Cardiovascular physiology in the twentieth century: great strides and missed opportunities

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Granger, Harris J. Cardiovascular physiology in the twentieth century: great strides and missed opportunities. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H1925–H1936, 1998.—In a broad sense, physiology is the study of the chemical and physical bases of life processes. Consequently, the evolution of our knowledge of cardiovascular functions is closely linked to the developments in many fields of science, including chemistry, physics, engineering, and biology. A cursory examination reveals that different “foundation” sciences predominated in different stages of the history of cardiovascular physiology. Today, cardiovascular physiology is poised to exploit new developments in all areas of scientific inquiry. However, cardiovascular physiologists have not always embraced the power of the multidisciplinary approach. In this brief overview of the history of cardiovascular physiology in the 20th century, the major focus is on some of the major advances in the field and the contributions of other disciplines to these developments. In addition, the forces that influenced cardiovascular science in this century and their impact on the evolution of the field in the new millennium are discussed.

IN CONJUNCTION with the centennial celebration of the inauguration of the American Journal of Physiology, I was asked to commission a historical article focusing on some aspect of cardiovascular physiology. As editor of AJP: Heart and Circulatory Physiology, I reached the naive conclusion that the responsibility for generating this historical account should be my own. After several false starts, I finally decided to approach the issue from three perspectives. The paper begins with a brief recounting of some of the many advances made in cardiovascular physiology in the 20th century. Several excellent monographs (25, 45, 176) are available that deal with this subject in a more thorough and authoritative fashion than that in the current effort. Perhaps access to this paper through electronic media may serve as a conduit to the aforementioned sources. After surveying the past and present state of cardiovascular physiology, I analyze the forces that influenced the course of the field in the last quarter of this century. The final section of the paper attempts to project the path of development of cardiovascular science in the early part of the 21st century.

Cardiac Physiology

Excitation. In 1623, William Harvey (71) published his classic treatise on the circulation of blood through the cardiovascular system. This thorough and provocative investigation of the route that the bloodstream takes through the heart and blood vessels demolished the classic viewpoint established nearly 1,500 years earlier. Harvey’s discovery unleashed a flood tide of interest in the heart and blood vessels, which led to detailed examination of the structure and function of the heart. We begin our brief overview of the major advances in cardiac physiology with the measurement of the first monophasic action potential with an extracellular electrode placed on the surface of the heart by Burdon-Sanderson and Page (19) in 1883. Their measurements revealed the long duration of the cardiac action potential. In 1913, Einthoven (39) designed a new string galvanometer for more precise measurements of faint electrical signals projected by the heart onto the surface of the body; from these recordings he developed a conceptual approach in determining the general motion of the cardiac excitation wave during a single heartbeat. Although His (76) and Purkinje (137) described the conduction tissues that bear their names in the first half of the 19th century, the atrioventricular node was not discovered until 1906 (171). In the following year, Keith and Flack (88) identified the sinoatrial node while tracing the source of electrical activity responsible for activation of the atrioventricular node. In 1915, Sir Thomas Lewis (102), using extracellular electrodes placed on the epicardium of the canine ventricle, mapped the temporal and spatial pattern of the spreading excitation wave. The ultrastructural basis of intercellular communication in the heart was revealed by Sjöstrand and colleagues (161) in 1958; specialized intercellular junctions between neighboring cardiac cells in the region of the intercalated disk...
implied intimate contact. Nearly a decade later, Revel and Karnovsky (141) used more sophisticated electron microscopy (EM) technology to resolve the hexagonal array of subunits that form gap junctions in the intercellular nexus. Since then much work has been done on the regulation of gap junction permeability in cardiac tissue and on the macromolecular structure and function of the junctional subunits known as connexins.

In 1951, Draper and Weidmann (36) and Woodbury and colleagues (183) reported the first intracellular recordings of action potentials from Purkinje fibers and ventricular myocytes, respectively. In the early 1960s, Noble used the Hodgkin-Huxley analysis to characterize the electrical activity of cardiac muscle (117) and established the role of inward rectification in the heart (118). In the late 1960s, Noble and Tsien (119, 120) characterized the outward potassium currents in cardiac Purkinje fibers. In 1976, Noma and Irisawa (122) elucidated the ionic mechanisms responsible for normal pacemaker activity in rabbit sinoatrial nodal preparations. Noma (121) discovered in 1983 the existence of a novel potassium channel in the heart that is regulated by intracellular ATP.

Electromechanical coupling. The importance of extracellular calcium in cardiac contraction had been appreciated since Ringer’s classic work in 1883 (142). However, the mechanism of this phenomenon was not determined until 1967 when Reuter (139) used the voltage-clamp technique to show that extracellular calcium contributed to the slow inward current observed during the plateau phase of the action potential. In the following year, the first demonstration that a surface transport protein exchanges sodium for calcium was made in guinea pig atria by Reuter and Seitz (140). In 1972, Fabiato and Fabiato (40) developed the skinned fiber preparation for studying excitation-contraction coupling in cardiac muscle; using this technique, they found that the calcium sensitivity of the sarcomeres and regulation of calcium release from the sarcoplasmic reticulum could be studied in the absence of the sarcolemmal barrier. By 1987, Inui (83) demonstrated a calcium-activated calcium channel in the junctional sarcoplasmic reticulum using ryanodine as an ultrastructural probe. Interest in the regulation of calcium removal from the cytosol was heightened when an ultrastructural probe. Interest in the regulation of calcium release from the sarcoplasmic reticulum could be studied in the absence of the sarcoplasmic reticulum calcium-activated ATPase.

The cardiac cycle. The ability to examine the sequence of events responsible for cardiac pumping was enhanced by the development of the electrocardiogram to follow electrical activity and pressure transducers to monitor mechanical activity. In the early 1920s, Lewis (101) and Wiggers (180–182) provided the first descriptions of the simultaneous electrical and mechanical events of the cardiac cycle. Interest in the minute output of the heart in animals and humans had remained high since the days of Hales. In 1870, Adolph Fick (44) had derived a relationship among oxygen consumption, arteriovenous oxygen difference, and total flow through the lungs that provided a means of calculating cardiac output. At the end of that decade, Forssmann (52) reported the first atrial catheterization in humans. In the 1940s, Cournand and colleagues (27, 28) used atrial sampling and Fick’s principle to measure cardiac output in humans.

Regulation of cardiac performance. At the close of the 19th century, Otto Frank (53) examined the response of the isolated frog heart to distension and found that the maximum systolic pressure generated during isometric contraction was a function of the presystolic ventricular pressure. In 1914, Ernest H. Starling (130, 131), using an isolated heart-lung preparation and an apparatus to monitor the volume of the ventricles, demonstrated that ventricular stroke volume was a direct function of the end-diastolic volume. Subsequent studies by Sarnoff (152) in 1954 revealed modulation of the stroke work-preload relationship with stimulation of the heart by inotropic agents. In 1966, ultrastructural studies of the impact of preload on sarcomere length revealed the fundamental basis of the Frank-Starling mechanism (164). In the same period, Sonnenblick (162, 163) used force-velocity relations for isolated papillary muscle to quantify changes in contractility, a term generally used to specify length-independent changes in force development. More specifically, the maximum velocity of muscle shortening at zero afterload was found to increase when positive inotropes such as norepinephrine were applied to the papillary muscle.

In the 1950s and 1960s, the relative importance of the intrinsic Frank-Starling mechanism and reflex control of cardiac contractility was the subject of intense investigation. During this period, the work of Rushmer (149, 150) on cardiac dynamics in chronically instrumented conscious dogs had a dramatic impact on our understanding of cardiac behavior under a variety of physiological conditions including exercise. With the development of the electromagnetic (90) and ultrasonic (54) flowmeters and implantable probes, continuous measurements of cardiac dimensions and pressures, wall thickness, and several regional blood flows could be made in the same conscious animal.

Vascular Physiology

Hemodynamics. In 1733, Stephen Hales (66), an ordained minister who had passionate interests in both the spiritual and natural worlds, built much of the basic foundation that underlies our current understanding of hemodynamics. In addition to measuring blood pressure in horses and humans, he determined the output of the heart and demonstrated the relationship among blood flow, cross-sectional area, and velocity. Hales also noted that the total cross-sectional area of all daughter vessels exceeded that of the parent artery,
and he proposed that the major site of vascular resistance was in the microscopic blood vessels. Poiseuille (133) developed the U-tube mercury manometer to measure arterial pressure in 1828, and he was surprised to find a negligible pressure drop from the aorta to arteries with diameters as small as 2 mm. In the absence of existing techniques to cannulate smaller vessels, he studied flow through artificial glass capillaries as small as 30 µm (134). Poiseuille found that flow varied directly with the fourth power of tube radius and inversely with vessel length and fluid viscosity.

In 1831, Hall (67) examined the microvasculature and divided the vessels into arterioles, capillaries, and venules; he also stated the criteria for identifying each of the microvessel types. In the early 1960s, Folkow, Mellander, and colleagues (51, 111) further differentiated the peripheral vasculature into series-coupled segments that matched function to anatomic classification: windkessel (aorta and its branches), precapillary resistance (arteriolar), exchange (capillaries), postcapillary resistance (small venules), and capacitance (larger venules and veins) vessels. In 1926, Landis (97) published the first reliable measurements of capillary pressure and found the values in the frog mesentery to be only a few millimeters of mercury higher than those of venous pressure. In the 1960s and early 1970s, Wiedenheil (179), Intaglietta (81, 82), and Wayland and Johnson (177) developed sophisticated electronic instrumentation to monitor micropressures, red blood cell velocity, and microvessel diameter. The widespread distribution of these instruments ignited intense examination of microhemodynamics and provided a means of probing the concepts of vascular control in a direct fashion. In more recent times, pressure profiles from the small artery to terminal arterioles indicate the existence of significant resistance above the level of the largest arterioles, highlighting the potential role of these larger vessels in the regulation of blood flow, especially after dilation of the terminal vessels (15). In addition, the exchange function is now known to extend upstream to arterioles for oxygen transport (136) and to the postcapillary venules for water and proteins (160). Thus functional differentiation of the vascular tree is more complex than originally conceived. In 1963, Permutt and Riley (132) described the vascular waterfall effect whereby extravascular pressure determines the motive force driving blood flow; this concept has contributed to our understanding of the patterns of local perfusion in the pulmonary and coronary circulations.

Capillary exchange and permeability. Three decades after Harvey (71) established the cardiovascular circuit and proposed that the small arteries and veins were connected by “porosities of the flesh,” Malpighi (107) demonstrated the existence of capillaries using a double-convex lens. With better optics, van Leeuwenhoek (173a) was the first person to see red blood cells flowing through individual capillaries of the frog lung. Using silver nitrate to stain the extracellular matrix, Auerbach (4) demonstrated that the capillary wall consisted of flattened endothelial cells. In the 19th century, Ludwig (105) presented his filtration hypothesis, which envisioned fluid movement through the capillary wall driven by the difference between intracapillary and extravascular hydrostatic pressures. In 1896, Starling (166) demonstrated that a transmembrane oncotic pressure differential counterbalanced the hydrostatic pressure difference; imbalances between these hydrostatic and oncotic forces lead to filtration or absorption across the capillary wall. The validity of Starling’s law of the capillaries was confirmed by Landis (98) in 1927. He used the recently developed micromanipulator and sharpened micropipettes to measure capillary hydrostatic pressure and the movement of red blood cells in occluded capillaries to quantify fluid movement across the wall. Using these quantities and the oncotic pressure of plasma, Landis showed that the rate of transcapillary fluid movement was directly proportional to the net difference between transmembrane hydrostatic and oncotic forces. In 1963, Guyton’s (63) finding of a subatmospheric hydrostatic pressure in the interstitium of subcutaneous tissue stimulated additional studies of transcapillary fluid balance, including a greater focus on the physicochemical properties of the extracellular matrix.

The permeability of the capillary wall to water and solutes has received much attention. In 1930, Rous and colleagues (145) demonstrated a gradient of permeability in the microvasculature using water-soluble dyes injected into the circulation; the venous capillaries and postcapillary venules were more permeable than the arterial end of the capillary bed. Drinker and Field (37) examined capillary permeability in whole organs using simultaneous sampling of plasma and lymph. They demonstrated that permeability was an inverse function of solute size. Chambers and Zweifach (23) proposed that water and solutes moved through the intercellular cement located between adjacent endothelial cells. In the early 1950s, Pappenheimer (128, 129) applied the power of physics, mathematics, and biology to the problem of capillary permeability and developed the groundbreaking pore theory. By raising intravascular pressure to maintain constant organ weight after an osmotically active solute was introduced into the arterial inflow, Pappenheimer was able to determine the effective osmotic pressure generated by the solute. Using solutes of different size and a mathematical model describing the physics of water and solute interaction with a cylindrical or slit pore, Pappenheimer predicted the dimensions of putative pores in the capillary wall. The pore model has dominated much of the subsequent work on capillary permeability (172). However, with the advent of the electron microscope, Palade (126, 127) discovered that vesicles or caveolae in the endothelium were capable of transporting materials, especially proteins, across the capillary wall. The relative importance of solute transport through fluid-filled channels or caveolae remains unresolved.

Interest in regulation of vascular permeability was heightened by the demonstration by Majno (106) of...
endothelial cell contraction/retraction in postcapillary venules exposed to inflammatory stimuli. With the introduction of techniques to label proteins and other macromolecules with fluorescent probes (3), leakage of solutes in different segments of the intact microcirculation could be monitored; these intravital studies established the importance of postcapillary venules as a major site for regulation of microvascular permeability (168). The development of quantitative fluorescence microscopy allowed quantification of solute extravasation in single exchange microvessels perfused in situ (79) or after isolation (186). Moreover, fluorescent dyes sensitive to calcium, voltage, and other physiological variables aided in probing key signaling pathways. These single-vessel studies demonstrated important roles for intracellular calcium (124) and nitric oxide (NO) (187) in signaling of venular hyperpermeability.

Intrinsic regulation of blood flow. The concept of a link between local blood flow and tissue metabolism is relatively new. In the 1870s, Gaskell (57) and Roy and Brown (146), and in the early part of this century Hooker (77), speculated on the possible role of tissue metabolites in reactive and functional hyperemia. The coupling between the vasculature and skeletal muscle metabolism during contraction was examined by Krogh (93, 94) in 1918. He discovered that the number of perfused capillaries increased after the onset of contraction and concluded that this reaction served to facilitate oxygen transport to hypermetabolic muscle cells. Krogh envisioned an arteriomotor reaction responsible for increased blood flow and oxygen delivery to the capillaries and a capillaromotor response capable of adjusting oxygen diffusion parameters such as capillary surface area and capillary-to-parenchymal cell diffusion distance. The metabolic view of local vasoregulation reached its zenith in the last third of this century with Berne's (13, 148) adenosine hypothesis. Adenosine, a powerful vasodilator and breakdown product of ATP hydrolysis, accumulates in the interstitium when ATP levels in parenchymal cells fall. Thus this chemical linkage between vascular tone and parenchymal metabolism provides an elegant system for regulating local blood flow in accordance with tissue oxygen demand. In 1902, William Bayliss (10), brother-in-law and colleague of Ernest Starling, discovered that blood vessels respond to distension by contracting with greater vigor, leading to lumen narrowing and greater vascular resistance. The Bayliss or myogenic response represented another mechanism for local control of blood flow. In addition, this pressure-dependent reaction of resistance vessels provided an explanation for the high intrinsic tone found in the arterioles. The Bayliss response received little attention until Folkow (48–50) and Johnson (86, 87) used the concept to explain autoregulation of blood flow in skeletal muscle and intestine. In 1968, Baez (5) provided the first direct evidence of myogenic vasoregulation in the intact microcirculation. In the same decade, Mellander (112) proposed that an important physiological role of the myogenic reaction is stabilization of transcapillary fluid movement through regulation of capillary hydrostatic pressure. The first insight into potential mechanisms underlying the myogenic response was the finding that isolated arteries subjected to distension exhibit depolarization (69). With the development of the isolated perfused arteriole in 1981 (38), the stage was set for a more careful examination of the cellular basis of the Bayliss response. In isolated coronary (95) and skeletal muscle (41) arterioles, the pressure-dependent constriction is an intrinsic property of the vascular smooth muscle cells alone; in addition, increased distension pressure leads to a rise in cytosolic calcium of smooth muscle cells of the arteriolar wall (110).

With the realization that arterial vessels larger than arterioles make a significant contribution to total vascular resistance in many organs, the question arises as to how these upstream vessels receive the message to dilate during hyperemia initiated at the arteriolar level. Schretzenmayr (155) reported in 1933 that increased flow through an artery causes the vessel to dilate. In a landmark paper published in 1980, Furchgott (55) demonstrated the capacity of endothelium to modulate the contractile state of surrounding vascular smooth muscle. A few years later, Ignarro (80) identified the endothelium-derived relaxing factor as NO. In vascular smooth muscle, cGMP inhibits myosin light chain kinase, leading to relaxation. Because NO production by endothelial cells is proportional to shear rate (147), the mechanism of flow-dependent vasodilation of arterial vessels was revealed. The link between shear rate and NO production appears to involve the activation of a shear-sensitive potassium channel that mediates endothelial hyperpolarization (123), calcium entry from the extracellular fluid, and calcium-dependent activation of NO synthase. Shear-induced vasodilation extends into the arteriolar tree as well (96). Another means of signaling upstream vessels to dilate when terminal arterioles relax is by electrical coupling of vascular cells; thus the vascular bed acts as a functional syncytium. In 1967, Rhodin (143) showed the existence of close appositions between adjacent smooth muscle cells, as well as myoendothelial junctions. In recent years, propagation of vasodilation initiated in small arterioles to larger upstream segments has been demonstrated (156).

Integrative Cardiovascular Physiology

Reflex control of the circulation. Overall regulation of the cardiovascular system requires neurogenic and endocrine mechanisms superimposed on intrinsic controllers of the heart and blood vessels. In 1866, deCyon and Ludwig (33) showed that stimulation of the aortic depressor nerve caused a reflex bradycardia and arterial hypotension, providing testimony to the ability of afferent nerves to influence the behavior of the cardio-

1 R. Furchgott, F. Murad, and L. Ignarro share the 1998 Nobel Prize in Medicine or Physiology.
vascular system. In 1903, Köster and von Tscharner (92) found that the frequency of action potentials measured in the depressor nerve increased with distension of the aorta. The carotid baroreceptors were discovered in 1923 by Hering (73). Subsequently, Bronk and Stella (18) established the proportional relationship between carotid sinus baroreceptor firing rate and distension of the vessel wall. The ability of the baroreceptors to adapt was demonstrated by Heymans (75) in 1950, using vasoactive agents applied to the baroreceptive surface. In 1957, McCubbin et al. (109) found resetting of the baroreceptors in chronic arterial hypertension, suggesting adaptation to chronic distension.

The concept of mechanoreceptor reflexes initiated in the atria was introduced by Bainbridge (7) in 1915. In 1956, Henry, Gauer, and Reeves (72) provided evidence for the atrial location of receptors influencing urine flow, suggesting a reflex mechanism for regulation of blood volume. The following year, Baissset and Montastruc (6) demonstrated that the renal effect was due to a reflex reduction in circulating levels of antidiuretic hormone. A more direct link between atrial distension and cardiovascular functions involving release of natriuretic proteins from granules stored in atrial myocytes was uncovered by Delhaut (32) in 1981.

In 1933, Heymans (74) discovered the chemoreceptor reflex and the ability of receptors located in the carotid sinus and aortic arch to monitor the chemical composition of arterial blood. A reflex arterial hypertension and cardioacceleration arising from nerve endings in skelatal muscle of humans were demonstrated by Alam and Smirk (1) in 1937. Mitchell (113) revisited this issue in 1977 and subsequently demonstrated the ability of muscle afferents to respond to the metabolites released from contracting muscle.

Claude Bernard (12) presented evidence for the existence of vasomotor nerves in 1851. Two years later, Schiff (154) sectioned the brain stem and spinal cord to show that neurogenic vasomotor activity originated from higher centers of the central nervous system. In 1908, Bayliss (see Ref. 9) proposed the existence of a vasomotor center that orchestrated vasoconstrictor and vasodilatory influences through efferent nerves. In the middle of this century, Shipley and Gregg (159) and Randall and colleagues (138) delineated the efferent pathways projecting to the heart. In 1946, Alexander (2) stressed the tonic and reflex functions of the medullary cardiovascular center. More recently, the loci of cardiovascular integration in the central nervous system have been extended from the medulla upward to hypothalamic, limbic, and other forebrain regions, as well as downward to the spinal cord (91).

Hormonal control of the circulation. The existence of a vasoconstrictor system dependent on the kidney was suggested by the studies of Goldblatt (60), who showed that impairment of renal perfusion led to arterial hypertension. In 1898, Tigerstedt and Bergman (173) discovered renin. Forty years later Braun-Menendez (17) and Page (125) independently identified angiotensin as the product of renin action.

Overall regulation of the circulation. The interactions of the heart and blood vessels required to achieve cardiovascular homeostasis are complex and difficult to conceive and explore without the help of conceptual and mathematical models. In 1955, Guyton (62a) developed a graphical analysis based on simultaneous plots of cardiac function and venous return curves. The point of intersection of the two graphs represented the steady-state level of cardiac output on the y-axis and the mean atrial filling pressure on the x-axis. The impact of neurogenic and hormonal influences on each of the cardiac function and venous return curves allowed the determination of the overall impact of the specific disturbances on cardiovascular behavior. In 1967, Guyton and Coleman (64) proposed a model to integrate the interactions of the heart, blood vessels, and kidney in the long-term regulation of arterial blood pressure. The concept emphasized the role of the kidney as the primary determinant of the arterial pressure over the long term. Later, intrinsic and extrinsic regulation of blood flow in different vascular beds, baroreceptor and chemoreceptor reflexes, and hormonal influences were added to the basic renal-body fluid loop to help predict cardiovascular responses to different perturbations (65).

Molecular and Cellular Basis of Cardiovascular Functions

Several developments in the second half of this century led to a shift in focus toward the cellular and molecular bases of cardiovascular functions. First, techniques for isolating and culturing cardiac and vascular cells were perfected. Second, advances in our understanding of cell signaling pathways provided a conceptual foundation for investigations of cardiac and vascular cell responses to specific disturbances of their physical and chemical environment. Third, new developments in light microscopy allowed optical monitoring of a wide variety of physiological variables in living cells in space and time. Simultaneously, the explosive growth in light and EM immunocytochemistry made it possible to precisely locate tagged antibodies to specific proteins in fixed cells and to examine the possibility of colocalization of any two proteins. Fourth, voltage- and patch-clamp technology fostered detailed analysis of the ion channels that govern electrical activity in cardiovascular cells. Finally, the revolution in molecular biology provided the tools to probe the impact of gene overexpression, deletion, and modification on cultured cardiac and vascular cells and the cardiovascular system of the intact animal. In this brief review, I present some examples of the power of cell and molecular biology as applied to problems in cardiovascular physiology.

Cardiac cell biology. In 1912, Burrows (21) observed single beating cardiac myocytes migrating from the edge of tissue isolated from the embryonic chick heart. The pulsation, migration, and division of chick embryo heart cells dispersed by trypsin digestion was reported in 1955 by Cavanaugh (22). In 1960, Harary and Farley (68) reported the organization of single beating cardiac myocytes isolated from postnatal rat hearts into beat-
ing fibers. Claycomb (26) cultured cardiac myocytes in serum-free medium in 1981, and the same year Dow (35) successfully isolated viable cardiac myocytes from the adult heart using enzymatic dispersion.

To date, the genes that encode for calcium, potassium, sodium, and chloride channels in cardiac muscle have been cloned (178). In addition, several ion transporters in cardiac sarcolemma and sarcoplasmic reticulum have been cloned (178). The insertion of these cloned genes into bacterial or viral DNA and the subsequent transfection of Xenopus oocytes or cardiac myocytes to overexpress the channel allow detailed electrophysiological analysis with patch-clamp techniques under conditions that overcome the problem of low channel density. Alternatively, the protein product of the cloned gene can be inserted into liposomes to examine channel behavior in the absence of the complexities of intracellular signaling. The rate at which information on these ion channels and transporters is accumulating is so rapid that several groups have developed mathematical models to integrate the findings and explore the physiological implications for the intact cardiac myocyte (99, 103).

Freshly dispersed myocytes or contractile proteins isolated from the myocardium played a major role in helping to elucidate sarcomere dynamics and the functions of each component of the contractile machine. Optical techniques for monitoring sarcomere length coupled with force measurements in single cardiac myocytes have provided insight into cardiac contraction at a level of precision that had not been possible with the papillary muscle preparation (184). With the use of surfaces coated with myosin, interactions between myosin and actin can now be observed by monitoring the motility of actin filaments added onto the myosin layer (70, 185). The impact of different chemical modulators or genetic modifications on the motility of the actin filaments provides insight into the maximum velocity of shortening. To probe force development in this environment, laser beams are used as optical tweezers to prevent the movement of the actin over the myosin layer; the energy required to prevent motion provides an index of maximum isometric force. The data from the motility assay are used to examine the dynamics of cross-bridge cycling.

Another powerful approach centers on modifying specific amino acids of individual contractile proteins using transgenic mice (144). Site-directed mutagenesis has been employed to examine the role of myosin, actin, troponin, and tropomyosin subunits in cardiac contraction and relaxation. The impact of the mutations are examined with sonocardiography in the intact mouse, the isolated perfused heart, and individual cardiac fibers or cells. In reverse fashion, gene hunting in human populations is based on identifying the genetic locus of cardiac abnormalities in conditions such as familial hypertrophic or electrogentic disorders using the family tree and the genetic record of the individuals composing the tree (11, 58). Several groups have used results from gene hunting in humans to generate similar genetic abnormalities in the mouse, thereby achieving a process for deeper exploration of basic pathophysiological mechanisms.

Vascular cell biology. Before the modern thrusts in understanding atherosclerosis and inflammation and the discovery of endothelium-dependent control of blood vessel caliber, studies in cell biology of the cardiovascular system focused mainly on the heart. Today, vascular cell biology has emerged as a robust entity that attracts the scientific efforts of pathologists, physiologists, pharmacologists, geneticists, morphologists, and biochemists. The number of papers on vascular endothelium and smooth muscle now match or exceed those examining cardiac functions. The structure of smooth muscle is very different from striated muscle; indeed, contractile elements are less organized, attach to the sarcolemma, and run diagonal to the long axis of the myocyte (42). Freshly dispersed vascular smooth muscle cells are used to determine the electrophysiological basis of vasoregulation and the intracellular signaling pathways that modulate cytosolic calcium and contractile functions. From the perspective of local control of blood flow, recently characterized ion channels are relevant. First, in single isolated vascular smooth muscle cells, extension leads to activation of a nonspecific stretch-activated cation channel that conducts calcium and sodium (31), providing a membrane mechanism for explanation of the Bayliss response. Second, the existence of an ATP-sensitive potassium channel in vascular smooth muscle may help explain the effect of hypoxia on vessel tone (84, 170). Calcium imaging in vascular smooth muscle cells has demonstrated global transients and localized areas of calcium release and membrane translocation, implying a more complex spatial distribution of intracellular signaling processes (116). Calcium modulates vascular myocyte contraction through a calcium/calmodulin-dependent activation of myosin light chain kinase (167). Sustained contraction of vascular muscle cells is maintained by slow cycling of cross bridges (i.e., the latch state), which accounts for the low oxygen requirements of the contracted cells (34).

Cultured vascular smooth muscle cells (24, 59) are used extensively to investigate mechanisms of cell proliferation and migration induced by chemical (175) and physical (20) stimuli. Many chemical mediators once thought to act exclusively as vasoconstrictors have been shown to modulate vascular muscle cell growth as well (14). Moreover, components of the basement membrane and extracellular matrix known to regulate proliferation now appear to have a significant impact on vascular tone (29). Thus previous notions of the actions of specific vasoactive agents need to be revised to include multiple effects.

The revolution in vascular cell biology has to a large extent been led by dramatic advances in our understanding of vascular endothelium. Not long ago, the endothelial monolayer of the vascular wall was viewed as a simple nonthrombogenic surface and passive filter. In 1963, Maruyama (108) isolated human umbilical vein endothelial cells using trypsin digestion, but these cells
failed to replicate and could not be passaged. In the same year, Pomerat and Slick (135) isolated and passaged endothelial cells from the rabbit aorta that were amenable to serial passage for a year. However, at high passage, spindle-shaped cells overtook the culture. A decade later, Jaffe (85) used collagenase to disperse human umbilical vein endothelial cells, and they succeeded in generating a pure culture capable of serial passage. In 1975, Booyse (16) applied Jaffe’s approach to bovine aorta. In the same year Wagner and Matthews (174) isolated and cultured capillary endothelial cells from rat epididymal fat pads. In 1979, Folkman and colleagues (46) achieved a long-term culture of capillary endothelium from human adrenal cortex. Using a different strategy, Ryan and colleagues (151) developed a bead perfusion technique to isolate endothelial cells from microscopic blood vessels of the lung.

Freshly dispersed and cultured endothelial cells have been utilized to investigate a large number of issues in the nascent field of vascular biology. Our understanding of inflammation, atherosclerosis, angiogenesis, capillary permeability, and endothelium-dependent vascular reactivity has evolved more rapidly due to the availability of these cells. For example, the ability to create continuous endothelial monolayers to mimic the inner lining of the blood vessel allowed detailed analysis of the molecular and cellular basis of leukocyte adhesion to, and migration across, the vascular wall (56, 78). Deletion or overexpression of genes encoding specific adhesion molecules directed to the surface of the endothelial cell or leukocyte during activation helped clarify the role of each adhesion molecule at different times during sticking and diapedesis (157). Techniques such as atomic force and tandem confocal microscopy provided clear evidence that the basal and apical surfaces of the endothelial monolayer are dynamically interacting with the basement membrane and flow stream on the opposite surface (8, 30). For example, focal adhesions are continuously forming and disengaging at the cell-to-matrix interface, although the average adhesion force across the entire surface remains relatively constant. From studies of endothelial monolayers, a compelling hypothesis has emerged that views vascular hyperpermeability as an imbalance between retractive forces that tend to cause cell rounding and adhesive forces that act to maintain a flattened morphology (114). From this perspective, inflammatory mediators cause increased capillary permeability by either enhanced cytoskeletal activation, reduced integrin-to-basement membrane interactions, or diminished cell-to-cell adhesion as a result of the decreased number of cadherin binding sites.

Interest in angiogenesis was greatly stimulated by the discovery of tumor angiogenesis factor by Folkman (47) in 1971. Cultured endothelial cells provide a valuable tool to probe the impact of growth factors on the various stages of angiogenesis, including detachment from the basement membrane, migration, proliferation, and tube formation. Vascular endothelial growth factor is of special interest, because its production in various cell types is increased when exposed to low oxygen tension (62). Moreover, vascular endothelial growth factor receptors are unique to endothelial cells (43) thus providing a mechanism for targeting growth of the vasculature per se. In recent years, several angiogenic and antiangiogenic factors, as well as their receptors, have been isolated and cloned (61). The excitement generated by these findings reflects the potential to use this knowledge to develop clinical strategies for controlling cancer and chronic ischemic disorders.

Developmental cardiovascular biology. In the past decade, great advances have been made in our understanding of the formation of the cardiovascular system in the embryo. The use of the zebrafish as a key model in these types of studies emphasizes the value of comparative biology (165). With the use of this model, genes have been identified that determine the orientation and form of the cardiac tube, electrical conduction in the heart, and formation of the major arteries. Similar studies have been conducted on mice embryos with specific gene deletions (144). For example, a transgenic mouse with no ability to produce the vascular endothelial growth factor receptor flk1 fails to develop a normal vasculature, supporting an important role for this growth factor in vasculogenesis (158). These advances in developmental biology are likely to improve our capabilities in cardiovascular tissue engineering for clinical use, as well as to provide greater insights into restructuring the heart and blood vessels in the mature organism.

Missed Opportunities: The Consequences of Parochialism

Today the tools and concepts of molecular biology are being rapidly assimilated into the armamentarium of the cardiovascular physiologist. However, this assimilation was delayed for at least a decade due to a number of factors. In general, the primary focus of the cardiovascular physiologist during the first half of this century was on physical techniques of cardiovascular investigation. With the development of physiological chemistry into the separate discipline of biochemistry, less emphasis was placed on uncovering the chemical basis of cardiovascular functions within a physiological setting. The predilection for physical analysis is supported by the close ties that remained with the biophysics and bioengineering communities as these fields became more independent of physiology.

The isolation of physiology from biochemistry precluded the timely integration of molecular biology into the continuous search for the basic chemical and physical principles underlying cardiovascular behavior. Moreover, the traditional organization of biomedical sciences along these discipline lines created barriers to rapid exchange of techniques and concepts between the disciplines. As a result of this situation, traditional physiologists initially viewed the evolving dominance of molecular biology as a threat rather than as an opportunity. By contrast, cardiovascular scientists in clinical departments were more receptive to the molecular revolution.
because their focus was problem based rather than discipline oriented. Moreover, in departments of internal medicine, where cardiovascular molecular biology initially took flight, cardiologists were in intimate contact with oncologists and other specialists who stood at the leading edge of the new biology.

As the center of gravity moved toward molecular and cell biology, the need for application of the techniques of this field to physiology gradually became compelling, and the composition of physiology departments changed rapidly. However, in human endeavors as in physiological systems, time delays lead to oscillation, and the proper balance between molecular and systems approaches remains elusive. Today as functional genomics becomes the new horizon in physiology and molecular biology, the availability of cardiovascular scientists with expertise in systems and whole animal physiology is limited. A strong argument can be made that this situation arose from the initial isolation of the physiology community from the mainstream of events driving the revolution in molecular biology.

The Future of Cardiovascular Research

There are lessons embedded in the history of cardiovascular physiology during the 20th century. The first and foremost is the need to return to the broad definition of physiology as the field of science that examines the chemical and physical basis of life processes. Arguments regarding the relative value of reductionist versus integrative approaches are counterproductive, because an understanding of the parts is required to illuminate the behavior of the whole. Second, cardiovascular science must stand in the stream of ideas that receives nourishment from all the traditional and nascent disciplines. Carl Ludwig, one of the greatest and most prolific physiologists of the 19th century, designed his Institute of Physiology with three fundamental components: a physiology unit in the center flanked by histology and biochemistry divisions. Similar patterns can be seen in the scientific approaches of Claude Bernard, Ernest Starling, and August Krogh. Today, a complete understanding of cardiovascular functions requires input from biophysics, biochemistry, pharmacology, structural biology, genetics, bioengineering, and other fields. To some extent, the era of the discipline-based organization of the biomedical sciences is inconsistent with the revolution in biology that has provided a common conceptual foundation now shared by all disciplines. The thematic and problem-based approaches are likely to dominate in the future, as evidenced by the current proliferation of multidisciplinary faculties and institutes. Third, we must train graduate students to develop a broad view of cardiovascular science that spans from molecular biology to systems physiology and fosters an appreciation for human biology as a whole. To achieve this goal, courses in the basic medical sciences will have to be totally reorganized to break down the barriers inadvertently created by the traditional disciplinary approach. Furthermore, the widespread availability of sophisticated technology to probe the functions of the heart and blood vessels in a clinical setting presents an important opportunity to tap a new resource for instructing graduate students and postdoctoral fellows in human cardiovascular physiology and pathophysiology. Finally, greater integration of basic sciences and clinical disciplines is expected to occur with an increased focus on thematic or problem-based organization of the entire medical enterprise. In the future we are likely to see a general trend toward placing cardiovascular scientists from basic and clinical disciplines under a single administrative entity. This approach will facilitate the translation of basic research into clinical strategies for care of patients with cardiovascular disease.

A central theme in any survey of physiology is the requirement for technological developments to facilitate breakthroughs in our understanding of human biology. A thorough examination of major advances in the past century supports this notion. Yet, as our ability to generate information increases in exponential fashion, there are signs that conceptual and theoretical developments are lagging behind the explosion in facts. For example, microarrays allow the simultaneous analysis of expression levels of thousands of genes (153). Changes in expression of these many genes over time can be monitored for specific physical or chemical stimuli. How will this information be used to enhance our understanding of the functions of the intact vessel, heart, or cardiovascular system? With necessity as a driving force, a new theoretical foundation for integrating information from the molecular and cellular through organ and system levels will emerge in the next century. The solution to this problem will require input from mathematicians, computer scientists, engineers, and biologists.

In summary, the history of cardiovascular physiology provides testimony to the ability of the human mind to reveal nature’s secrets and to use this knowledge to improve the human condition. Great strides were made in the 20th century with a primary focus on the tissue, organ, and system levels of cardiovascular integration. In the last quarter of this century, the emphasis has been on the molecular and cellular bases of cardiovascular functions. At the dawn of the new millennium, there are important signs pointing to another major shift in the fundamental approach to cardiovascular research and training. A renewed emphasis on the multidisciplinary approach that served the early masters so well will liberate cardiovascular science from the confines of the discipline-based paradigm.

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