The history of the capillary wall: doctors, discoveries, and debates

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Hwa C, Aird WC. The history of the capillary wall: doctors, discoveries, and debates. Am J Physiol Heart Circ Physiol 293: H2667–H2679, 2007. First published August 10, 2007; doi:10.1152/ajpheart.00704.2007.—In 1628, William Harvey provided definitive evidence that blood circulates. The notion that blood travels around the body in a circle raised the important question of how nutrients pass between blood and underlying tissue. Perhaps, Harvey posited, arterial blood pours into the flesh as into a sponge, only then to find its way into the veins. Far from solving this problem, Marcello Malpighi’s discovery of the capillaries in 1661 only added to the dilemma: surely, some argued, these entities are little more than channels drilled into tissues around them. As we discuss in this review, it would take over 200 years to arrive at a consensus on the basic structure and function of the capillary wall. A consideration of the history of this period provides interesting insights into not only the central importance of the capillary as a focus of investigation, but also the enormous challenges associated with studying these elusive structures.

ANCIENT GREEK PHYSICIANS, having limited knowledge of the vasculature, believed that arteries and veins were separated by a fluid called “parenchyma” that effused from the blood (Fig. 1A). After William Harvey (1578–1657) published his discovery of the circulation of the blood in 1628, scientists further investigated the mechanisms by which blood traversed from arteries to veins. Harvey himself did not know of the existence of the capillaries. While some texts state that he “surmised” or “postulated” the existence of these minute vessels, Harvey preferred the notion that blood simply “percolated through the tissues somewhat as water permeated the earth” (quoted in Ref. 30) (Fig. 1B). Indeed, many of his contemporaries refused to believe that the arteries and veins were directly connected; like Harvey, they thought that blood leaked out of one vessel and filtered through the organ tissue to the other vessel. Still others argued that a direct vascular connection from arteries to veins had to exist but acknowledged that this connection remained unproven.

At first glance, Marcello Malpighi’s (1628–1694) discovery of the capillaries in 1661 should have settled the question. Indeed, most modern accounts of the history of the capillary begin with a passing reference to Malpighi’s observations and then jump in time to the work of the great physiologists of the late nineteenth century and early twentieth century, including Ernest Starling, August Krogh, and Eugene Landis. In fact, Malpighi’s discovery was followed by 200 years of uncertainty as to the true nature of capillary structure (Fig. 2 shows time line). In the present review, we explore the basis for this uncertainty.

Why revisit the events that led to the discovery of what today seems so obvious, that capillaries possess a wall? There are, of course, many advances in the history of the microcirculation that might seem to bear greater immediate relevance (and perhaps interest) to the modern-day biomedical practitioner, including the seminal contributions of the aforementioned late nineteenth-/early twentieth-century physiologists, the use of electron microscopy in the 1950s and 1960s to gain insights into the ultrastructure of the endothelium, the first successful culture of primary endothelial cells in the early 1970s, and the discovery of nitric oxide. There are two reasons for turning our attention to the capillary wall. First, in contrast to the more familiar milestones of the late nineteenth century and early to mid-twentieth century, little has been written about the history of the capillaries in the preceding era. More importantly, a consideration of the process by which investigators arrived at the consensus that capillaries have walls yields insights into how science progresses.

Proving the Existence of Capillaries

In 1640, William Harvey had boiled organs, including the liver, spleen, lungs, and kidney, and then dissected the tissue until he could see “capillamenta,” or capillary threads. The minute vessels he saw were actually small arteries or veins, as opposed to true capillaries, which are invisible to the naked eye. Harvey posited that

...in the members and in the extremities the blood passes from the arteries into the veins either directly by means of an anastomosis or indirectly through the porosities of the flesh, or by both these means. (Ref. 16, p. 88)

In their paper on “Harvey and the problem of the ‘capillaries,’” Elkana and Goodfield (14) explain the rationale for Harvey’s hypothesis that “blood might pour into the flesh as into a sponge”:

A hypothesis of this form is a very rational one, both for reasons of structure and for reasons of function. For, if one postulated a system of blood circulating through a series of completely closed vessels—which is what a capillary connection entails—then one must face the question: how do the heat and the nutritive substances carried in the blood reach the flesh where they are required? Harvey believed that blood had both these functions. (Though during Harvey’s time heat was treated as an incorporeal substance, which might perhaps be expected to go through the walls of the blood vessels easily, food substances certainly were not regarded as incorporeal.) (Ref. 14, p. 67)

Just four years after Harvey’s death, Malpighi, a professor of medicine at the University of Bologna, discovered the capillaries while studying the lungs of frogs with a compound

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double-convex lens microscope (Fig. 3A). In 1661, Malpighi detailed his observations in a letter written to his advisor, Giovanni Borelli, a mathematics professor at the University of Pisa:

From this I could clearly see that the blood is divided and flows through tortuous vessels and that it is not poured out into spaces, but is always driven through tubules and distributed by the manifold bendings of the vessels [Malpighi commented on the presence of particles in the blood, but did not appreciate the existence of red blood corpuscles, which had been described by Jan Swammerdam in 1658]. (Ref. 1, vol. 1, p. 194)

Malpighi had a contemporary in the Netherlands, Antoni van Leeuwenhoek (1632–1723), who devoted his time and skill to microscopy. Leeuwenhoek’s microscopes were simple single lenses that he ground himself—they were composed only of a piece of metal that held the magnifying glass and screws that allowed the adjustment of eyepiece and objective. He used his microscopes to study the architecture of tadpoles, fish, rooster combs, rabbit ears, and bat wings (Fig. 3B shows the tail of an eel). Leeuwenhoek did not see capillaries as structurally distinct structures, but rather as functional entities defined by the direction of blood flow, relative to the heart:

Hereby it plainly appeared to me, that the blood-vessels I now saw in this animal, and which bear the names of arteries and veins, are, in fact, one and the same, that is to say, that they are properly termed arteries as long as they convey the blood to the farthest extremities of its vessels, and veins when they bring it back towards the heart. (Ref. 19, p. 92)

Anatomic injections were used extensively in the seventeenth century to demonstrate the internal structure of the body and particularly the vasculature, including the capillaries (35). For example, Malpighi injected vessels with ink, urine colored with ink, and black-colored liquid mixed with wine. He demonstrated that such medium injected into the renal artery (but not the renal vein) reaches the smallest branches of the artery and the internal glands (Malpighi bodies, or glomeruli) so as to produce the appearance of “a beautiful tree loaded with apples” (Fig. 4A). Employing wax injections, the Dutch investigator Frederik Ruysch (1638–1731) demonstrated the presence of blood vessels in virtually all tissues and organs of the body, including the vasa vasorum and the bronchial capillaries. In 1696, Ruysch suggested that “tissues were only vascular networks variously arranged” (quoted in Ref. 35, p. 305). According to this view of “vascular autocracy,” in which “the whole body is almost nothing more than a prodigious assemblage of lymphatic and blood vessels” (quoted in Ref. 35, p. 321), differences in function between tissues were attributed to variations in the arrangement of the vascular networks. Despite the growing body of evidence that capillaries existed, remarkably little attention was devoted to these minute

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1 According to L. J. Rather, Malpighi: “...did not see the circulation as a whole in action, as one might stand back and see, for example, the pump-driven circulation of a coloured fluid in a system of pipes all accessible to simultaneous observation. What he saw as was follows: 1) movement of blood in one direction in progressively more finely branching arteries (in the mesentery of living animals), 2) movement in the opposite direction of presumably the same stream in the veins; 3) a gap between, in which he was unable to make out what was taking place, and finally 4) in the dried lungs of frogs the newly discovered minute blood-vessels. He then inferred that the same vessels were present throughout the bodies of animals” (Ref. 30, p. 56).

2 In a letter from Delft, Leeuwenhoek extrapolated his observations of capillaries in tadpoles to the circulation of humans: “If we now plainly perceive, that the passage of the blood from the arteries into the veins of the tadpole, is not performed in any other than those vessels, which are so minute as only to admit the passage of a single globule at a time, we may conclude that the same is performed in like manner in our own bodies, and in those of other animals” (Leeuwenhoek A von. The Select Works of Antony van Leeuwenhoek: Containing His Microscopical Discoveries in Many of the Works of Nature. New York: Arno, 1977).
vessels in the 1700s. Eighteenth-century investigators viewed the microscope as unreliable and untrustworthy. The early lenses were flawed, creating artifacts and optical illusions. Moreover, a perceptual consensus was lacking: each observer saw what he wanted through the microscope. It was not until compound achromatic lenses were introduced in the 1830s that microscopy became widely adopted as a research tool (Ref. 10, p. 58).

**Defining the Capillary**

By the nineteenth century, it was generally agreed that the arteries and veins were connected by capillaries. However, there was no immediate consensus as to what defined a capillary. In Robley Dunglison’s 1856 publication *Human Physiology* (11) the capillaries were synonymous with the term “intermediate vessels,” described as vessels of “extreme minuteness... by some considered to be formed by the terminations of arteries and the commencement of veins; by others to be a distinct set of vessels” (Ref. 11, p. 343).

Marshall Hall (1790–1857) from London was among those who believed that capillaries were independent entities (Fig. 5A). He was one of the first to distinguish between the minutest arteries/veins and the true capillaries on anatomic grounds. In 1831, he wrote:

> The last branches of the arterial system and the first roots of the venous, may be denominated minute; but the term capillary must be reserved and appropriated to designate vessels of a distinct character and order, and of an intermediate station, carrying red globules, and perfectly visible by means of the microscope. (Ref. 15, p. 18)

Hall was also among the first to emphasize the functional significance of capillaries:

> At this point there is an obvious and remarkable change in the appearance of the circulation: the course of the blood becomes of only half its former velocity, and the globules, consequently, instead of moving too rapidly to be seen, become distinctly visible. If the vessel be traced, it is next observed, not to subdivide, but to unite with other branches, and to pass into that distinct system and net-work of vessels to which I would restrict and appropriate the term capillary. The object of this peculiar distinction and character of the capillary vessels is very obvious: a more diffused and slower circulation is required for administering to the nutrient vessels or functions, than that of the arteries... the capillaries... anastomose continually, so as to form a complete net-work of vessels of uniform character and dimensions. [Emphasis added] (Ref. 15, p. 29 and 31)

In 1835, Allen Thomson [writing in Todd’s *Cyclopaedia of Anatomy and Physiology* (38)] countered that it was best to include all the minute vessels as capillaries, because

> the communicating vessels are not every where of the same kind and that from the use already made of the term by physiological writers its meaning will thus be more easily
understood. The vessels which lead from arteries to veins are of very various sizes, some admitting only one globule at once, others being so large as to allow the passage of three, four, or even a greater number of red globules together. (Ref. 38, p. 669)

The following description by Thomson of the capillaries was reminiscent of Leeuwenhoek’s functional definition:

The small arteries pass into veins quite in a gradual manner, the ramifications of each class of vessel becoming more and more minute until they meet, the two kinds of vessel presenting no difference of character other than the change of direction assumed by the moving blood, which enables us to say with certainty where the artery terminates, and at what point the vein begins, and affording thus no reason to consider the continuous tube by which they join as different in structure from either the minute artery or vein. (Ref. 38, p. 669)

In his book *Elements of Physiology* (24), Johannes Müller (1801–1858), a renowned professor of anatomy and physiology at the University of Berlin, defined capillaries as “a network of microscopic vessels, in the meshes of which the proper substance of tissue lies... the reticulated vessels connecting the arteries and veins” (Ref. 24, p. 217–218). Although Müller could not precisely define “the point at which arteries terminate and the minute veins commence,” he noted that within a given vascular bed, the capillaries had a uniform diameter (“the small vessels which compose [the intermediate network] maintain the same diameter throughout”; Ref. 24, p. 218). According to Müller, capillaries differed only in “size of the meshes” (i.e., density) and their configuration (see Fig. 4). He employed colorful metaphors to describe organ-specific configurations of the capillary networks:

...the mode of ramification in the small intestines resembles a tree which is not in leaf, in the placenta a tuft, in the spleen an asperge or sprinkling brush, in the muscles a branch of twigs, and in the choroid plexus of the brain a lock of hair, in the Schneiderian membrane a trellis-work. These meshes are smallest in the lung, liver and kidneys; largest and most sparse in ligaments and tendons. (Ref. 24, p. 219)

The lack of uniformity in the definition of a capillary persisted past the mid-nineteenth century and was brought to attention by H. N. Eastman of Geneva Medical College in a paper published in New York in 1867 (13). He criticized physiologists for “confound[ing] the terms ‘capillaries,’ ‘capillary arteries,’ ‘capillary veins,’ and ‘minute vessels’”. Eastman suggested using terms such as “terminal or distal arteries, or arterioles, and radical veins, venules or veinlets” in place of “capillaries”: “Not only the student is confused, and fails to acquire any precise idea of the author’s meaning, but the more advanced scholar is led to doubt whether the writer really recognizes any distinctive difference in the terms he employs”.

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3 Previous investigators had argued that in addition to capillaries, arterioles also gave rise to open-ended vessels “of a particular order, whose office it is to pour out, by their free extremity, the materials of nutrition” (Ref. 11, p. 349). These were commonly termed “exhalants,” “exhalant vessels,” or “nutrient vessels.” Thomson argued that “arteries terminate always by direct continuity of tube in the veins, and that no other visible passages are connected with the minute vessels... we must suppose that the various interchanges of materials occurring between the blood and the organized textures... as in nutrition, secretion, respiration, transpiration etc. must take place by some process of organic transduction through invisible apertures of the minute vessels” (Ref. 38, p. 671).

4 Like Thomson, Müller denied the existence of minute arteries with open mouths: “Microscopic observations and minute injections have shown that the capillary vessels are merely fine tubes which form the medium of transition from arteries to veins, and that no other kind of vessel arises from them; that the minute arteries have no other mode of termination than the communication with the veins by means of the capillaries; in a word, that there are no vessels terminating by open extremities” (Ref. 24, p. 221).
He theorized along the lines of Hall and Müller: the capillaries are distinct in structure and function from both arteries and veins.

From Tissue Theory to Cell Theory

Prior to the 1800s human physiology and pathology was solidly rooted in Hippocratic principles. Disease arose from an imbalance of the four humors, and therapy (e.g., bloodletting, laxatives, and emetics) was directed toward reestablishing equilibrium. In the early 1800s, physicians began to see patients not as sick individuals but as diseases. Advances in anatomic pathology (particularly in France) served to localize disease to discrete regions of the body. Marie François Xavier Bichat (1771–1802), who is considered the “father of descriptive anatomy,” emphasized the importance of tissues. In his book, *Treatise on Membranes*, Bichat wrote that organs “are themselves composed of several tissues of very different nature, which truly form the elements of these organs” (quoted in Ref. 34, p. 59).

The focus on gross pathology in early to mid-eighteenth-century France contrasted with the growing use of light microscopy in Germany and Britain. The microscope provided investigators with the means to resolve organs and tissues into finer units of structure. Until that time, tissues were believed to consist of fibers. Early microscopy did little to change that view. As Everard Home reported in 1820, the tissue fibers appeared to be composed of a string of beads, or “minute globules connected together by a gelatinous substance” (Ref. 18, p. 25). During the second quarter of the nineteenth century, improvements in the light microscope led to the gradual recognition of the cell as the primary structural unit of tissue, culminating in Theodor Schwann’s (1810–1882) founding of the cell theory in 1839. He wrote that all organic tissues have . . .one common principle of development as their basis, viz. the formation of cells; that is to say, nature never unites molecules immediately into a fibre, a tube, and so forth, but she always in the first instance forms a round cell, or changes, when it is requisite, cells into the various primary tissues. (quoted in Ref. 40, p. 222)

According to early iterations of the cell theory, an amorphous substance (termed the blastema or cytoblastema) gave rise to cells in the tissues. In 1855, Rudolph Virchow (1821–1902), a student of Müller’s, published a paper entitled *Cellu-
lar Pathology in which he refuted the blastema theory (to which he had originally subscribed) and instead famously advocated cytogenesis (“all cells are derived from other cells”). Although this essay represented a synthesis of old ideas espoused by some of Virchow’s colleagues, he proposed the novel and important hypothesis that cells were autonomous or “sovereign.” Moreover, the body consisted of “anatomical units” containing cells, blood vessels, and nerves. Finally, Virchow introduced the paradigm of cellular pathology:

All diseases are in the last analysis reducible to disturbances, either active or passive, of large or small groups of living units whose functional capacity is altered in accordance with the state of their molecular composition and is thus dependent on physical and chemical changes of their contents. . . . All pathological formations are either degenerations, transformations, or repetitions of typical physiological structures. (Quoted in Ref. 30, p. 132)

As we discuss below, these changing perceptions of tissue organization in health and disease were integral to the recognition of the capillary wall.

The Capillary Wall

Progress in delineating the true nature of the capillary wall in the 1800s proceeded along two distinct, though overlapping lines of investigation: anatomy/histology and physiology/pathophysiology.

The anatomists and histologists. It was typical for authors of medical texts to first acknowledge Malpighi and Leeuwenhoek for discovering the capillaries and to then voice their own opinions on whether or not capillaries actually had walls.

A sampling of major publications in the first half of the nineteenth century suggests that many investigators believed that capillaries were wall-less canals or channels in the tissues, paths carved out by dying cells. In describing these structures, authors often appealed to metaphors. For example, in 1835, J. W. Earle (12) described capillaries as membraneless channels, “like brooks in the moist earth,” and referred to the observations of a Dr. Wedemeyer from Hanover:

At length [arteries] gradually terminate altogether in membraneless canals formed in the substance of the tissues. The blood in the finest capillaries no longer flows within actual vessels whose parietes are formed by a membranous substance, distinguished from the adjoining cellular tissue by its texture and compactness, but in simple furrows, or canals, whose walls are formed by the surrounding cellular tissue. (Ref. 12, p. 8)

In a publication covering his work from 1818 to 1820, Ignaz Döllinger (1770–1841) stated that the blood was walled in by mucus, the fundamental substance of tissues, “just as a stream receives a bed of earth and does not have to be enclosed in a tube” (quoted in Ref. 30, p. 60). In 1831, Hall wrote that capillaries were “mere canals” as opposed to “real tubes” (Ref. 15, p. 47). He did not elaborate on his “many reasons” for his thinking, but did point out that more research was needed in this area. In 1833, Müller claimed in the first edition of Elements of Physiology that “one must think of the capillary vascular walls as mere thickened margins of the substance, not however as very self-sufficient membranes” (quoted in Ref. 30, p. 63).

The claim that capillaries lacked walls was not idle theory, but rather was based on inductive reasoning. Earle pointed to
the inability to detect a membrane with light microscopy; the facility with which globules (cells) passed between blood and tissues; the “rapidity with which the blood is seen to work out for itself a new passage, or canal, in the tissues”; the impossibility of detecting any “pores” or “openings” in the sides of vessels; and the “impossibility of the processes of nutrition and absorption being carried on through the coats of vessels” (Ref. 12, p. 8). Thompson cited the rapidity with which new capillaries formed (seen by some to be incompatible with capillaries having fully formed walls) and the ease with which the blood appeared to pass out of the larger vessels and take an “irregular and indeterminate course through the non-vascular parenchyma of the organ” (quoted in Ref. 38, p. 670).

Although visual proof was lacking, some investigators correctly inferred the presence of the capillary wall. For example, in their 1829 text A Manual of General Anatomy, A. L. Bayle, H. Hollard, and H. Storer stated that:

The capillaries of capillary vessels can scarcely be distinguished from the substance of other organs, and we know not, therefore, anything certain respecting their texture: we can only suppose that they are formed by the continuation of the internal membrane of the arteries and veins. (Ref. 2, p. 42)

In 1835, Allen Thomson noted that injected capillary vessels in the ears of birds and reptiles could be separated from the neighboring tissue and argued that the “active properties of the capillary vessels [might] belong to parietes as in the larger vessels” (quoted in Ref. 38, p. 670).

In 1839, Theodor Schwann was the first to describe what would later be named the endothelium:

The capillary vessels, in the tail both of the fully developed and young tadpoles, are seen to be surrounded by a thin, but distinctly perceptible membrane, which does not exhibit any fibrous arrangement. The variety in the thickness of this membrane in different instances sufficiently explains why we cannot distinguish it in all capillary vessels, just as we cannot detect the cell-membrane even in the blood-corpuscles, although there can be no doubt of its existence. (Ref. 33, p. 154)

Schwann further details this thin membrane by stating that “very distinct cell-nuclei occur at different spots upon the walls of the capillaries. . . they are either the nuclei of the primary cells of the capillaries, or nuclei of epithelial cells, which invest the capillary vessels. . . these nuclei frequently seemed to lie free upon the internal wall of the vessel. . . that these are the nuclei of the primary cells of the capillaries is, therefore, most probable” (Ref. 33, p. 155) (Figs. 1D and 6).

Müller, who in the first edition of Elements of Physiology had expressed doubts about the existence of the capillary wall, was evidently convinced by Schwann’s findings. In a later edition of his book (translated from German in 1843), he commented:

[Theodor] Schwann has recently ascertained [1839], by means of the microscope, that the capillaries have not merely membranous parietes, but a coat in which circular fibres are arranged as in the arteries can be distinguished. . . even where no transverse fibres are visible, the capillaries seem to have delicate membranous parietes, in the substance of which oval bodies resembling nuclei of epithelium scales appear at intervals. (Ref. 24, p. 225)

Müller now cites additional evidence for the existence of a true wall: 1) fluids injected into arteries passed into veins without extravasation; 2) blood currents were able to cross above and below each other without uniting; and 3) the solid matter in between the currents was not involved in the bloodstream, despite the high number of currents and the smallness of the sections of solid matter between them (Ref. 24, p. 224).

In 1842, the British surgeon and pathologist James Paget (1814–1899) likewise stated that capillaries were not “mere channels drilled into tissues around them” but rather had distinct walls (Ref. 27, p. 287). The capillaries were “composed of a completely structureless membrane, in which no fibres or striae are ever discernible, but which bears minute oval corpuscles, the persistent nuclei of the cells from which the capillaries are formed. . . this may be named the primary vascular membrane” (Ref. 27, p. 287). A year later, William Carpenter at the Bristol Medical School added in his book Principles of Human Physiology that “there can be no doubt that [capillaries] are produced. . . by the formation of communications among certain cells, whose cavities become connected with each other, so as to constitute a plexus of tubes, of which the original cell-walls become the parietes” (Ref. 5, p. 349). Finally, in the fourth (1844) edition of his book, Müller definitively stated that “capillary vessels are not mere channels in the substance, they also possess membranous walls” composed of cells (quoted in Ref. 30, p. 63).

It would seem as though the debate over the presence of capillary walls was settled. However, the controversy continued. In 1849, Bennett Dowler from New Orleans wrote with incredulity:

Professor Carpenter’s anatomical history of the capillaries does not seem embarrassed with any doubts whatever, though it bears on its face very little that can be called absolute certainty. He says that “the capillary circulation is carried on through tubes which have distinct membranous parietes—originating in cells”—in another place, he says that “the capillaries arise from a minutely anastomosing network, into which the blood is brought by the ramifications of the arteries on one side and from which it is returned by the radicles of the veins on the other.” Cells! network! ramifications! radicles! one side! and the other side! (Ref. 8, p. 455)

In 1856, the question still remained open in the eighth edition of Robley Dunglison’s Human Physiology. Some investigators, Dunglison explained, believed that the bloodstream “is seen to work out for itself, easily and rapidly, a new passage in the tissues, and it is esteemed certain, that in the liver venosa of the egg, the blood is not surrounded by vascular parietes” (Ref. 11, p. 347). On the other hand, for histologists, capillaries did seem to be provided with walls, “and it has even been announced, that they are composed of a fibrous structure, analogous to the muscular” (Ref. 11, p. 348). H. N. Eastman of Geneva Medical College (who, as mentioned above, had suggested the need to clarify the definition of the word “capillary”) opined in 1867 that capillaries were channels, “mere repositories of the blood” with walls formed from the “tissues themselves” and a “tenuous structureless membrane” (Ref. 13, p. 77).

In the later 1800s, it was not unusual for authors to take an intermediate position regarding the presence of walls. For example, in the 1875 publication A Manual of Physiology: Being a Course of Lectures, Professor Küss at the Medical School of Strasbourg stated that
The capillaries are generally formed of coats of very simple structure: their tissue is apparently amorphous, but traces of cellular structure are found in them, in the shape of laminated flattened plates, the remains of ancient cells, which have lost the principal physiological properties of the globular element when losing its form. The capillaries have not, however, perhaps, always distinct walls: this is probably the case with the capillaries of the liver, which are, apparently, only lacunae hollowed out in the substance of this organ (interstices between groups of hepatic cells). (Ref. 22, p. 159)

The physiologists and pathologists. Capillary structure was by no means restricted to discussions among anatomists. In fact, notions of capillary structure were directly related to how physiologists viewed the process of tissue growth and nutrition, and how pathologists thought blood cells traversed the capillary wall during inflammation.

It has long been recognized that substances utilized and/or secreted by the cells of the body must pass between blood and tissue. By the 1800s, this process of “nutrition” was appropriately ascribed to the capillaries. In 1835 Allen Thomson stated that “all those alterations of composition which accompany nutrition, growth, secretion, and other organic processes connected with the systemic vessels, occur in the smallest ramifications of the pulmonic and systemic circulation” (quoted in Ref. 38, p. 669).

In the first half of the nineteenth century, two schools of thought regarding nutrition and inflammation bore particular relevance to capillary structure. One school of thought was that blastema, which included albumin and fibrin, left the bloodstream to feed and form tissue cells (in normal states) or pus cells (in states of inflammation). The second school of thought, the corpuscular theory, held that circulating cells (some believed them to be red blood cells, others white blood cells) constantly trafficked between blood and extravascular space, giving rise to tissue cells and pus cells in normal and pathological conditions, respectively.

As a proponent of the blastema theory, John Hughes Bennett (1812–1875), an English physiologist, physician, and pathologist, argued in 1841 that the vascular changes that had for so long occupied center stage in inflammation were a prelude to inflammation.
the exudation of plasma. Bennett described the capillary wall as "composed of a simple, transparent, yet very firm membrane, without the smallest openings, studded at irregular intervals with nuclei of various shapes... fine filters... retaining the solid corpuscles and allowing only the fluid to transude" (quoted in Ref. 30, p. 85–86). Bennett believed that the exudate represented a blastema, transforming into tissue or pus cells.

Among the foremost advocates of the corpuscular theory was William Addison (1802–1881), a medical practitioner from England. Addison denied the existence of a membranous coat or wall in the capillaries: "Capillary vessels are channels running in a determinate manner around and among little fixed islets of living tissue... take away the islets and no vessels remain" (quoted in Ref. 30, p. 85–86). Bennett believed that the exudate represented a blastema, transforming into tissue or pus cells.

Augustus Waller (1814–1870), a contemporary of Addison’s from London, developed a technique for studying the microcirculation in the tongue of the living frog, in which inflammation was induced by long exposure to air (Fig. 5, B and C). In 1846, Waller stated: "Let us now examine the admirable manner in which nature has solved the apparent paradox, of eliminating, from a fluid circulating in closed

8 Over time Addison would modify his views. Faced with growing evidence that capillaries had walls, Addison backed off from his contention that leukocytes continually pass from the bloodstream to tissue. Instead, he suggested that in states of inflammation the capillary walls undergo cellular transformation (termed retrograde metamorphosis) in which white blood cells replace the fibrous walls of capillaries, thus opening a passageway to the underlying tissues. Even then, Addison’s view that pus cells are derived from circulating white blood cells was met with skepticism. An anonymous review of his work in 1844 stated that “we cannot agree with him [Addison] in considering the pus-corpuscles as metamorphosed white corpuscles (because we cannot think it probable that bodies so large as these can pass out of the capillaries, without an absolute rupture of their wall).” (Anonymous. Principles of medicine.... Br Foreign Med Rev 18: 91–115, 1844, p. 112). In another review: “Not only have the capillaries a permanent tubular coat, but it performs most important functions. It is impossible that corpuscles of the blood can pass through it without rupture” [Anonymous. Reviews. Lancet 43: 586–588, 1844, p. 588].
tubes, certain particles floating in it, without causing any rupture or perforation in the tubes..." (Ref. 41, p. 398). Waller made the novel observation (where others before him had only theorized) that at sites of inflammation some leukocytes were at times "protruding half out of the vessel" and concluded that corpuscles passed through the capillary wall. In a follow-up paper, Waller supported Addison’s claim that circulating white blood corpuscles were identical to the corpuscles of pus and hypothesized that the corpuscles might release a substance that dissolved the substance of the capillary walls.

Julius Cohnheim (1839–1884), one of Virchow’s students, greatly expanded on the findings of his predecessors. Using a combination of colloidal aniline blue injections and microscopy, Cohnheim proved what Addison hypothesized many 23 years earlier, namely, that white blood cells cross blood vessels to become pus cells (7). Cohnheim summarized the process as “an emerging of colourless blood corpuscles from the interior of a vein to the outside, completely through the intact wall.” He ultimately hypothesized that leukocyte passage was passive and secondary to alterations in the blood vessel wall induced by chemical or mechanical “agencies.” At that time, he seemed to view inflammation not as an adaptive response, but rather as a pathological response to small vessel damage. Only later would Ilya Metchnikov introduce the biological theory of inflammation, emphasizing the importance of diapedesis as a defense against microorganisms.9 In the 1900s, Eliot Clark and Eleanor Clark (a husband and wife team from the University of Pennsylvania) were the first to prove a critical role of the endothelium in mediating this process (“[we have] demonstrated that a change in the endothelium itself is an essential preliminary to the sticking of leukocytes”; Ref. 6, p. 428).10

In addition to diapedesis, increasing attention was being paid to the mechanisms of transudation and exudation across the capillary wall. In 1858, Joseph Lister (1827–1912), a surgeon from England with an interest in inflammation, described the capillaries as consisting of “a delicate homogeneous membrane beset with occasional nuclei. . . . The thinness of the walls of the capillaries, as compared with the small arteries, is doubtless, calculated to favour the mutual interchanges which must take place between the blood in them and the tissues in their vicinity” (23).11

In 1884, G. Hare Philipson, from the University of Durham, wrote:

By means of the blood the constituent elements of the body are supplied with the nutrient substances and the oxygen which they require. By the blood and the lymph are conveyed away the waste and surplus matters which have ceased to be useful to the tissues. (Ref. 29, p. 310)

In describing the pathogenesis of edema, Philipson stated that

The quantity and nature of the liquid which escapes from the capillaries and veins depend not only on the intravascular pressure and the resistance of the flow, but also to a great extent on the character and condition of the vessel wall, and especially in their endothelial lining. . . . Cohnheim has shown that the vessel wall is not to be regarded as a dead membrane, but as a living organ. (Ref. 29, p. 310)

In the late 1800s and early 1900s, the problem of how substances cross the capillary wall was a central question in human physiology and pathology. August Krogh, a professor of zoophysiology at the University of Copenhagen who focused his investigation on the regulation of capillary density and oxygen delivery (for which he was awarded a Nobel Prize in physiology or medicine in 1920), was the first to present a comprehensive theory of capillary function in his 1922 book, *The Anatomy and Physiology of Capillaries* (20). In discussing the exchange of substances through the capillary wall, Krogh stated:

...the chief function of the capillaries [is] the exchange of substances between the blood and the tissues, or tissue fluids, taking place through the capillary wall. Apparently, at least, this function of exchange is a very complex one: gases, water, inorganic salts, organic crystalloids of the most varied description, and, in certain tissues, even colloids are constantly passing through the capillary endothelium, and not infrequently the direction of the passage changes. . . . In the capillary blood vessels we have, just as in the osmometer, a membrane which is permeable to crystalloids and impermeable to colloids. An absorption of isotonic salt solution, can, therefore, take place, and, indeed, must take place, when the hydrostatic pressure in the vessels—the capillary blood pressure—is lower than the osmotic pressure of the proteins. (Ref. 20, pp. 266 and 282)

Together with Frank Starling’s observations in 1894 (36), Krogh’s work would open the door to all of microvascular physiology concerning capillary function, including that of Eugene Landis, John Pappenheimer, Robert Chambers, Benjamin Zweifach, Eugene Renkin, Silvio Baez, Ephraim Shorr, and others.

The essence of this work was captured by Pappenheimer in his description of vectorial transport as an “ultramicroscopic circulation”:

...visible flow of blood through the capillaries is, in fact, very small in comparison with the invisible flow of water and dissolved materials back and forth through the capillary walls. . . . This invisible component of the circulation takes place at a rate which is many times greater than that of the entire cardiac output. Indeed, it is by means of this “ultramicroscopic circulation” through the capillary wall that the circulatory system as a whole fulfills its ultimate function in the transport of materials to and from the cells of the body. (28)
Discovery of Endothelial Cells and Rouget Cells

A discussion of the capillary wall would not be complete without reference to the discovery of its cellular components. The endothelial cell and the Rouget cell (pericyte) would become a focal point of capillary research in the twentieth century. While a comprehensive history of endothelial and pericyte biology falls outside the scope of the present review, a brief discussion of their discovery bears relevance to early concepts of the capillary wall.

Following the widespread use of light microscopy, but before the development of histological stains, the capillary wall had the appearance of a bland membrane or syncytium. In the mid-nineteenth century, the introduction of silver nitrate staining by several German investigators definitively established the presence of a cellular lining and hence the existence of a capillary wall (Fig. 6, E and F). After the vessels were injected with the silver solution and the tissue was exposed to light, a dark brown precipitate lining the cell boundaries could be observed (the rest of the cells were left relatively unstained). The usefulness of silver nitrate as a histological stain was first proposed by Friedrich von Recklinghausen (1833–1910) of Berlin in 1860 (39). Two years later, von Recklinghausen used the reagent to stain the lymphatics and was the first to note that they were lined with cells (see Ref. 32). In 1863, Oedmannson of Stockholm applied silver nitrate to epithelial surfaces and theorized that stomata (or openings in the vessel wall) had the appearance of a bland membrane or syncytium. In the mid-nineteenth century, the introduction of silver nitrate staining by several German investigators definitively established the presence of a cellular lining and hence the existence of a capillary wall (Fig. 6, E and F). After the vessels were injected with the silver solution and the tissue was exposed to light, a dark brown precipitate lining the cell boundaries could be observed (the rest of the cells were left relatively unstained). The usefulness of silver nitrate as a histological stain was first proposed by Friedrich von Recklinghausen (1833–1910) of Berlin in 1860 (39). Two years later, von Recklinghausen used the reagent to stain the lymphatics and was the first to note that they were lined with cells (see Ref. 32). In 1863, Oedmannson of Stockholm applied silver nitrate to epithelial surfaces and theorized that stomata (or openings in the vessel wall) had to exist (26). Finally, in 1865 and 1866, several German investigators settled the debate over whether capillary walls exist when they stained the capillaries with a solution of silver nitrate mixed with gelatin to keep the capillaries distended (42).

The resolving power of silver nitrate was captured by Stowell in 1882:

Without the aid of reagents the structure of the capillaries appears very simple. A few nuclei with nucleoli are seen scattered along the walls of an elastic hyaline membrane. Nitrate of silver dispels this illusion and resolves this homogeneous membrane into a single layer of nucleated cell-plates, united together by a “cement substance” which is stained a deep black by the solution. (Ref. 37, p. 113)

In 1865, the Swiss anatomist Wilhelm His (1831–1904) introduced the term “endothelium” in a programmatic essay titled “Die Häute und Höhlen des Körpers (The membranes and cavities of the body)” (17).

In 1873, Rouget described contractile elements on the capillaries of the hyaloid membrane of the frog as oval nuclei arranged longitudinally with surrounding irregular cell bodies, the branched processes of which encircled the capillaries (31). Rouget was unable to stain these cells. He observed them in several vascular beds and concluded that they were muscle cells. Staining of Rouget cells was successfully carried out by Zimmerman in 1886 (with silver nitrate) and Meyer in 1902 (with methylene blue) (reviewed in Ref. 3). Zimmerman, who ultimately coined the term “pericytes” (43), held that contraction of these cells controlled capillary permeability (others such as Krogh would later counter that capillary dilation, as opposed to contraction, was necessary for regulating permeability).

Conclusion

In this review, we have focused on a relatively unknown and unheralded era in the history of the vascular research, which culminated in the discovery that capillaries had walls. As we alluded to in the introduction, the value of considering this period of history is not so much in the discovery itself as it is in the lessons that may be drawn from it. In this final section, we wish to move beyond pure historical narrative and formulate what we believe are the important lessons from this period.

Technology as an engine of progress. Progress in understanding the identity and nature of the capillary wall has mirrored advances in technology. Imagine the challenge of piecing together the structure and function of the circulation with the naked eye. In some cases, investigators relied on sheer creativity. Harvey, for example, studied cold-blooded animals in which the circulation was considerably slowed. In other cases, the development of new techniques afforded incremental improvements in resolution. For example, the injection of the vascular system with dyes revealed the existence of yet smaller vessels (though the capillaries remained invisible). The development of the light microscope represented a major technological breakthrough. Initial studies were limited by the poor quality of the lenses and the lack of vital stains, and yielded only vague images of the capillary beds. Later, the use of special stains, particularly silver nitrate, provided a clearer view. In the twentieth century, many new tools—including the electron microscope, the culture of primary endothelial cells, and molecular biology—contributed to our present-day understanding of the capillary.

Today, perhaps more than ever, technology continues to drive discovery in the field of microcirculation. One of the great challenges inherent in studying the capillaries—whether 200 years ago or today—is their invisibility to the human eye. An additional complexity is the extent to which their component parts, particularly the endothelial cells, undergo phenotypic drift in vitro. New proteomic approaches have revealed that capillaries and their endothelial lining comprise a mosaic of phenotypes, which have been variously described as “vas-

12 In his original paper, a mere 8 lines in length, von Recklinghausen did not describe specifically the endothelium. The following is an English translation of the paper, entitled “A method to differ between microscopical cavernous (empty) and solid structures” (39): “If you put fresh or even better dried animal pieces (tissue) into a light solution of argentum nitricum (so-called hellstone-solution), put them into a low-concentrated solution of sodium chloride and expose to the impact of light, you get a dispersed, tight, black silver precipitation in those areas (of the tissue), that contain essentially watery processes running nearly circularly around the capillary. Rouget was not able to stain these cells; only their nuclei were tinged with carmine. . . . According to Rouget, these perivascular cells were to be found regularly on capillaries in various organs of the body, and he presumed that they were real plain muscle cells and acted as such” (Ref. 3, p. 38).

13 In 1873, Rouget stated that these cells: “...are arranged in the direction of the tubes, and surrounded by a zone of protoplasm with branched elongations which embrace the capillaries in a number of places like so many loops” (quoted in Jones T. The structure and mode of innervation of capillary blood vessels. Am J Anat 58: 227–250, 1936, p. 229).

14 As recounted by Bensley and Vintrup in 1928: “In the hyaloid membrane of the frog these cells were fairly regularly arranged, their protoplasmic processes running nearly circularly around the capillary. Rouget was not able to stain these cells; only their nuclei were tinged with carmine. . . . According to Rouget, these perivascular cells were to be found regularly on capillaries in various organs of the body, and he presumed that they were real plain muscle cells and acted as such” (Ref. 3, p. 38).
cular addresses” or “zip codes” (25). An exciting prospect for the future is to identify these addresses and to leverage the information for delivering drugs to specific vascular beds. However, the success of site-specific targeting awaits further advances in technology, including increased resolution of phenotyping tools and improvements in the specificity and safety of gene/protein delivery systems.

Judging scientific progress in the context of the times. At first glance, it is surprising that the very existence of the capillary wall was ever in doubt. However, as we hope to have conveyed in this review, the controversy was perfectly understandable in the context of eighteenth- and nineteenth-century science. Other debates that followed seem no less unusual by today’s standards. For example, is mature endothelium the source of red blood cells and/or leukocytes? Do capillaries have the power to elaborate hemoglobin, form lymph, or make antibodies? Do capillaries constrict by means of endothelial cell swelling? Each of these questions was posed and debated by highly capable investigators who, while schooled in the methods of experimental inquiry, were limited (relative to later generations) in their knowledge base and technical repertoire. Referring to the path on which Western medicine has moved over the past two and a half thousand years, Lelland Rather, a Stanford pathologist and medical historian, points out that it culminates not only in our present, but also in an “infinite number of presents, each one as confident as our own that it had scaled the heights of time” (Ref. 30, p. 16). Extrapolating into the future, our present-day concepts of the microvasculature promise to undergo significant, unanticipated changes.

The nonlinearity of progress in the field. A consideration of the history of the capillary wall reminds us that the impact of new discoveries is rarely, if ever, immediate. Thomas Kuhn, a philosopher of science who was responsible for popularizing the term “paradigm,” argued that mature science develops from the successive transition from one paradigm to another through a process of revolution (21). By definition, new paradigms are at odds with existing notions of health and disease. Consider, for example, Harvey’s discovery of the circulation in 1628. At the time, the prevailing view of the vasculature was that veins delivered blood and arteries delivered air or pneuma to the various organs of the body. These were open-ended systems in which blood and air were expelled into and consumed by the tissues. Harvey’s critics (the “Galenic Brigade”) could not accept that blood circulated, because Nature would never be so wasteful as to have the heart make more blood than is consumed by the tissues.15 Once blood was seen to circulate rather than dissipate, it was necessary to determine how substances (including solutes and cells) passed between blood and underlying tissue. For those who subscribed to the corpuscular theory of nutrition, the presence of a true capillary wall was simply incompatible with their belief that circulating cells constitutively migrated from blood to tissue. While they would ultimately acknowledge the indisputable evidence that capillaries had walls, they did so at the expense of abandoning the important concept of leukocyte transmigration.

A modern-day illustration of the difficulty in accepting new ideas is provided by Judah Folkman’s hypothesis that tumors require blood vessels to grow. Folkman faced “‘wall-to-wall critics’. . . the dogma back then was that tumors do not need a new blood supply since they grow on existing vessels, and that redness associated with tumors was inflammation from dying tumor cells, not vascular tissue” (quoted in Ref. 4).

According to Kuhn, “novelty emerges only with difficulty, manifested by resistance, against a background provided by expectation” (Ref. 21, p. 64). Once the novelty is accepted as fact, we may be less inclined to explore the events around that discovery. For the unquestioning modern-day practitioner, the acceptance of the capillary wall as simple fact belies a rich history of methodological, technical, and conceptual advances.

The study of the capillary wall: dead science or viable pursuit? We have learned from this historical survey that the capillary (and by extension its wall) was very much at the forefront of studies in human physiology and pathology. As the principal site of exchange between blood and tissues, the capillary underlay fundamental principles of nutrition, metabolism, tissue growth, and repair. Harvey, who applied Bacon’s scientific method and quantitative physiology to the discovery of the circulation, was the first to consider the existence of these tiny connections between arteries and veins. In their pioneering work with the microscope, Malpighi and Leeuwenhoek focused their lens on the capillary system. Schwann, the founder of the cell theory, was the first to illustrate cells lining the capillary. Virchow, who famously introduced the theory of cytogenesis, struggled for years with the concept of leukocyte transmigration and pus formation. The focus on capillaries would continue into the early to mid-twentieth century. It was not until the recognition of atherosclerosis as a distinct disease entity in the 1900s that attention shifted from the capillaries to the large arteries (reviewed in Ref. 9).

These considerations beg the question of whether the capillary wall has lost its appeal as a subject for scientific inquiry. We believe that the answer is no. While progress in this field continues unabated, it occurs under different guises. Over the past 50 years, the field of capillary research has largely splintered along the lines of organ-specific disciplines. Rather than constituting an integrated system, the capillaries from different organs fall under the domain of subspecialists whose frame of reference is not the vasculature per se, but rather the physiology and pathology of the organ in which the capillaries reside. Progress in understanding the capillary wall has become similarly compartmentalized. For example, neuroscientists are primarily concerned with improving drug delivery across the walls of brain capillaries, pulmonary researchers are focused on reducing transfer of solutes and fluids across the walls of lung capillaries in acute lung injury, and hepatologists are interested in preventing the loss of endothelial fenestrations in liver capillaries in diseases such as cirrhosis. The paucity of cross-disciplinary interactions notwithstanding, these efforts, when considered collectively, suggest that the modern-day study of the capillary wall continues to thrive. More than that, there is a growing appreciation that site-specific endothelial...

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15 In referring to Harvey’s inference regarding the large quantity of arterial blood, Hoffman (a Galenist) stated: “A few words, but weighty! But how will you prove it my dear Harvey? For you would seem to accuse Nature of stupidity in that she went so far astray in a work of almost prime importance as is the making and distributing of food. . . . You would seem to impose upon Nature the character of a most rude and idle artificer who destroys the work she has done and perfected so that, forsooth, she should not be at a loss for anything to do, for, to make raw again the blood that has been perfected by all her agents and then again to concoct it, what is it other than this?” (quoted in Whitteridge G. William Harvey and the circulation of the blood. London: Macdonald; New York: American Elsevier, 1971, p. 162).
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