules do not penetrate the capillaries through pores, but are actually taken up by vesicles that transport the molecules across the capillaries and extruded. Both of these explanations are plausible based on all the information presented in this publication.

Another major finding is the regional differences in capillary permeability that were observed in different organs in the same animal. The authors measured lymph/plasma ratios of dextran and radioactive albumin over time to calculate the relative permeability of each organ. Interestingly, the liver was found to be the most permeable, followed by the intestine and the cervical region. These permeability data fit very well with our current understanding of the ultrastructural characteristics of the capillaries from these organs. At the time of this report by Mayerson and colleagues, other scientists were using newly developed technologies to describe the endothelial cell types in the microcirculation of a number of organs. It is interesting to see how physiological function of the microcirculatory permeability fits well with the anatomy of capillary endothelium.

This paper has been referenced time and again since it was published in 1960. The methods presented in it have been used by scientists the world over and are still in use today. The use of the lymph/plasma ratio represented a novel approach to examining the exchange of macromolecules. This technique has been used as a method to try to understand the mechanisms of tissue edema in a number of diseases and is a highly valuable tool for the modern physiologist. This paper also represents the first quantitative study of capillary permeability in various organs, and its results have been confirmed many times by others.

1960

"Lady Chatterley's Lover"
is ruled not to be an obscene book;
East Germany closes the border with West Germany;
and "Ben Hur" wins ten Oscars,
to be equaled by "Titanic" in 1998

Endothelial contraction induced by histamine-type mediators: an electron microscopic study

G. Majno, S.M. Shea, M. Leventhal

J Cell Biol. 1969;42:647-672

One of the important physiological functions of the microcirculation is the regulation of permeability. Blood vessels may become leaky as a result of direct injury to the endothelium such as that produced by physical trauma. Permeability increases may also be triggered by a variety of chemical mediators. Previous research had suggested that endogenous chemical mediators such as histamine increased microvessel permeability by creating small gaps between venular endothelial cells. The mechanism responsible for the formation of gaps between endothelial cells remained a mystery despite the interesting hypotheses put forth by a number of laboratories. Majno and colleagues had reviewed electron micrographs of histamine-injected tissue and noticed that the endothelial cells underwent contraction, possibly thus accounting for the increase in permeability induced by histamine. In this seminal paper, the authors describe in detail the cellular effects of a number of mediators on endothelial cell contraction using state-of-the-art electron microscopy techniques.

To do this, they injected histamine, serotonin, and bradykinin into the scrotum of rats. In order to maintain normal vessel dimensions, the root of the cremaster was constricted prior to sacrifice, which resulted in a mild degree of vascular congestion. The investigators then examined electron micrographs from 599 nuclei of endothelial cells which represented the various experimental conditions. A series of outstanding electron micrographs is presented that beautifully illustrate the changes within endothelial cells following administration of a number of chemical mediators. The most salient finding is the pronounced nuclear pinching that occurs in leaky vessels. The authors very astutely conclude that the only acceptable explanation for nuclear pinching is endothelial cell contraction. They were the first to discover that contraction of endothelial cells can result in increased vascular permeability secondary to exposure of vessels to the chemical mediators histamine, serotonin, and bradykinin. This observation represents a major step in our understanding of the process of increased vascular permeability that occurs in the microcirculation during pathophysiological conditions.

Perhaps one of the most underappreciated aspects of microcirculatory research is the area of vascular permeability. One possible explanation for this is the difficulty in direct measurement of microvascular permeability in intact organs. However, increases in blood vessel leakiness are a major consequence of a number of diseases in the heart. For example, edema formation occurring in the ischemic-reperfused myocardium is a result of increased permeability of coronary microvessels. Increased tissue edema exacerbates myocardial dysfunction and tissue necrosis by a number of mechanisms. Fluid accumulation in the interstitium can compress coronary vessels and further limit blood flow to injured cardiac myocytes. It is also now appreciated that therapies that limit microvascular permeability can limit tissue injury in a number of models of tissue injury. Taken together, a more complete understanding of how microvessels modulate permeability is extremely important for the development of therapeutic strategies of the treatment of cardiovascular diseases. This first-rate paper by Majno and associates provides very convincing evidence that the contraction of microvascular endothelial cells promotes the permeability increases that are mediated by chemical mediators independent of direct endothelial cell disruption.

1969

Neil Armstrong takes “one small step for man, one giant leap for mankind”;
Butch Cassidy and the Sundance Kid ride lawlessly across cinema screens;
and Samuel Beckett is awarded the Nobel Prize for Literature

Isolation of a tumor factor responsible for angiogenesis

J. Folkman, E. Merler, C. Abernathy, G. Williams

J Exp Med. 1971;133:275-288

In this now legendary paper, Folkman and colleagues describe the isolation and characterization of a substance derived from tumors that promotes the growth of blood vessels. It was well known that tumors required a constant blood supply in order to grow in size, hence the development of new vessels. Folkman and his group capitalized on this observation and isolated a soluble factor from both human and animal neoplasms that caused capillary endothelium to grow. This substance was called tumor angiogenesis factor (TAF) and represents the genesis of modern day research in the field of angiogenesis.

In initial experiments, these investigators injected rats with tumor cells and then isolated the tumors, which were subjected to a number of steps designed to isolate a number of factors that could then be assayed for angiogenic activity in a biological assay system. The isolation procedure resulted in six fractions that were assayed for biological activity in a dorsal air sac produced in the rats by the injection of air. The investigators placed a specially designed tube into the air sac and injected a test fraction obtained from the solid tumors. The extent of vascular proliferation in the tube was measured 48 hours later. A number of the supernatants from rat tumors produced angiogenesis, and the authors reported that fractions obtained from mouse B16 melanomas, human neuroblastomas, Wilms tumors, and hepatoblastomas also produced neovascularization. Histological evaluation of subcutaneous tissues stimulated with tumor angiogenesis factor revealed the presence of serpentine veins and venules at first, followed by the development of dense capillary beds that resembled regenerating endothelium.

The authors then provided preliminary characterization of the tumor angiogenesis factor and indicated that this soluble factor was comprised of 25% RNA, 10% protein, 50% carbohydrate, with the remainder presumed to be lipid material. The authors also reported that tumor angiogenesis was relatively stable, and they believed that the protein moiety associated with RNA was essential for the mitogenic effect of this factor on capillary endothelium.

In the summary of the paper, the authors suggest that inhibition of this newly discovered angiogenic factor might arrest solid tumors at a very early stage.

This exciting paper precedes the discovery and description of a host of growth factors such as vascular endothelial cell growth factor (VEGF), which is currently the focus of a number of investigations. Agents that inhibit the growth of tumor blood vessels are being evaluated clinically for the treatment of a number of cancerous tumors in man. In addition, the application of vascular growth factors such as VEGF to ischemic hearts is currently being investigated in humans as a means to supply blood to regions of the heart suffering from poor perfusion. The development of agents that either inhibit the development of new vessels or promote neovascularization can be directly traced to this seminal paper by Folkman's research team. It has taken nearly 30 years for this brilliant research to come into the mainstream and to stimulate other scientists and clinicians to pursue the modulation of angiogenesis as a means of therapy. At present there is an explosion of research into vascular growth factors and agents that inhibit vessel proliferation, and it is almost certain that the result of these efforts will be novel therapies for cancer and cardiovascular diseases.

1971

The Aswan High Dam is opened;
Decimal currency is introduced into the UK;
and Louis Armstrong,
legendary jazz trumpet-player, dies aged 71 years

Quantitative investigations of the adhesiveness of circulating polymorphonuclear leukocytes to blood vessel walls

A. Atherton G.V. Born

J Physiol (Lond). 1972;222:447-474

Much of today's research into the microcirculation deals with the interactions between circulating leukocytes and the endothelium. A plethora of studies have been aimed at the elucidation of the mechanisms responsible for the adhesive interactions between these two cell types. It is believed that understanding how leukocytes become adherent to the blood vessel wall will lead to the development of novel anti-inflammatory agents that could be used to treat a number of diseases in man. Atherton and Born describe for the first time the use of intravital videomicroscopy to quantify leukocyte rolling in the microcirculation. Their revolutionary paper describes the techniques required to visualize leukocyte–endothelial cell interactions in the hamster cheek pouch and in the mouse mesentery.

Atherton and Born first detail the two animal preparations utilized as well as the techniques developed to count the number of emigrated granulocytes within histological sections of tissue. Baseline leukocyte rolling in the microcirculation is measured. The surgical techniques required to exteriorize the mouse mesentery or the hamster cheek pouch induced a transient rolling response that rapidly subsided. Both of these intravital preparations remained stable for several hours following the surgical procedures and a short equilibration period. Thus, the techniques required to investigate leukocyte function in the microcirculation in situ do not significantly alter normal leukocyte behavior.

Subsequent experiments were conducted to determine the effects of various inflammatory mediators on leukocyte rolling in the microcirculation of the mouse mesentery and hamster cheek pouch. In the venules of the mouse mesentery, ethylenediaminetetraacetic acid (EDTA) rapidly and totally abolished granulocyte rolling. The authors suggest that calcium and/or magnesium ions are essential for granulocyte rolling in venules. Human serum albumin was shown to have no effect on leukocyte rolling in the hamster cheek pouch. Similarly, both trypsin and histamine failed to exert any significant effects on leukocyte rolling in the microcirculation. Finally, experiments were also performed with agents that were believed to stimulate granulocyte function, including Hammarsten casein, plasma permeability factors, and culture filtrate from *Escherichia coli.* As expected, all of these agents increased leukocyte rolling in the microcirculation.

While the data presented in this paper do not reveal the cellular mechanisms that modulate leukocyte rolling, they do lay the foundation for subsequent studies investigating leukocyte–endothelial cell interactions. Since the publication of this paper, a number of laboratories throughout the world have utilized intravital microscopy techniques as a means of investigating leukocyte adhesion. It is now well appreciated that leukocyte rolling along the endothelium of blood vessels is largely mediated by a family of adhesion molecules called selectins. It is possible that various selectins may have been responsible for the rolling response that was observed in the mouse mesentery and hamster cheek pouch. Regardless of the rolling mechanism described in this paper, there is no doubt that modern microcirculation scientists are indebted to Atherton and Born for their innovation in instrumentation, surgery, and scientific thinking that resulted in the development of modern intravital videomicroscopy.

1972

US swimmer Mark Spitz wins seven gold medals at the Munich olympics;
President Idi Amin Dada expels 50 000 Asians from Uganda;
and US troops finally leave Vietnam

The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine

R.F. Furchgott, J.V. Zawadzki

Nature. 1980;288:373-376

This clearly revolutionary article set the stage for modern vascular biology. A very interesting aspect is the use of traditional pharmacological methods in a highly elegant manner to discover a novel vascular mediator with far-reaching implications. This study investigated isolated segments of rabbit aorta in response to acetylcholine (ACh). It had previously been reported that ACh produced graded contractions of rabbit thoracic aorta that were mediated via interactions with muscarinic receptors on the vascular smooth muscle. It had also been reported that injection of ACh in vivo resulted in a rapid and marked vasodilatory response. The central observation was that ACh induced vasorelaxation of the isolated rabbit aorta if care was taken to preserve endothelial cell integrity. The authors demonstrated for the first time that the vascular endothelium releases a substance that mediates vasorelaxation, and this substance was later named endothelium-derived relaxing factor (EDRF).

The data presented in this paper include the vascular reactivity profile of vascular rings and transverse strips of rabbit thoracic aorta. Initial experiments documented the potent vasorelaxation effect of low concentrations of ACh on both of these preparations. Denudation of the endothelium by rubbing was shown to abolish the vasorelaxant effect of ACh and resulted in a modest degree of vasocontraction of the vascular segments. In addition, collagenase treatment to remove endothelial cells also abolished the vasorelaxation previously observed with ACh. Furchgott and Zawadzki also utilized a novel vascular sandwich method to confirm that endothelial cells were in fact the source of the relaxing factor that was released following the application of ACh. These authors also demonstrated that when blood vessels were exposed to anoxia, the vasorelaxant effects of ACh were attenuated. This represented the first demonstration of selective endothelial cell injury following anoxic conditions. All of the data presented in this landmark paper point to the existence of a vasodilator substance released by healthy endothelial cells to regulate vascular tone.

It has been almost 20 years since the initial discovery of EDRF by Furchgott and Zawadzki and, since that time, thousands of papers have been published that emphasize the biological significance of this highly labile substance. The identity of EDRF was later determined to be nitric oxide (NO), and three different isoforms of an enzyme, NO synthase, capable of regulating the formation of NO, were evidenced. A number of studies have determined that endothelial cell–derived NO is essential for the maintenance of vascular homeostasis throughout the circulatory system. In this regard, NO is an important regulator of blood flow and arterial pressure, leukocyte and platelet adhesion, blood vessel growth and repair, and permeability of the microcirculation. Endothelial cell production of NO is now well known to be dramatically reduced in pathological states such as hypertension, atherosclerosis, and ischemia/reperfusion syndromes, and it is believed that loss of NO is a major contributor to organ injury in these diseases. This has prompted major research efforts aimed at developing NO-releasing compounds that might be useful for NO supplementation for patients suffering from cardiovascular diseases. It is remarkable that a straightforward paper investigating the vascular reactivity of rabbit aorta could serve to reveal a major regulatory pathway in the circulation that has kept molecular biologists, biochemists, pharmacologists, and physiologists busy for all these years.

1980

UK's Nigel Short, aged 14 years,
becomes the world's youngest Chess Master;
Björn Borg wins his fifth consecutive
Men's Singles title at Wimbledon;
and San Salvador's Archbishop, Oscar Romero,
is assassinated

Superoxide radicals in feline intestinal ischemia

D.N. Granger, G. Rutili, J. McCord

Gastroenterology. 1981;81:22-29

A major breakthrough in our understanding of the mechanisms of ischemia/reperfusion injury to the microcirculation was provided by this elegant study. Previous studies by this group had clearly determined that permeability of intestinal capillaries was dramatically increased following 1 hour of regional intestinal ischemia and reperfusion in the cat. In the present study, the authors sought to uncover the mechanisms responsible for ischemia-mediated increases in intestinal capillary permeability. Granger and colleagues focused on the potential effects of a number of vasoactive substances, including histamine, bradykinin, and prostaglandin, using specific pharmacological antagonists of these mediators. The various agents were administered intravenously at 1 hour, and at 15 minutes prior to reperfusion of the intestine.

Interestingly, pretreatment with indomethacin (a prostaglandin synthesis inhibitor) or cimetidine and benadryl (specific H_1 and H_2 histamine antagonists) failed to significantly alter the vascular permeability changes resulting from intestinal ischemia/reperfusion. Based on data presented in previous studies utilizing other experimental models, it would seem logical that both prostanoids and histamine contribute to the vascular permeability changes observed in the intestinal microcirculation following ischemia/reperfusion. However, this study clearly indicates that prostaglandins and histamine do not mediate microcirculatory injury following ischemia/reperfusion.

In an additional set of experiments, the effects of superoxide dismutase (SOD) on postischemic intestinal capillary permeability was also investigated in this same model of ischemia/reperfusion. These experiments are truly groundbreaking, since the investigation of the effects of this potent and specific superoxide scavenger had not been studied in any in vivo model prior to this study. The data presented in this paper regarding treatment with SOD very convincingly suggest that the superoxide anion generated following intestinal ischemia/reperfusion contributes to increased capillary permeability. Remarkably, the postischemic lymph-to-plasma protein concentration ratio (L/p) in SOD-treated animals is very similar to that observed under control conditions in the same animals. This paper represents the first report that SOD treatment protects the microcirculation from in vivo ischemia/reperfusion injury and laid the foundation for subsequent investigations of the potential protective actions of various forms of SOD and other free-radical scavengers in a host of animal and human ischemic syndromes.

Clearly, the most arresting information provided by this excellent research paper relates to SOD therapy in the setting of ischemia/reperfusion injury. Following the publication of this paper, a number of investigators began to study the effects of SOD therapy on tissue injury in a variety of animal models of ischemia/reperfusion injury. The majority of experimental studies of SOD that followed the work of Granger et al dealt with the potential cardioprotective actions of SOD in myocardial ischemia/reperfusion injury. A host of studies of SOD and myocardial ischemia/reperfusion injury were performed during the next 10 years by numerous laboratories, each using different forms of SOD in different animal models. Interestingly, despite a large number of reports suggesting that SOD can indeed protect ischemic myocardium, there was an equal number of publications that failed to demonstrate any protective effects of this free-radical scavenger. As a result, the period of the 1980s was marked by frequent controversies at international meetings, where investigators would present totally contradictory data, resulting in heated discussions relating to the actions of SOD. To date, these actions in the setting of myocardial ischemia/reperfusion remain an unsolved mystery.

1981

Picasso's "Guernica" returns to Spain after spending 40 years in New York; Egyptian President Anwar el-Sadat is assassinated during a military parade; and Bob Marley, king of reggae music, dies aged 36 years

Leukocyte capillary plugging in myocardial ischemia and reperfusion in the dog

R.L. Engler, G.W. Schmid-Schonbein, R.S. Pavlec

Am J Pathol. 1983;111:98-111

In this paper, Engler and colleagues present the very first experimental report that the low flow or "no-reflow" that occurs following myocardial ischemia/reperfusion is mediated at least in part by leukocyte plugging in myocardial capillaries. The authors point out that leukocytes are large, stiff cells that naturally adhere to the vascular endothelium, but despite this obvious rationale for implicating leukocytes in the pathogenesis of "no-reflow," there was a paucity of data suggesting the involvement of circulating white blood cells in this problematic microvascular disorder. The experimental design utilized for these studies was remarkably simple yet highly elegant. Results presented in this seminal paper served to launch the concept of "leukocyte-mediated" reperfusion injury in a variety of tissues including the heart.

The direct observation that leukocytes can occlude capillaries in skeletal muscle following hypotension was the starting point for this study. In order to investigate the potential role of circulating leukocytes in postischemic capillary obstruction, the authors subjected anesthetized, open-chest dogs to left anterior descending coronary artery ischemia for periods of 1, 3, or 5 hours, followed by reperfusion. Additional animals were subjected to either 1 or 3 hours of ischemia in the absence of reperfusion. Hearts were perfused with Ringer's lactate containing a carbon suspension as a marker for capillary patency. In addition, the presence of leukocytes remaining within capillaries was also determined from the numerous myocardial tissue biopsy samples that were obtained from both nonischemic and ischemic regions of the heart.

Engler and colleagues demonstrated that the vast majority (98%) of the capillaries within nonischemic myocardial tissue contained carbon, very few leukocytes, and few erythrocytes. Whereas tissue harvested from the ischemic-reperfused zone revealed that 60% of the capillaries had no carbon, high hematocrits, and approximately one leukocyte per unbranched capillary, 40% of the capillaries demonstrated reflow and no leukocytes. Furthermore, a significant correlation was demonstrated between capillaries with no reflow (without carbon) and the frequency of leukocytes remaining in these capillaries.

This study provided clear-cut evidence of leukocyte capillary plugging in myocardial tissue following ischemia and reperfusion.

This thorough and exciting study triggered subsequent studies that examined the involvement of leukocytes in myocardial reperfusion injury in terms of their role in capillary "no-reflow," coronary endothelial cell injury, and myocardial cell "stunning" and necrosis.

1983

US troops invade the Caribbean island of Grenada;
Polish Solidarity leader Lech Walesa wins the Nobel Peace Prize;
and Ben Kingsley wins the Best Actor Oscar for his portrayal of Gandhi

A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1

R. Rothlein, M.L. Dustin, S.D. Marlin, T.A. Springer

J Immunol. 1986;137:1270-1274

Rothlein and colleagues describe the discovery and characterization of a novel leukocyte–endothelial cell adhesion molecule in this now classic paper. These investigators had discovered that homotypic aggregation of isolated lymphocytes required CD11a/CD18 [leukocyte function associated antingen–1 (LFA-1)] but did not involve like-like interactions between LFA-1 molecules on adjacent cells. This led to the idea that a second, yet undiscovered, molecule might be responsible for adhesive interactions between lymphocytes. Rothlein generated a monoclonal antibody using LFA-1–deficient Epstein-Barr virus (EBV)–transformed lymphoblastoid cells and determined that this antibody inhibited aggregation of LFA-1–positive EBV cells. The antibody that was produced characterized a new cell adhesion molecule that was termed intercellular adhesion molecule–1 (ICAM-1). We now know that ICAM-1 is a highly important cell adhesion molecule expressed on the surface of a number of cells, including endothelial cells, and that it participates in the cellular pathology that results from both acute and prolonged inflammation.

Rothlein et al not only described the development of an antibody (RR 1/1) that recognized ICAM-1, but also described the immunoprecipitation of ICAM-1, which indicated that ICAM-1 was a single chain with a molecular weight of 90 000. Rothlein and colleagues also report on the expression of LFA-1 and ICAM-1 on healthy and LFA-1–deficient human cells. Based on all of the data presented in the paper, the authors conclude that ICAM-1 is a ligand in many, but not all, LFA-1–dependent adhesive interactions. Data presented in this elegant publication provide clear evidence for the existence of a new cell adhesion molecule and provide the foundation for additional studies investigating the expression and function of ICAM-1 throughout the cardiovascular system.

In subsequent studies, it was discovered that ICAM-1 was constitutively expressed on the surface of inactivated endothelial cells and also served as the ligand for CD11b/CD18 (MAC-1). It was also determined that endothelial cell ICAM-1 expression could be markedly upregulated by a variety of stimuli, including cytokines, and that elevated ICAM-1 expression contributes to neutrophil infiltration in inflamed tissues. The principal action of ICAM-1 is to mediate the firm adhesion of leukocytes to the endothelium and to allow them to transmigrate out of the vasculature and produce tissue injury. It was also later reported that cardiac myocytes are capable of expressing ICAM-1 following cytokine stimulation, and it has been proposed that myocyte ICAM-1 is critical for cardiac cell injury. As a result of the discovery of ICAM-1 by Rothlein and colleagues, there has been an explosion of research into the contribution of endothelial cell ICAM-1 in inflammatory disease states such as myocardial ischemia/reperfusion injury and other cardiac disorders. A number of ICAM-1 monoclonal antibodies have been generated in the years following the original description of this important adhesion molecule and have been used as tools to probe the actions of ICAM-1. Furthermore, a number of ICAM-1 antibodies have been employed as pharmaceutical agents in the clinical setting to determine the feasibility of anti–ICAM-1 therapy in a number of conditions including renal transplantation. Clearly, ICAM-1 is a central molecule in the inflammatory response of the vasculature in a wide range of disease states.

1986

The US space shuttle Challenger explodes on takeoff; Spain and Portugal join the European Union; and Nigerian Wole Soyinka wins the Nobel Prize for Literature

Leukocyte rolling and extravasation are severely compromised in P-selectin–deficient mice

T.N. Mayadas, R.C. Johnson, H. Rayburn, R.O. Hynes, D.D. Wagner

Cell. 1993;74:541-554

We are treated here to a marvelous blend of cutting-edge molecular biology, cell physiology, and classic physiology in one. P-selectin is an adhesion glycoprotein expressed by activated platelets and endothelial cells, which serves to tether circulating leukocytes to the endothelium as well as modulating platelet-leukocyte interactions. Prior to this breakthrough paper, there were a number of reports that described the actions of P-selectin and suggested that it might play a pivotal role in various cardiovascular diseases.
Much of this information was gained through the use of monoclonal antibodies that inhibited P-selectin–mediated cell adhesion. Mayadas and colleagues reported on the development of a P-selectin null mouse that was generated by gene-targeting in embryonic stem cells. The authors then utilized this novel gene-targeted mouse to investigate leukocyte behavior in vivo.

The authors carefully describe the procedures employed to develop the P-selectin–deficient mice and rigorously test these animals to fully ensure that they are indeed P-selectin knockouts.The authors performed Northern blot analysis for P-selectin and E-selectin in the wild type– and P-selectin–deficient mice and confirmed that the gene-targeted animals do not have P-selectin RNA but do possess E-selectin RNA. Immunostaining of platelets indicated that only wild type–mice contained P-selectin within the alpha granules of the platelets but not in the mutants. In addition, staining of lipopolysaccharide-treated lung tissue with antibodies directed against P-selectin also revealed a lack of P-selectin expression in lung endothelium of the mutant mice. Interestingly, the P-selectin–deficient mice displayed a significant elevation of circulating peripheral neutrophil counts when compared to wild type–mice. Most importantly, this paper very elegantly describes the rolling of leukocytes in the mesentery of wild type and P-selectin mutant mice under basal conditions and following activation of P-selectin in mesenteric venules. Mutant mice failed to demonstrate any significant degree of leukocyte rolling at baseline or following stimulation with the calcium ionophore A23187.

The authors also examined the degree of peritoneal neutrophil influx following thioglycollate administration. Mutant mice exhibited a significant reduction in the number of neutrophils in the lavage despite the significant increase in circulating neutrophils counts. Thus, disruption of the P-selectin gene results in a profound deficiency of leukocyte rolling within the microcirculation, which is accompanied by an attenuated recruitment of neutrophils at sites of inflammation.

This paper set the stage for the use of gene-targeted animals as a means to explore cardiovascular physiology and pathophysiology. Techniques described in this paper have been used in recent years by a number of laboratories to generate a host of animals in which a gene for a particular protein of interest has been disrupted, or in which gene manipulation results in overexpression of a protein. In this manner, cardiovascular scientists are able to examine the precise actions of a protein under normal conditions or in a pathological state such as ischemic heart disease. Gene-targeted animals are ideal to further our understanding of cardiovascular disease, since animals in which a gene is disrupted provide a highly pure experimental model free of the complexities that one may encounter when utilizing pharmacological agents or monoclonal antibodies to neutralize a protein of interest. The use of gene-targeted animals has recently dramatically increased, and we should begin to reap the benefit of these excellent research tools for years to come.

1993

Harley-Davidson celebrates its 90th birthday;
A bomb explodes at the World trade Center in New York;
and Algerian Nourredine Morceli sets a new world record for running the mile

Microcirculation

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