Endothelial Biomedicine

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The Endothelium in History

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The endothelium is only now beginning to gain acceptance as a physiologically relevant organ with potential clinical significance. Yet the cell layer called the endothelium was first identified well over a century ago. In this chapter, we explore the circumstances leading to the slow recognition of the endothelium as a system with untapped diagnostic and therapeutic potential. We trace historically important steps toward increased interest in the endothelium, beginning with ancient discussions of the heart and blood vessels, and the conviction that blood derives from nutrition and is continually used up by the body. We see that, in Western medicine, the dominant culture of the Catholic Church impeded new discovery and instead emphasized reliance on accepted ideas for nearly 1,500 years. Only in the context of the Scientific Revolution of the seventeenth century could anatomists such as William Harvey challenge prevailing dogma and reach the conclusion that blood circulates and that it does so through a system of connected vascular vessels.

In this chapter, we examine those contributions and the developments that followed, slowly and gradually, the rise of new technologies for observation and the framing of new questions. We ask what caused researchers to focus on cells and tissues, and then, during the last part of the nineteenth century, to identify endothelial cells (ECs) as a unique structure, distinguishable structurally, physiologically, and developmentally from the epithelium that researchers initially had seen as closely connected to it. Then, we explore what implications the identification and naming of this particular set of cells had for the biomedical sciences.

The potential for applying principles of endothelial biology to the clinic has been much less well developed, and one goal of this book is to help change that. We return to the current research situation and to the medical potential of EC research in the final chapter (see Chapter 196).

PRE-ENDOTHELIAL HISTORY

Medical researchers often blame the second-century physician Galen for holding back progress in understanding the vascular system. These same researchers point to seventeenth-century physician William Harvey as the heroic founder of modern medical research. Galen certainly did maintain a theory-driven interpretation of arteries and veins as conduits for all manner of things. Inhaled air, expended air, nutriment, and blood all flow through the same blood vessels, according to Galen, responding to the needs of the body as a whole (Figure 1.1, *left side*).

Almost inevitably, medical researchers and textbooks refer to Galen as "in error." Of course, from our twenty-first century perspective of accumulated knowledge, he *was* wrong. However, such a clear-cut judgment ignores the context of the times, Galen's reasoning, and his potentially positive contributions. As surgeon and historian Sherwin B. Nuland explains, what really matters to historical judgment is whether Galen should have known better (1). Given that he did not dissect humans, but relied on animal studies alone, we can excuse some of his descriptions, which deviate from what he would have seen had he been able to look as carefully at humans as we can today. And, given that he could not see the microscopic capillaries and that what he *could* see showed differently colored and textured arteries and veins, we can understand his descriptions of the arterial and venous networks as two largely separate vascular systems. After all, the two systems do look different. Arteries are thick, pulsate, lie deep within tissues and carry red-colored (as we now know oxygenated) blood; veins have thin walls, do not pulsate, are often superficial (such as those on the back of the hand), and contain bluish (deoxygenated) blood.

But later studies, often praised as exemplary (notably William Harvey's), did not differ significantly from Galen's in the physical observations that they were based on; these studies also relied on animal models and naked-eye observation. The difference lay in the questions asked, the assumptions made, and in the nature of the search for additional new information. Harvey drew on a diverse mix of experimentation, observation, and calculation in a way that Galen only argued that researchers should do. When Nuland calls Galen "The Paradox of Pergamon," he emphasizes the irony that, during

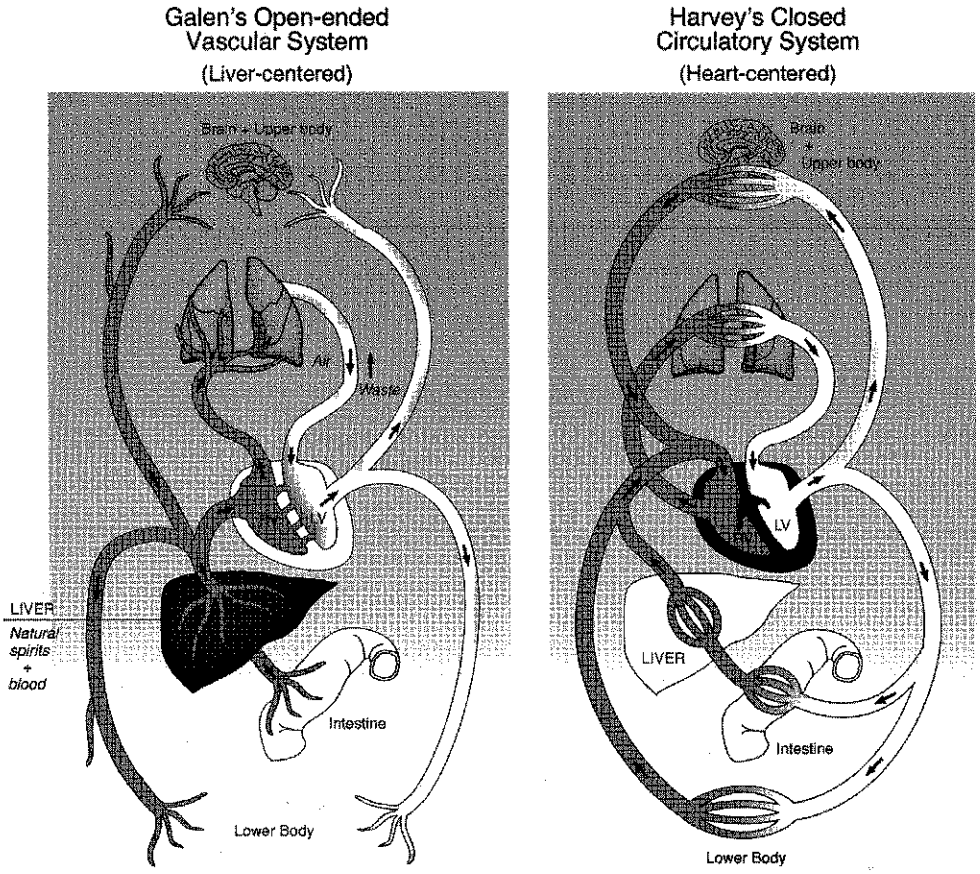


Figure 1.1. Schematic of the vasculature system as viewed by Galen (*left*) and Harvey (*right*). Galen did not recognize blood as circulating. He believed that arteries and veins functioned as distinct, open-ended systems, with veins carrying blood (synthesized in the liver), and arteries carrying both blood (derived from venous blood through invisible interventricular holes) and pneuma (derived from the lungs). Harvey employed simple yet elegant physiological experiments, including ligating arteries and veins, to prove his hypothesis that blood circulates.

his lifetime, this physician, noted for his progressive demand for evidence based on experience and for his questioning the authority of others, did not allow similar questioning of his own authority and did not question his own experiences and interpretations further. Thus, Galen was "wrong," both at the time and in retrospect, in his inconsistent application of his own evidence-based epistemology.

These faults had a lasting effect. It is no exaggeration to say that Galen's ideas, his insistence on adhering to them, and their unquestioning acceptance and promulgation by Catholic Church–run medieval universities effectively held back Western biomedical discovery for about 1,500 years. The universities adopted the ancient learning of Galen, Aristotle, and others ex cathedra, to be taught through rote lecture and memorization and without question. Medical students did not carry out their own dissections, nor did they question existing knowledge or add new discoveries. Although Galen did not create this climate of uncritical acceptance of dogmatic ideas, his own attitudes and writings did not discourage such blind acceptance – as long as it was acceptance of his own ideas.

What, then, was the impact of Galen's interpretation? We can ask whether his "mistakes" actually captured something worth noting, and whether they were reasonable in the context in which he worked. In his insistence that the arteries and veins allowed blood, air, and nutriment all to flow in the same vessels and in both directions, as needed by the body for nutritive reasons, he actually assigned the blood vessels an active role in helping to determine which direction and at what rate the flow would occur. In this, he saw something that those "moderns" missed who viewed the system as passive plumbing that merely allowed fluids to pass through the body.

For Galen, as for the already legendary Hippocrates of the fifth century B.C., the arteries and veins both play important regulatory roles in maintaining function in a balanced, healthy body. Although we know little about Hippocrates the individual, or even about Hippocratic ideas about blood and vessels, we do know that the Hippocratic ideal retained its attraction well into the twentieth century. With its system of interacting humors and responses to the environment, the Hippocratic body was active, with an observable structure, a function that

responded to environmental conditions, and developed over the life of the individual as the baby grew into the adult. Structure, function, and developmental responses to environment are all parts of the Hippocratic body, and Galen largely adopted that set of assumptions. This ancient model, which dominated medicine for nearly 2,000 years, was internally active and reactive within its environment in ways often ignored in later times.

CIRCULATION

Galen insisted that the heart has invisible pores that allow the movement of blood through the thick walls of the septum (see Figure 1.1, *left*). This must be the case, he surmised, because he could not see how fluids could travel from the arterial to the venous system otherwise, as they surely do. Generations of medical students absorbed this lesson as their professors read from the Galenic texts. When they looked at bodies, it was to read off the lessons of the texts: "See, here we observe exactly what the great Galen tells us that we must see."

Only in the early sixteenth century did Andreas Vesalius join a small number of anatomists who were beginning, especially in Padua, to actually look at the body with their own eyes and to ask questions that went beyond Galen's doctrines. At first, these questions focused mainly on filling in details and correcting small errors. Vesalius began by asking how it is that the blood can pass through the presumably small pores that Galen had described in the heart's septum. In 1541, Vesalius contributed to a new edition of Galen. Two years later, in 1543, *De Fabrica* appeared under his name. There he wrote that:

> The septum is formed from the very densest substance of the heart. It abounds on both sides with pits. Of these none, so far as the senses can perceived, penetrate from the right to the left ventricle. We wonder at the art of the Creator which causes blood to pass from right to left ventricle through invisible pores (2).

Although Vesalius had made many new observations that disagreed with Galen, he did not challenge Galen's interpretation of the blood's movement. If Galen said that the blood passes through pores in the heart's septum, even if those pores are invisible, then it must be so.

Vesalius continued looking and continued thinking, however. By the second edition of his book, he concluded that he had not seen what Galen said he should see and that, therefore, the pores through the septum are simply not there. Galen was simply wrong about this. As Vesalius wrote in his 1555 edition:

> Not long ago I would not have dared to turn even a hair's breadth from Galen. But it seems to me that the septum of the heart is as thick, dense and compact as the rest of the heart. I do not see, therefore, how even the smallest particle can be transferred from the right to the left ventricle through the septum (2).

This was a tremendous breakthrough. Despite the attacks he received for the impertinence and even perceived sacrilege in challenging Galenic authority, Vesalius and his contemporaries had opened the door for further questioning of anatomical and physiological details. They also laid the groundwork for the basic methods of biomedical science: Start with one's own observations rather than blindly accepting established doctrine. In particular, Vesalius opened the way for the study of the blood system of heart and vessels, and this focused attention on the anatomical structures that seemed important for physiological function. Medicine moved away from the idea of Hippocratic humors that run throughout the body and serve as a unifying holistic tie. Instead, a new emphasis on blood began a trend toward breaking the body into smaller and smaller units, looking for localization of function within defined structures and, eventually, localization of disease within specific structures and functions.

William Harvey carried the investigation further. Building on Vesalius's work (and his questioning the existence of pores in the septum) and on the observations of Hieronymous Fabricius of Aquapendente (who had discovered valves in the veins but not the arteries and had asked why), Harvey found the Galenic interpretation of the movement of blood through the heart and vessels unsupportable. As he noted in the opening section of *De motu cordis* in 1628:

> When they say that the left ventricle draws material, namely air and blood, from the lungs and the right sinus of the heart for the formation of spirits, and likewise distributes spirituous blood into the aorta; that sooty vapours are sent back to the lungs through the vein-like artery and spirit forwards into the aorta; what is it that keeps the two streams apart? And how do the spirits and the sooty vapours pass in opposite directions without mixing or getting into disorder (3)?

And so on to the point that they "would have it that the mitral valves should hinder its return. Good God! How do the mitral valves hinder the return of air, and not of blood?" (3). The fact that, in the same introduction, Harvey also apologized for having to challenge Galen's authority almost 1,500 years later shows just how long Galen's grip on medical theory lasted during the Middle Ages and the Early Modern Period. But that grip was loosening as Vesalius, Harvey, and others opened their own eyes and trusted their own senses.

Harvey famously went on to outline his arguments that blood must circulate through the body, moving out through the arteries and back through the veins after having passed through the tiny anastomoses that connected the two systems. Even though these connections and the passage of blood through them was not yet visible, for Harvey, the overwhelming accumulation of evidence compelled him to the conclusion that blood must move from one system to the other and that, therefore, the connections must exist (Figure 1.1, *right*).

The overpowering logic, the diversity of converging types of empirical evidence relating to blood's quantity and movement, and the accumulated anatomical evidence eventually carried the day in favor of Harvey's interpretation, although not without a fight. Gradually, after 1628, blood was accepted as circulating through an essentially closed system of blood vessels. In connection with interpretations from mechanical philosophers such as René Descartes in 1632, the heart came to be seen as a pump or a furnace, pushing blood out into the arteries by the action of contraction (4,5). For the mechanists, blood flows along its constrained path until it finally reaches the heart again, and it flows in from the veins to fill the void left by yet another contraction that has sent out yet more blood into the arteries.

The weight of argument in favor of the circulation model was overwhelming, even though Harvey himself could not actually see the connections between the arterial and venous systems. They must be there, but it would take new technology to see them. Sure enough, when Italian anatomist Marcello Malpighi used the newly available compound microscope to look at blood flow in the lungs of frogs in 1661, he directly observed the connecting capillaries (6). His reports drew on the direct, meticulous observation of diverse tissues and experimental manipulations to enhance observation—for example, injecting colored fluids into the vessels to observe their paths. Malpighi's capillaries were so small and so important in allowing the blood to circulate that they naturally became a focal point for understanding how the transmission of blood from arteries to veins works. With Antoni van Leeuwenhoek's confirmation using his higher-powered single-lens microscopes in the 1670s, the circulation of blood was largely accepted.

By the mid-seventeenth century, then, a very neat anatomical picture formed that was clearly "right" in the sense of accurately describing the physical phenomena of blood flow. But it largely missed the physiological action and life of the system, and it also lacked any sense of how the system develops or whether it simply exists, already connected from the very earliest stages of any individual. The focus remained primarily on structure: Harvey's followers had turned Galen's active and reactive system into a machine, with arteries and veins serving as mere passive plumbing. That Harvey himself did not hold such a mechanistic view is evident from his vision of the blood as the body's revitalizing agent. For Harvey, the circulation of blood brings renewal, similar to the cycle of evaporation and spring rains that renew the soil, or like the heavenly bodies orbiting and returning every year. Circulation brings life, and the parallels between circulation in the macrocosm of the heavens and in the microcosm of man stamped a sort of confirmation on the circulation hypothesis.

Yet Harvey's vitalistic picture had given way to a largely mechanistic world view in medicine. The mechanistic conceptions of the body also resonated with the emergence and increasing popularity and importance of sophisticated mechanical contraptions, such as clocks or pumps (7). Indeed, it can be argued that the prevailing images and metaphors of the organism during the seventeenth and eighteenth century were all derived from the technologies of these times, which provided both the instruments for studying biological phenomena as well as the interpretative framework for its understanding. The best known and one of the more far-reaching analyses is Julian Offray de la Mettrie's "Man a Machine." Although he did not focus on anatomy as such, La Mettrie (8) saw a close connection between the fluids circulating in the vessels and the maintenance of the "elasticity of the blood vessels on which their own circulation depends."

The emerging new world order of early globalization and increased trade also contributed to the prevailing view of the importance of circulation and well-defined channels of transport. Here, as in most instances of the development of scientific ideas, the exchange of metaphors went both ways: On the one hand, the existing social and economic order shaped ideas about the organism (including concepts of pathology), while on the other hand, the biological conception of the organism also became a model for ideas about the organization of the state and the economy (9,10).

The mechanistic conception of the organism, together with the increased understanding of anatomy, also contributed to the development of a new conception of disease as a localized deficiency in a particular part of the body. Not unlike a broken machine, a sick body was considered to have a broken part. Pathology emerged in the nineteenth century as a scientific discipline that investigated both the symptoms and causes of disease within this framework of machine-like organisms (11).

SPECIALIZATION IN BIOMEDICAL RESEARCH

The nineteenth century brought a new view of the circulatory system and its blood vessels, in terms of tissues and then cells. Rather than seeing vessels as long, essentially unstructured pipes through which the blood passively flows, researchers began to see the vessels as structured and constructed of parts. In particular, cells came to be seen as making function possible and developing over time.

The new view arose partially because of increased knowledge. Improved achromatic microscopes and microscopic techniques made it possible to observe smaller and smaller parts of the organism. Technology and inquiry reinforced each other: The desire to see more stimulated the push to develop new technologies and, simultaneously, new technologies stimulated new questions. At the same time, biology was emerging as a field of study, with an emphasis on examining structure (through anatomy and cytology), function (through physiology), and development (focused on cells and organisms). Although "biology" as a field by that name only emerged in the nineteenth century, and only fully developed in the early twentieth century, already the study of life was beginning to be differentiated into specialized subfields of study, localized in different specialties within medical schools and research institutes.

Development

Early in the nineteenth century, Karl Ernst von Baer and others had carefully examined eggs and discovered that mammals have eggs too (first seen in a family dog sacrificed for the cause of science) (12). Observing the processes of development, they saw an emergence of form from what appeared to be unformed matter. That is, they saw form coming into being only gradually (or epigenetically), with the egg developing layers and only then differentiating into organs and systems.

Von Baer joined fellow embryologist Christian Heinrich Pander in noticing that the process of development forms "germ layers" (13). These connected but distinct layers of matter then become the various parts of the differentiating organism. Perhaps the embryo was always divided into the outer ectoderm, inner endoderm, and middle mesoderm layers, or perhaps the layers arise epigenetically through the developmental process? That remained to be determined, and some researchers held each position. (It was not until the late nineteenth century that researchers understood that these layers arise only at the gastrulation stage.) In addition, the biological significance of the layers remained to be determined: Did they provide the start of differentiated body parts, and therefore have embryological significance? Did they represent tissues that would give rise to different functions, and therefore have physiological significance? Or were they just structurally different, and changing with time? These were central questions for early nineteenth-century biologists.

Cells

Early in the nineteenth century, Matthias Schleiden and Theodor Schwann focused on cells (14,15). They saw cells as the vital units that make up organisms, and they offered a theory of cell development whereby accumulating cells make up a growing and differentiating organism. The history of ideas about the formation of new cells during the mid-nineteenth century shows how contemporary philosophical and theoretical conceptions can shape the interpretation of observations. Schwann, who was committed to a unified theory of nature, first conceived of the formation of new cells as analogous to crystallization, which was an established mode for the emergence of new forms. He thought that existing cells secrete material, and new cells emerge through a process analogous to crystallization. It took several decades of painstaking and detailed observation to establish the mechanisms of nuclear and cell division.

By mid-century, with advancing microscopic techniques, a growing community of biological researchers had generated a picture of the embryo as a fertilized egg cell that undergoes cell divisions, develops germ layers, and then differentiates into specialized types of cells and tissues (16–20).

Cell Pathology

Cells also assumed the central role in understanding disease, with Rudolph Virchow presenting the case for *cellular pathology* (21,22). Although the "morbid anatomists," as the early pathologists were called in Paris (led by Pierre Louis, Xavier Bichat, and others), had emphasized localization of disease in organs, Virchow localized disease in the cells. Medical science needed to understand which cellular changes were associated with which diseases, he urged, and also how cells contributed to causing disease. Cells work together at times to form membranes, Virchow asserted, including that lining the capillary (21): "A capillary vessel is a simple tube, in which we have, with the aid of our present appliances, hitherto only been able to discover a simple membrane, best at intervals with flattened nuclei..." This is "a membrane as simple as any that is ever met with in the body." Although he did not call this membrane the endothelium, it was, in effect, what he was describing. And, as in later contributions, he argued that the "simple membrane" results from the cells working together. For Virchow, medicine should focus on cells and how they work together to make up functional tissues and organs. Pathology should examine the failures that occur at each level, down to the cellular.

Pathologists also began to distinguish even more finely among different types of cells and tissues. For example, diseased linings of organs and parts called for identification; Viennese surgeon Theodor Billroth used the prefix endo- to describe as an "endothelioma" those tumors occurring in what came to be known as *endothelial cells* (23).

Connecting the Pieces

In the dissecting rooms and in pathology labs, researchers were looking at ever finer distinctions in their search to link disease with localized material. Physiologists sought to link functions to the localized parts of organs and cells, asking how the parts cause the observed responses. Embryologists wondered how the parts and their functions arise, although they had no way to make much progress in studying human development as yet. Structure, function, and development began to hold their specialized places in medical education. Meanwhile, the clinical ideal remained largely Hippocratic, focused on the whole organism and its interactions.

William Osler exemplified the clinician's perspective on and wish for – if not the reality of – holism and integration. He did not look inside vessels for an endothelial lining, but instead emphasized the whole system and its actions and failures. As he wrote in *Diseases of the Arteries*, the arteries reflect the whole of life, with its "wear and tear." For "Among organs, the bloodvessels (sic) alone enjoy no rest . . . like other organs, they live under three great laws – use maintains and in a measure sustains structure; overuse leads to degeneration; in time they grow old, in threescore or in fourscore years the limit of their endurance is reached and they wear out (24)." Osler's remained largely a structural view, but one that saw the organism as an organic whole:

> The stability of tubing of any sort depends on the structure and on the sort of material used; and so it is with the human tubing. With a poor variety of elastic and

> muscular fibers in the bloodvessels, some are unable to resist the wear and tear of everyday life, and have at forty years of age arteries as old as those of others at sixty . . . not only are there individuals, but whole families with "shoddy" bloodvessels. Hence the truth to the old saying attributed to Cazalis, "a man is as old as his arteries." In the building of the human body, as of chaises, there is, as Autocrat says, "always somewhere a weakest spot," and too frequently this is in the circulatory system. The conditions of modern life favor arteriosclerosis, as a man is apt to work his body machine at high pressure . . . Living quieter lives and with less stress and strain, women are not so frequently the subject of arterial changes, and in consequence they last longer (24).

THE ENDOTHELIUM

The Swiss anatomist Wilhelm His introduced the term *endothelium* in 1865, in a programmatic essay titled "Die Häute und Höhlen des Körpers (The membranes and cavities of the body)" (Figure 1.2). Halfway into his tenure as professor of anatomy and physiology in Basel (from 1857 to 1872) His introduced an academic research program that became the foundation for his work in developmental mechanics. It was based on the conviction of a "tight connection between histological embryology and the most fundamental problems of general physiology" (see Ref. 25, p. 33). Programmatically, His continued the work of Xavier Bichat, who began his short but extremely productive career with a monograph on the membranes of the human body (26). Following Bichat, His's program was to identify the embryological origins and further developmental differentiation of tissues that have structural and functional meaning for the organism.

> Nobody doubts, as was first recognized by Bichat, that all the capacities of the living body can, in the end, be explained by the coordinated interactions of the capacities of its tissues. These capacities of the tissues are, however, a direct consequence of their organization . . . A cell, even though it is endowed with rich internal capacities, only develops in closest dependency of its external conditions, it even responds promptly to the most fleeting external cause, either through changes in its vegetative state, or through other changes in its vital functions. . . . These phenomena will be revealed by means of pathological–anatomical and experimental as well as embryological analysis (25, p. 34).

During the following decades, His constantly refined his initial program, always ready to adopt new technologies. After the mid-1880s, these included advanced apochromatic microscopes and microtomes (that His helped to refine) that allowed meticulous serial sectioning as well as new methods for the three-dimensional representation of anatomical structures. As a result, anatomical details became observable both in adult and in embryonic specimens. His's program sought theoretical generality, but was based on observed particulars in both human and vertebrate (mostly chick) specimens.

His triggered an immediate and at times rather heated debate about the appropriateness of this new concept of endothelium. His's specific focus was on the cavities and membranes of the third germ layer, the mesoderm, which include the vascular system, pleural spaces, and the pericardium and peritoneum. He focused especially on the importance of developmental history (*Entwicklungsgeschichte* or descriptive embryology) in understanding histology and anatomy. During that time, the respective contributions of different germ layers to various organs systems were still debated, as were the actual mechanisms of organogenesis. His's own program emphasized the movements and foldings of germ layers as a strictly mechanical and material cause for differentiation, development, and function. His's focus remained on early developmental stages, rather than on the later anatomical results and their biological and medical implications. In the context of increasing specialization, this mattered, because many medical researchers did not yet hold the early developmental stages as important. Researchers questioned his claims about the developmental process, about observations based on manipulative techniques that necessarily destroyed the organism being studied, and about the claims that these cells and tissues were really distinct and deserving of special consideration.

One of the peculiar features of the mesoderm, which His and other embryologists clearly recognized, is the formation of inner cavities within the differentiating mesodermal tissues (e.g., the vascular system, the lymphatic system, or the pleural spaces) and the histological differentiations associated with these structures. Among the differentiated structures connected to these cavities were so-called inner membranes, which show a remarkable diversity and thus proved to be a serious challenge for microscopic anatomists and histologists.

One problem was conceptual. How should one refer to those cell layers that line these inner cavities of the mesoderm? Common practice at that time was to refer to them as an epithelium, in strict analogy to the epithelia covering the outer surfaces of organs (e.g., the keratinocytes that cover the skin or epithelial cells that form the inner lining of the digestive system) and protecting these organ systems from their environment. In this case, the generic term *epithelium* simply meant a layer of cells serving as a lining. But, as His pointed out, the cells that line the cavities of the inner germ layer (mesoderm) exhibit certain characteristics that differentiate them from those epithelial cells that originate from the two outer germ layers (endoderm and ectoderm). Therefore, these structures should be identified by their own designation.

One alternative was to call them "false epithelia." His found that unsatisfactory and instead introduced a new term, *endothelia*:

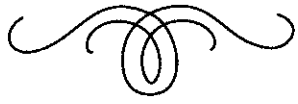
DIE

HÄUTE UND HÖHLEN DES KÖRPERS.

ACADEMISCHES PROGRAMM

VON

WILHELM HIS



BASEIL,
SCILWEIGHAUSERISCHE UNIVERSITYTH-BUCHDRUCKEREL,
1865.

Figure 1.2. Frontispiece of Wilhelm His: *Die Häute und Höhlen des Körpers,* published in 1865. In this publication, an outline of His's research program, he first defined the endothelium as the lining of the vasculature and the lymphatic system.

It is customary to refer to the cell layer that lines the vascular and inner cavities as an epithelium. The same designation is also used for the inner cellular linings of the joint cavities and those on the back of the cornea. However, all these cellular layers that line the cavities of the inner germ layer [mesoderm] display such a large number of similarities and, from their first appearance during development, they differ from those cellular layers that have their origin in one of the outer layers [endoderm and ectoderm] to such a degree that it is well justified, especially with respect to understanding their physiological functions, to identify those by means of a special designation, either referring to them as "false" epithelia in opposition to the "true" epithelia, or by calling them endothelia [sic], thus reflecting linguistically their relationship to inner membranes (25, p. 18).

His went on to describe the differences between endothelial and epithelial cells:

> Beginning with early development, the contrast between serous and vascular endothelia on the one hand and true epithelia on the other hand is already visible. The former develop, as we have seen, from lymphoid cells, the least differentiated cell type that the inner germ layer [mesoderm] can produce and which are also the precursor (Mutterform) for all others. Soon they take on their characteristic flattened shape and become transparent and after reaching this stage they barely change anymore nor do they participate in any significant way in growth processes within the body (25, p. 18).

From these statements, it is clear that, by 1865, His did not recognize the participation of ECs in blood vessel formation. The perceived passivity of ECs in development is also in stark contrast to the activity of epithelial cells, which were already recognized to continue to grow and participate in changes during development.

A second, physiological difference between endothelial and epithelial cells was recognized by His. Whereas epithelial cells produce all those substances that form the secretions of the various glands in the body, in contrast, ECs were not seen to produce any form of secretions. As His emphasized, "we have no reason to ascribe to endothelia any secretatory functions" (25, p. 18). The final difference between endothelial and epithelial cells that His mentioned relates to their function as barriers: Although blood serum can pass freely through ECs, which therefore do not provide a clear separation between the blood in the vessel and the surrounding intercellular substrate, epithelial cells act as a much stronger barrier, especially with regard to larger molecules:

> There is another aspect in which true epithelia and endothelia are in stark contrast to each other; serum can freely pass through the latter at any place; sometimes serum filters through the endothelia and leaves the blood vessels in order to nourish the surrounding tissues; sometimes it passes from the tissues into the lymphatic system or the serous cavities, following a simple pressure gradient. This implies that endothelia do not provide a strict boundary between cavities and intercellular substances of the inner germ layer [mesoderm]; therefore physiologically these have to be seen as a whole, as they equally contribute to the function of containing the general nutritional fluids. The situation is different with true epithelia (25, p. 19).

Summarizing His's arguments, which we present here at some length because of their historical significance, we see that the concept of the endothelium as a separate and clearly distinguishable part of the body arose as a consequence of three different considerations. First, the endothelium can be distinguished because of its embryological origin from the mesoderm, becoming a layer of cells that covers the cavities of the inner germ layer (mesoderm). Second, ECs have a clearly recognizable structure, with the endothelial layer clearly identified as a connected layer of flattened cells. And third, ECs were not considered to be active in physiological secretion. Instead of having an active role that would have been considered physiological at the time, the endothelial layer was seen as providing a somewhat porous lining for the vascular system and related mesodermal cavities. Endothelium was more a matter of providing structure to support the vascular plumbing system, rather than as anything more active.

The New-Found Endothelium

In the years following His's introduction of the term, not everyone immediately adopted his proposal to identify the endothelium as a separate entity. Arguments continued about the usefulness of separating the endothelium from epithelium. Was there really something different here and, if so, did it deserve its own name? A survey of textbooks and published articles from the later nineteenth century suggests that leading anatomists such as Joseph Hyrtl, Carl Gegenbaur, and Philipp Stöhr – who all argued against the separateness of the endothelium – seemed to have the upper hand. For them, the epithelium and presumed endothelium had fundamental similarities in function and in morphology. If it was important to make distinctions of type, they preferred using additional descriptive terms to specify the origin of these "thelia," such as mesenchymal epithelium. This interpretation was codified in some histology textbooks, which typically defined an epithelium as a connected layer of cells covering the surface of the body, an organ, or an inner cavity. Under this definition, endothelium was simply a specific form of epithelium consisting of flattened cells (*Plattenepithelium*) that lined the blood vessels.

Narrowing the Endothelium to Blood and Lymphatic Cells

Increasingly, however, others did take seriously the differences, because the term *endothelium* had its uses. Increasing acceptance that something specialized called the endothelium existed was reinforced after the 1880s and 1890s because of the advanced microscopic and histological techniques and improved equipment that made possible a much more detailed and wider range of observations. Specifically, researchers began to reliably distinguish the endothelium as a layer of cells that together serves as a membrane lining blood vessels, the lymphatic system, and (for some) parts of the nervous or other systems. The influential Heinrich Wilhelm Gottfried von Waldeyer, for example, suggested restricting the term to those cells that make up the innermost layer of blood and lymph vessels and the posterior lining of the cornea. He thus excluded some of the other "thelia" also derived from the mesoderm and that His had included in his definition of the endothelium

(27). Waldeyer's approach, based on detailed observations, provided considerable clarification about what should be included as endothelium.

The types of observations on which Waldeyer drew illuminated fine anatomical structure, and discussion of function followed. If this layer of cells is truly differentiated from others, then why? What function does it serve? Also, questions arose about how ECs develop and whether that developmental history might provide clues to their identity and use.

By 1908, knowledge about the endothelium and its use had already begun to achieve standardization and become accepted within the body of established scientific knowledge. Even such a general standard source as *Gray's Anatomy* reflected the new understanding about an endothelial lining for the vascular system. What had been called the epithelial layer was now the endothelium, and the capillaries, in particular, were seen as "very small endothelial tubes which connect the venous system with the arterial system." "The nucleated endothelial cells which constitute the wall of a capillary are flat, irregular in outline, and are united by a cement material." This passage reflects the state of knowledge: Endothelium is connection, the cells together make up the vessel walls, and they are united by some unknown material. Furthermore, they make up vast connected networks or systems of "endothelial tubes throughout the entire blood-vascular system. The heart is a great muscular thickening around a portion of the system of endothelial tubes" (28, pp. 586–587).

In 1910, A. A. Böhm and colleagues' textbook was one of many specialized texts to appear on the study of histology, which had become a standard field in medical education. For these authors, the message was clear about what types of cells constituted ECs and the significance of their embryonic origins from the mesodermal germ layer: "*Endothelial* cells are differentiated mesenchymal cells. They line the blood- and lymph-vessels and lymph-spaces." It was also important to describe them: "Endothelial cells are in structure like those of the mesothelium. In blood- and lymph-vessels they are of irregular, oblong shape, with serrated borders" (29). By the beginning of the twentieth century, although debate might continue about the existence or importance of the serrations or about the ways in which the cells connected into tissue, histologists largely followed His's initial characterization. They saw endothelial tissue as lining vessels and providing more nuance to the structural plumbing system for blood circulation.

Thus, the beginning of the twentieth century brought a growing awareness of the complexity of what had once looked like such simple tissues and cell structure. Now, it was seen that capillaries consisted of a complex coating, including ECs that themselves seemed to be connected. The understanding of structure had changed dramatically, and it remained for others to interpret what this meant for function. It now seemed that vessels do more than passively carry blood or lymphatic fluid around the body: They play some more active functional role as well. But how? Most importantly, are the vessels made of permeable cells, or do they have porous passages to allow fluids to pass from the vessels to the body? If so, how? To answer such questions, researchers studied both the fine structure and the related function of the ECs under both normal and pathological conditions.

Physiology and Pathology: The Endothelium in Action

With accepted structure came ascribed function. Pathologists and physiologists each worked to understand endothelial function. Already in 1884, pathologist G. Hare Philipson discussed, in his Bradshawe Lecture "On the Pathological Relations of the Absorbent System," the endothelium, which he defined as the lymphatic and circulatory vessels. As he put it, "Alterations in the amount of transudation may thus be referable, not to disturbance of the circulation, but to changes in the vessel wall, and especially in their endothelial lining" (30). The vessel wall is not a passive or essentially dead membrane, but is living and active. It helps regulate the flow of fluids either along the vessels or perhaps even through the vascular walls. But how?

Physiological studies undertaken during the first half of the twentieth century supported the concept that, in addition to the visible flow of blood through capillaries, there existed an "invisible flow of water and dissolved materials back and forth through the capillary walls," and that this "invisible component of the circulation takes place at a rate which is many times greater than that of the entire cardiac output" (31). In 1927, Eugene M. Landis demonstrated that flow through the capillary wall is directly proportional to the difference between hydrostatic and osmotic forces acting across the membrane (32). Other investigators compared the flow of graded-size molecules through artificial porous membranes and capillaries and used mathematical formulas to calculate capillary filtration coefficients and pore dimensions.

These studies gave rise to a pore theory of capillary permeability, which predicted the presence of two distinct pathways across capillaries: a small pore system for the passage of small hydrophilic solutes through water-filled channels, and a large pore system for the passage of macromolecules. Proof for the existence of these structures would have to wait for the development of higher-resolution microscopy; the advent of new microscopic techniques clarified the structure of individual cells and, especially, how cells interact.

More than a Layer Lining Blood Vessels: Form and Function Together

The discussions about the very existence and then the nature and role of the endothelium during the first decades of the twentieth century reflect epistemological and methodological discussions within medicine and biology more generally. A developmental approach reflected the belief that biological understanding required knowledge of the origin and genesis of structures. A morphological/histological approach argued for the comparative evaluation of the internal configuration and external appearances of structures, whereas a functional/pathological approach emphasized the role that certain structures have in the orderly operations of the body.

In the endothelium, we see all three at work, as researchers sought to discover whether, what, and why endothelia exist. Even though the picture became much clearer as research moved into the early twentieth century and the endothelium was accepted as a specific entity, continuing debates about its structure and role reveal the competing underlying assumptions of the researchers involved. These debates also reveal the tendency of researchers in different specialty areas to work at different levels of analysis.

Ramon y Cajal's histology textbook in Spanish, revised and translated in 1933, provides an example. Ramon y Cajal emphasized the structural flatness of the endothelial layer of cells. Those flat cells that form a thin, pavement-like layer over a surface are endothelial, he maintained, wherever we find them and whatever their origin. The metaphor of the pavement not only conveyed that this was a flat covering, but also that it contained a sort of cement that held the individual cells together to do their job of providing a solid lining (33). This raised the question of how the cells adhere. Histologists, especially in the 1920s and 1930s, used silver nitrate preparations to discover detailed cell appearance. In the blood vessels, ECs were seen as being connected by a "resistant cement," but in the corneal surface they seemed to have connecting filaments holding the individual cells together. Details of these phenomena were laid out in textbook after textbook, each recognizing that the meaning and role of these cells remained largely unknown, and each emphasizing different aspects of what was known.

Edmund Cowdry's widely used textbook of the next year (1934) sought to bring the structure and function closer together, looking at the "functional significance of cells and intercellular substances." Cowdry pointed to a debate about the importance of ECs with respect to red blood cells. Florence Sabin and others believed that red blood cells must be produced intravascularly, because the endothelium forms a tight barrier to control the movement of cells. Alternatively, if blood cells form outside vessels, then this requires movement across the endothelial layer into the vessels. Cowdry felt that the issue was not resolved but that, in either case, the endothelium played a central role (34).

In his discussion, Cowdry recognized that it was crucial to understand the nature and extent of the endothelial layer's permeability as well as what regulates movement across that layer. Are the individual cells penetrable, or are there pores between cells that either allow, enable, or restrict movement? And how? As details about the structure became clearer, these functional questions came more sharply into focus as well.

A few years later, in Cowdry's 1938 edition, he noted that the endothelium of capillaries in the brain "holds back protein quite effectively; for the cerebrospinal fluid – a special tissue fluid – is remarkably free from protein. Permeability naturally increases with the inevitable thinning of the endothelium in capillary dilatation or stretching. This happens when the liver swells on receipt of absorbed substances from the digestive tract after meals; but the brain cannot expand to anything like the same degree because it is limited by a bony case" (35, pp. 125–126). By the end of the 1930s, questions about the extent, nature, and effects of endothelial permeability assumed increasing importance.

Leading histologist Alfred Kohn and others argued that, especially for the vascular and lymphatic system, the notion of the endothelium as a cover and barrier facing a hollow space is misguided. Rather, because these vessels are always filled with blood and other internal substances, the vascular system is actually a complex unit composed of moving mesenchymal elements (blood and lymphatic fluids), a layer of cells (endothelium), and the vascular wall. Functionally, these cells all interact; furthermore, the ECs mediate important exchanges between the blood and other tissues (36).

According to Kohn, the functional role of the endothelium to mediate the important material exchanges within the body also accounts for the differentiation among ECs. "Fatter" ECs will be found in those areas of the body where a more intensive exchange occurs, whereas more flattened cells will occur in areas of less functional importance. Some authors referred to this as the reticulo–endothelial system, whereas others rejected the idea and continued to look at individual cells as the mechanism of control. The seeming clarity of the twentieth-century's early decades was once again giving way to debates and alternative interpretations, and evolving and expanding questions. Different levels of analysis and new techniques would bring new information, new insights, new questions, and new debates, as new technologies allowed new observations.

TECHNOLOGY AND ITS IMPACTS AND IMPLICATIONS

Modern science is characterized by a close connection between the development of scientific concepts and theories and the available technologies and methods. Indeed, we find this connection at the birth of modern science during the so-called Scientific Revolution, which was greatly aided by telescopes, microscopes, and shortly thereafter by clocks and the pendulum. These instruments and their associated new methodologies not only allowed for increased precision in measurement, they also carried with them (or in a sense embodied, as the modern historiography of science would have it) a specific conceptual structure. Instruments and research methods are therefore not just a more precise extension of our senses. Rather, through their interactions and intervention with "nature," they constitute – or in a very real sense create – scientific objects. And these scientific objects are not just material objects "out there in nature" with specific physical properties; they are simultaneously conceptual abstractions that also have theoretical significance.

The history of endothelial biomedicine is a good illustration of these patterns. We have already seen how early ideas of the circulatory system and the introduction of the concept of the endothelium reflected larger scientific trends of the times.

The innovations included contributions of then-prevalent research methodologies and technologies. His's recognition of the endothelium as a separate and distinct entity of the body drew both on his theoretical ideas about the role of germ layers during embryogenesis and also on the newly available microscopes, microtomes, and histological techniques that revealed the morphological characteristics of ECs and their mesodermal origins. The interactions of ideas, questions, and technologies accelerated during the course of the twentieth century (37–39). As we discuss below, physiological approaches gave way to ultrastructural studies; electron microscopy, in turn, was largely supplanted by cell culture.

New Technologies, New Discoveries: Electron Microscopy

Electron microscopy brought great advances in understanding structure. In doing so, however, it also moved away from an appreciation for development and dynamic interactions of the cells and their surroundings. Much of the initial excitement generated by electron microscopy was due to its ability to reveal details about the internal structures of cells and resolve some of the long-enduring arguments over whether these were artifacts. Many of the crucial techniques for employing electron microscopy in the study of cells were developed to advance the understanding of cytoplasmic structures. But electron microscopy also helped reveal some of the distinctive features of ECs.

Although a number of new procedures for improving the resolution of the light microscope were developed during the 1940s (e.g., phase contrast and ultraviolet microscopy [40,41]), the electron microscope offered the greatest promise for generating images at finer resolutions that could reveal structures in cell cytoplasm. The electron microscope depended on the idea that electrons have wave properties, with wavelength inversely proportional to electron velocity, advanced by Louis de Broglie in 1924. Several investigators began to develop electron microscopes in the 1930s, and the first commercial microscopes were introduced by Siemens in 1939. Over the ensuing years, advances in microtome sectioning, embedding protocols, and fixation techniques set the stage for a new generation of investigators who employed electron microscopy to generate new information about the structure of cells. Discoveries included George Palade's description of mitochondrial cristae and endoplasmic reticulum–associated ribosomes (42,43).

At the meetings of the Electron Microscope Society of America in 1953, Palade presented a paper entitled "Fine Structure of Blood Capillaries," in which he presented micrographs of capillaries in different organs such as skeletal muscle, heart, intestine, and pancreas. He reported that, in these organs, "the endothelial cells form a continuous lining," and that the cells contained a "large number of vesicles concentrated immediately under the cell membranes facing both the capillary lumen and the pericapillary spaces." Surprisingly, electron microscopy studies failed to reveal pores of the dimensions postulated by physiologists. Palade proposed that the vesicles "may represent a system for transporting fluids across the capillary wall and may account for the high permeability rate of the capillaries." In a subsequent study employing ferritin as a molecular tracer, Palade concluded that "endothelial cell vesicles are the structural equivalent of the large pore system postulated in the pore theory of capillary permeability" (42,43).

A major result of the early electron micrographic studies of ECs was the discovery, in 1964, of Weibel-Palade bodies, which are unique to ECs. These organelles, now known to store and secrete von Willebrand factor and P-selectin, were described by George Palade and Ewald Weibel as "hitherto unknown rod-shaped cytoplasmic component which consists of a bundle of fine tubules, enveloped by a tightly fitted membrane." They concluded that, due to the regularity of its appearance in ECs, the rod-shaped bodies must have "functional significance which for the moment remains obscure" but suggested that they figure in vascular or blood physiology (44).

Another important consequence of electron microscopic studies during the 1950s was the finding of structural differences between capillaries in different organs. As noted by Hibbs and colleagues in 1958, "some variation in the structure of capillaries and arterioles normally occurs from one organ to another, and even among vessels of the same organ" (45). In the 1960s, electron microscopy was used to confirm the functional relevance of structural heterogeneity, demonstrating, for example, that the tight junctions between ECs in the brain form a highly functional blood–brain barrier, whereas the loose, somewhat disorganized junctions of postcapillary venular endothelium correspond to their predilection for solute and leukocyte trafficking during inflammation.

Cell Culture

It may be argued that physiological and morphological studies – in and of themselves – were beginning to yield diminishing returns. That would change in the early 1970s, when Eric Jaffe and Michael Gimbrone independently reported the first successful isolation and primary culture of human ECs from the umbilical vein (46,47). The cells, which were obtained by collagenase digestion, could be maintained in culture for weeks to months, and were identified as endothelium by the presence of Weibel-Palade bodies and von Willebrand factor (VIII-associated antigen). The capacity to culture ECs provided researchers with a new and powerful tool to dissect cell structure and function under controlled (albeit artificial) conditions. These studies have addressed every conceivable in vitro property of ECs including – but certainly not limited to – subcellular organelles (e.g., Weibel-Palade bodies, mitochondria, Golgi apparatus, and endoplasmic reticulum); membrane microdomains such as caveolae or lipid rafts; cell signaling (from cell surface receptor to the level of gene transcription and posttranscriptional regulation); and the cytoskeleton.

While the cultured EC became a focal point for research in vascular biology, increasing evidence pointed to the highly complex topology of the intact endothelium. In the 1980s, several groups carried out systematic immunohistochemical analyses of the endothelium in various organs (48–50). These studies, which built on the earlier results of electron microscopy, revealed differential expression of lectins and antigens in the intact endothelium. In other words, the endothelium displayed *molecular* heterogeneity. Implicit in these observations is a critical – though largely overlooked – message which was articulated by Robert Auerbach in 1985:

> The concept that vascular endothelial cells are not all alike is not a new one to either morphologists or physiologists. Yet laboratory experiments almost always employ endothelial cells from large vessels such as the human umbilical vein or the bovine dorsal aorta, since these are easy to obtain and can be readily isolated and grown in culture. The tacit assumption has been that the basic properties of all endothelial cells are similar enough to warrant the use of the cells as in vitro correlates of endothelial cell activities in vivo (51).

According to Auerbach, a key to understanding structural and functional heterogeneity was to isolate and study microvascular ECs from different organs. This approach makes the most sense if site-specific phenotypes are retained in culture. However, as we will see throughout this book, this assumption is only partially correct. For example, many site-specific properties of ECs depend on extracellular signals. Thus, when ECs are removed from their native microenvironment and cultured in vitro, they undergo phenotypic drift and lose many of their original properties.

Beyond Electron Microscopy and Cell Culture

Although electron microscopy and cell cultures largely contributed to the structural and functional characterization of the endothelium, other techniques have provided new insights into the endothelium. For example, during the past few years, investigators have employed novel genomic and proteomic techniques to uncover an enormous array of site-specific properties (so-called vascular addresses or zip codes) of the endothelium. Together, these studies suggest that "far from being a giant monopoly of homogenous cells, the endothelium represents a consortium of smaller enterprises of cells located within blood vessels of different tissues . . . while united in certain common functions, each enterprise is uniquely adapted to meet the demands of the underlying tissues" (52). The modern techniques of fate mapping emphasize their developmental perspective. Again, differences in methods matter, enabling researchers to highlight different aspects of development. Similarly, the availability of new model organisms for research in endothelial biology, such as genetically modified mice and zebrafish, have had a profound impact on theoretical conceptions of the endothelium.

One consequence of science's dependence on methods and technologies is that knowledge accumulation is rarely continuous. We see this in the gap existing between the availability of a large number of empirical details about ECs and our limited understanding of the endothelium as a functional whole with clinical importance. We lack relevant theoretical models in systems biology that could help illuminate this bigger and more complex system in which ECs reside. As a result of the specialized thinking within separated lines of study, the dominant view of the endothelium is still rooted more in the conceptual structure of isolated cell cultures, which has implications for the clinical applications of endothelial biology.

ENDOTHELIUM IN DISEASE

The first evidence of a potential role for the endothelium in disease (as victim, if not perpetrator) is found in published reports from late nineteenth century, which describe the abnormal morphology of ECs in a number of disease states, including tuberculosis and malaria. In the 1870s, Julius Cohnheim studied the frog tongue to demonstrate that leukocytes adhere to the blood vessel wall of venules (so-called pavementing of leukocytes), many of which passed through the wall into the extravascular tissues (leukocyte emigration). These observations were later confirmed in mammalian species. Studies in an ear chamber model demonstrated that leukocytes adhere to the damaged side of a blood vessel, suggesting that the blood vessel wall – as distinct from the leukocyte – is primarily responsible for mediating adhesion (53). However, the mechanisms underlying inflammation-induced leukocyte adhesion remained elusive for decades. According to one theory, the endothelium secreted a gelatinous substance that trapped leukocytes (54). Others claimed that electrostatic forces were responsible for mediating the endothelial–leukocyte interactions (55).

As with so many other aspects of EC biology, the elucidation of the role for endothelium in inflammation and the molecular basis of leukocyte trafficking would wait for the successful culture of ECs. In the 1980s, several investigators demonstrated that treatment of cultured ECs with inflammatory mediators resulted in phenotypic changes that included increased expression of cell adhesion molecules, leukocyte adhesion, antigen presentation, and procoagulant activities. These changes, many of which were subsequently shown to occur in vivo, were termed *endothelial activation.*

Another term that came into favor in the late twentieth century was *endothelial dysfunction.* In the 1970s, subsequent to Russell Ross's response-to-injury hypothesis to explain the mechanisms of atherosclerosis, there was a growing appreciation that the *intact* endothelium may actively contribute to disease initiation and/or progression (56). Although the term *endothelial cell dysfunction* was first coined in 1980 to describe

the hyperadhesiveness of the endothelium to platelets, it was quickly adopted by the field of cardiology to describe abnormal endothelial vasodilator function (57). Indeed, it is not uncommon today to find full-length publications on endothelial dysfunction that refer exclusively to altered vasomotor tone in coronary arteries. Of course, the endothelium has many functions beyond the control of vasomotor tone, and it is distributed widely throughout the body. Thus, endothelial dysfunction is not restricted anatomically to the heart, nor is it limited in disease scope to atherosclerosis.

CONCLUSION

Since Galen, we have determined that a layer called the endothelium exists, and that it is made up of cells called *endothelial cells.* The structure is derived from the mesodermal germ layer, is widely distributed throughout the body, is highly active with multiple functions, and is remarkably heterogeneous in structure and function. New techniques have illuminated more detail while simultaneously raising questions about the earlier simple assumptions.

Despite breathtaking advances in EC biology and a growing appreciation for its role in disease states, endothelial biomedicine has made little progress as a field. There are several possible reasons for this. First, overspecialization in medicine hampers cross-disciplinary approaches to the endothelium and keeps researchers in one area from learning about the ideas and approaches of another. Second, because the endothelium displays emergent properties and is so highly adapted to its microenvironment, it must be approached in the context of the whole organism. As long as cell culture studies are sufficient for publication and funding, there is little incentive to study endothelial biomedicine as a whole, or to look at the diverse and dispersed roles of endothelium in the body. Finally, it may be argued that by figuratively stripping the endothelium from the blood vessel and employing it as a frame of reference (i.e., "endothelial biomedicine") we are "inventing" a field with little clinical value.

Some might argue that the endothelium should not be considered outside the context of the blood vessel. They might point out, for example, that endothelial–smooth muscle cell interactions are essential and should constitute the minimal unit of investigation and inquiry. Admittedly, this argument stands for conduit and resistance blood vessels. Alternatively, it may be argued that the functional unit of capillaries (which comprise the vast majority of the surface area of the vasculature) is the endothelial–pericyte–extracellular matrix interaction. An investigator in an organ-specific discipline may make a case for studying the endothelium in the context of the whole tissue – for example, the nephrologist, who wishes to understand the role that the glomerular endothelium plays in urinary excretion.

We are beginning to appreciate previously hidden levels of communication between ECs and other cell types and systems. For example, increasing evidence suggests a tight developmental, structural, and functional link between the endothelium and the nervous system. Perhaps, one day, these endothelial–neural connections will define the term *vascular.*

So, it is really a matter of perspective. In choosing the endothelium as our frame of reference, we acknowledge the importance of the endothelial–mural cell interaction and the unique value of each and every vascular bed/organ (including the heart), but treat them as one of many configurations of the endothelium.

This book provides collective knowledge about the endothelium. It strives to answer the following questions:

- What do we know about the biology of the endothelium – for humans as well as other animals?
- What do we know about its structure, function, and development, including evolutionary development?
- What do we know about the clinical implications of endothelial function and the foundations on which those rest?
- How do we go about interpreting what we know, through metaphors, models, and methods?

This volume examines all these issues. In the final chapter, we return to reflect on the knowledge provided by the whole and on what it means for endothelial biomedicine.

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