PART I

CONTEXT

History of Medicine

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The Endothelium in History

Manfred D. Laubichler*, William C. Aird†, and Jane Maienschein*

*Center for Biology and Society, Arizona State University, Tempe; †Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts

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

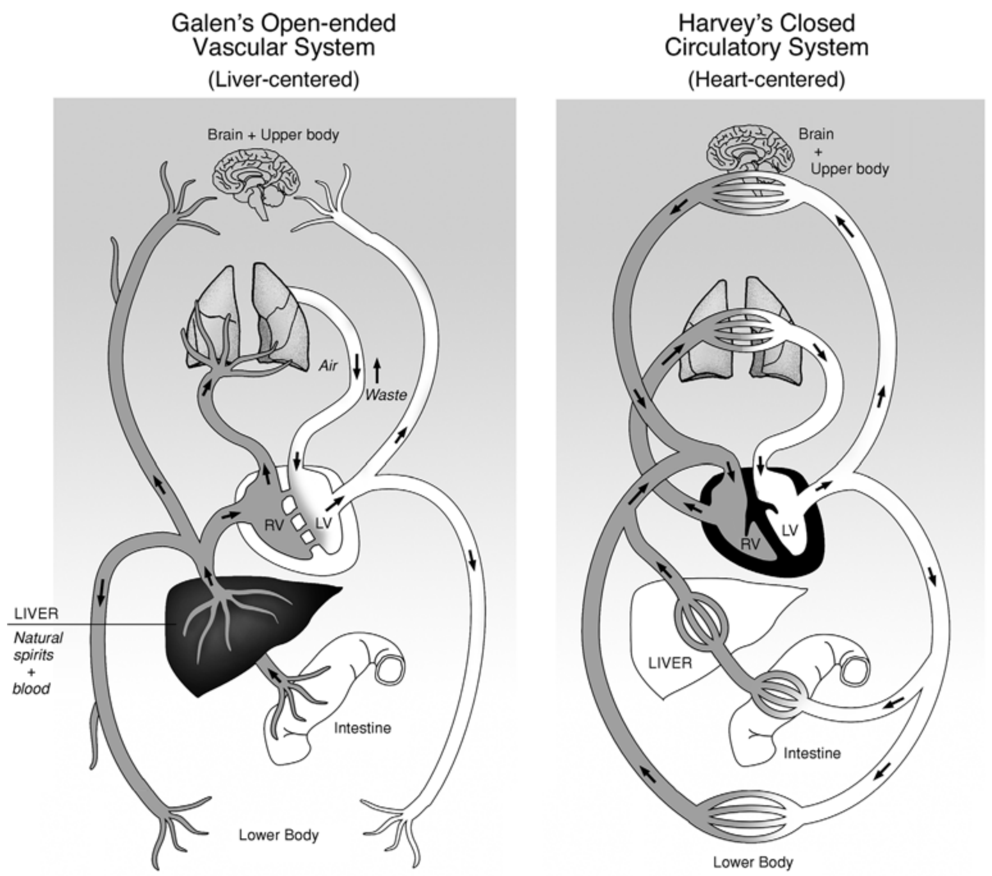

Figure 1.1. Schematic of the vasculature system as viewed by Galen (*left*) and Harvey (*right*). Galen did not recognize blood as circulating. He believed that arteries and veins functioned as distinct, open-ended systems, with veins carrying blood (synthesized in the liver), and arteries carrying both 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