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Five Examples Illustrating the Study of Form and Function

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INTRODUCTION

Observations of form, in humans and other animals, began in prehistoric times and must have been associated, often or always, with speculations about living activities and perhaps about life itself. Such associations seem to cast light on the careful representation of the body and limbs, and especially of the heart, in the cave paintings of Paleolithic times. Knowledge of anatomy and primitive ideas about illness and healing—the lore of function applied—were definitely formulated in ancient times and codified by Greek and other early scholars including Aristotle, Hippocrates, and Galen. And it is true today, as well, that even the most uninformed person has sometimes made observations on, and felt concern about the structure and function of his or her body. Form and function, then, are areas of deep and intrinsic interest to people. The powerful methods of science have extended our knowledge and produced a great array of new questions, and new ways for seeking answers. In the discussion to follow, five specific examples will be developed as background for an approach to present knowledge of form and function, but also as reminders of the problems that beset early workers, and are still with us today as we try to reach a comprehensive understanding of this central area of biological science.

Clearly, one of the greatest obstacles to understanding form and function is the extreme complexity of biological processes and organization, as compared with any other aspect of the natural world. Function, in particular, is often hard to analyze. Whereas simple and direct observations early yielded solid knowledge of morphology, especially of human anatomy, little real progress could be made in understanding physiology until a firm basis of physics and chemistry had been established. Biological systems are not only intricately organized but are also acutely sensitive to disturbance, and this, too, made difficult the analysis of function. And a further obstacle to real understanding, throughout the history of our species, has been the development of frameworks of ideas, or dogmas—sometimes based on religion or mysticism, sometimes on speculation and logic—which inhibit further exploration. New ideas must not only be validated on their own merits, but must also fight for a place in such established frameworks. This is illustrated in the following examples, as in William Harvey's opposition to the Galenic tradition, and in Karl Ludwig's opposition to the pervasive concepts of vitalism. Science is a way of knowing, not a structure of solid, immutable facts but rather a growing, changing system of observations, hypotheses and concepts constantly subject to experimental test, refinement, possible rejection, and progress in new directions. As the concepts and techniques of mathematics, physics and chemistry continue to evolve with time, their new developments must be reflected by changes in biological science as well. We are only beginning the process of realigning biological science with respect to current advances in the physical sciences, and inevitably great changes will occur in the problems, approaches, and structure of biology in the course of the next few decades.

Perhaps it would be well at this point to consider explicitly the appropriateness of developing our central theme, science as a way of knowing, in the context of the historical background of biological science. Since this program is directed primarily at the problems and challenges of teaching students in introductory biology courses at the college level, the interests of these students are of prime concern. And most instructors will agree, in all probability, that first year biology students are not capti-

vated by the history of the subject. The conscientious professor may present a carefully structured first or last lecture "encapsulating" the sweep of biological thought from Aristotle to the present day. Generally, this is seen by students as just another mass of facts to be stored in short-term memory until examination time. Nevertheless, in biological science as in all other aspects of human knowledge, history has many important lessons for the present. Reverting to ideas proposed years ago by the great chemist and teacher James B. Conant (1893–1978) we suggest that a few case histories—which are here called examples—can illustrate major aspects of science as a way of knowing against the background of knowledge developing through the last few centuries, and before scientific understanding had grown to the immense, complex, and awesome dimensions of its present state (Conant and Nash, 1948–1957).

Needless to say, such a treatment of individual examples cannot be comprehensive. In giving a reasonably brief account of specific discoveries, we must omit reference to the vast majority of scholars, scientists, and physicians who made basic discoveries and influenced the way of thinking of their contemporaries and successors. For example, in developing the background of William Harvey's discovery of the circulation, detailed reference is not made to the beautiful work of Andreas Vesalius (1515–1564—Moore, p. 523) who described and illustrated human anatomy with correction of certain Galenical errors. So a generalization permeates this section—that each of the scientists whose work is discussed drew from the past as he needed to, just as he contributed to the future such of his work as would endure (Foster, 1901; Franklin, 1949).

The basic problems of flow and function of blood that William Harvey addressed in the 17th century were the same that puzzle and motivate today's heart surgeon or circulation physiologist. People had struggled to solve these problems since before recorded history, and the consistent structure of Galenical tradition seemed, for a thousand years and more, to provide adequate or at least useful answers to their questions. Harvey's great contribution was to return to the basic questions and to apply to them the methods of science as a way of knowing—observation leading to hypothesis, to experiment, to rejection or refinement of hypothesis, to further experiments and so on. His formulation of the occurrence of circulation was the first real step in modern scientific study of function in relation to form.

Two centuries later Claude Bernard, recognized by many as the greatest of French physiologists, made many specific contributions to the field. More importantly, perhaps, he represents for us today a scientist who contributed significantly to the conceptual basis for the study of function by his development of the ideas of an internal environment in metazoan animals, and its precise regulation.

The third and fourth examples to be presented here continue to emphasize the point that consideration of structure is central to the discovery of important aspects of function. These examples are: (1) Processes of excretion and conservation of water by the kidneys of mammals that enable these animals to live in arid places on earth. (2) Processes of oxidative phosphorylation that make it possible for eukaryotic cells to extract maximum energy from nutrient sources.

Finally, the last example (3) concerns a general phenomenon—not only do biological processes depend on the structure of living material, but also it is of the greatest significance that organisms characteristically build non-living structures about themselves, and their lives are dependent on these external structures. The specific example presented here is the form and function of the eggshell of birds, but equally useful discussions could be developed about the carefully structured, function-determined building of calcareous or siliceous shells by protozoans and molluscs, of coral reefs, bee hives, skate egg cases, and a myriad of other external constructions of living organisms.

The central concept which emerges from the study of structure and function is that they are not separate though related, but

rather aspects of one and the same biological organization. Morphology—the study of structure per se—emerged as a valid discourse long before it was possible for observers to obtain real understanding of function. Thus there came to be a working practice dividing these two aspects between different disciplines—morphology (anatomy) and physiology. But in truth neither is valid as it stands alone. The crucial dependence of function on structure is obvious from the outset. No muscle contraction can occur without the muscle to contract. But structure may seem to persist as at least a relatively stable attribute of organisms even after death, the termination of function—a dead rabbit looks much like a living rabbit. Strikingly, this is not true at the level of cells and organs. The organization of cell membranes, mitochondria, and subcellular structures in general begins to dissipate as soon as the cell's energy sources and controls are interrupted. This is true of all living forms, but occurs especially rapidly in the case of organisms with fast metabolism, such as ourselves and other mammals. Thus the great English physiologist J. S. Haldane (1860–1936) wrote:

> . . . examination shows us that death is no mere stoppage of an engine owing to lack of air or fuel, but also total ruin of what we took to be the machinery. It is a mysterious dissolution in the association together of the infinitely complex normals which constitute . . . the life of an organism; and an examination of the fragments left has thrown no light on why the association should have existed at all, or endured so long (Haldane, 1922).

Even in the case of organs and tissues such as skin, muscles, and bones which persist apparently intact after death, when this occurs details of structure fade out. It would be impossible to know, from the dead bone in hand, that this was an active, dynamic structure building and remodelling continuously during the course of life. Nor would the dead skin be recognized as having, as it did in life, the rapidly fluctuating blood supply, and layers of cells actively dividing to replace the surface during use, and migrating, dividing, and repairing after injury. We who are charged with teaching biology to students should be constantly alert to the error of separating, by our manner of presentation, structure and function. Each of these aspects of biological organization loses its source of reference and much of its interest and validity when seen without its complement.

References

Conant and Nash (1948–1957), Foster (1901), Franklin (1949), Haldane (1922).

WILLIAM HARVEY AND THE DISCOVERY OF CIRCULATION

Any system of explanations must make use of accumulated observations and experience, and the Greek, Roman, and Islamic physicians and scholars who were the chief source of ideas of human anatomy and function had dissected human bodies and had made many observations of critical aspects of life. Life depended on air, food, and water. Warmth of the body—the presence of heat—was a key to life. When blood poured from a wound, death could soon ensue. These and many other observations were woven, in time, into elaborate explanations of form and function, and systematized by the eminent Greek physician Galen of Pergamum and Rome (*ca.* 129 to 200 A.D.). Galen's teachings, accepted and authorized by the Church, dominated Western medicine as long as 1,500 years later (see Moore, pp. 502–505). As Galenical doctrines were repeated, taught, argued about, and set down in treatises, they were altered and elaborated to become a fully coherent and convincing account of bodily structure and function. By the beginning of the 17th century, when William Harvey undertook his studies, according to Frank (1980, p. 3)

> The physiological concepts embedded in the Galenical anatomical tradition are best seen in two texts widely used throughout Europe in the early seventeenth century, Andre du Lauren's *Historia anatomica humani corporis* (1600) and Caspar Bauhin's *Theatrum anatomicum*

> (1605). Both were certainly well known to Harvey. . . . Traditional physiology perceived the function of the human body as tripartite, based upon the principal cavities ('venters' or 'bellies') observed in dissection—the abdomen, the thorax, and the head. Each venter had its primary function and dominant organ, which exerted its effects through a system of vessels containing an appropriate fluid.

Briefly, the three fluids associated with the body cavities were: (1) Venous blood, formed in the liver from nutrients drawn from the stomach, and flowing by the hepatic vein into the great vena cava for delivery through veins to all parts of the body. This venous blood also contained wastes, drawn off and discarded from the body in bile and urine. Thus it subserved the "natural" (or supportive) functions of the body—nutrition and excretion, in addition to reproduction. (2) Arterial blood carried the vital spirits, essential for life, from the heart to every part of the body. The function of the heart was complex. In the left ventricle there originated heat (innate heat, *calidum innatum*). Blood was driven from the heart in systole to the lungs, where excess heat was discharged and air taken up. Air mixed with blood was sucked back to the heart (by expansion of the heart in diastole) where the motion and heat worked on the air and blood to produce the vital spirits contained in the bright red arterial blood. This was delivered through the arteries, bringing heat and life to all parts of the body. In some regions of the body, and in animals without lungs, the pulsing of the arteries could draw air directly through the skin in the formation of vital spirits. (3) The third fluid, animal spirits, was formed in the brain and carried throughout the body by special tubes, the nerves. The flow of animal spirits was necessary for movement and sensation, functions uniquely characteristic of animals.

At this point it may be well to pause to consider the merits of the Galenical tradition as transmitted and of course somewhat modified by generations of physicians and professors. It provided a rational framework for explanation of the many observations that could be made by a thoughtful person without any of the concepts or tools of modern science—that may even be quite familiar to a student in a first-year biology course. It could provide ready answers to questions such as these: Why is breathing necessary for life? Why does the heart beat? Why, when a tight band is bound about the arm, does the hand grow pale, then cold and numb? Why does an unfortunate person lose blood rapidly through a severed artery and, if the artery is large, soon die? Why, in fact, is there so close a link between blood and life? What is the source of the heat of the body? Why does damage to the brain cause loss of motion, sensation, and even of life itself? These are questions based on relatively common events, and the Galenical doctrines went far beyond, giving background for explanations of a myriad of specific problems and questions that arise, even rarely, in the course of birth, health, illness, injury, and death. This doctrine gave confidence in coping with the horrifying events of real life. Ailing or wounded kings and commoners alike were treated by physicians on the principles of disturbed humors and the spirits animal, natural, and vital. Today, in the knowledgeable 20th century, we may look with complacency at the obvious fallacies and imaginative extravagances of Greek, Roman, Islamic, and early European doctrines. Nevertheless, they developed into a powerful and consistent framework for the scholar's thought and the physician's action. They were fully accepted and indeed strongly and even harshly enforced by both religious and lay teaching.

William Harvey, by his remarkable work establishing that blood circulates, destroyed the central consistency and power of the Galenical tradition. He is recognized today as the founder of modern physiology. He was born in Folkestone, England, in 1578 during the reign of Queen Elizabeth I. After study at Cambridge and in Padua, where he heard lectures by the great anatomist Hieronymus Fabricius, he returned to England and began practicing medicine. He was appointed physician at St. Barthol-

omew's Hospital in London, and lecturer in the College of Physicians. In due course Harvey was named court physician, serving both King James I and King Charles I. He was a vigorous investigator and lecturer, as well as physician. In 1628 he published his great work *Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus* (*Anatomical Studies on the Motion of the Heart and Blood in Animals*) (Fig. 1).

Trapped in the chaos and tragedy of the Civil War (1642 to 1646), Harvey lost much of his scientific work. After the War ended he lived in retirement, but before his death in 1657 he published two important works. *De Circulatione* (1649) extended his ideas on the circulation, and *De Generatione Animalium* (1651) was a treatise on development (SAAWOK—IV, pp. 19–22).

The questions William Harvey set out to answer, and his purpose and methods of seeking answers are spelled out clearly in *de Motu Cordis.*

> In discussing the movements and functions of the heart and arteries, we should first consider what others have said in these matters. . . . Then by anatomical study, repeated experiment, and careful observation we may confirm what is correctly stated, but what is false make right (p. 7; all quotations are from the 1941 edition of the translation of *de Motu Cordis* by C. D. Leake).

Among the questions which Harvey posed were these: What was the actual structure of the heart, and what were the functions of the movements that it made—systole (contraction) and diastole (expansion)? How did blood flow in the arteries and veins? What part did the lungs play in the flow of blood? Did the heart and the arteries beat simultaneously and independently?

> When I first tried animal experimentation for the purpose of discovering the motions and functions of the heart by actual inspection and not by other people's books, I found it so truly difficult that I almost believed with Frascatorius, that the motion of the heart was to be understood by God alone. I could not really tell when systole or diastole took place, or when and where dilatation or constriction occurred, because of the quickness of the movement. In many animals this takes place in a twinkling of an eye, like a flash of lightning. Systole seemed at one time here, diastole there, then all reversed, varied and confused. . . . Finally, using greater care every day, with very frequent experimentation, observing a variety of animals, and comparing many observations, I felt my way out of this labyrinth, and gained accurate information. . . . The path is open for others, starting here, to progress more fortunately and more correctly under a more propitious genius (pp. 25–26).

FIG. 1. William Harvey in 1628. From *de Motu Cordis,* translation by C. D. Leake.

The treatise *de Motu Cordis* has two major parts. In the first part Harvey questioned the function of the heart. He saw that the contractions of the heart chambers were not simultaneous, but that the auricles (atria) contracted first, so blood flowed into the ventricles, and that thereafter the ventricles contracted, driving blood into the pulmonary artery and aorta. Thus the action of the heart when it expanded was not to dilate and suck blood into it, as gen-

erally supposed. Rather, the active movement of the heart was contraction, like a muscle, decreasing the size of its chambers and driving blood from it. The valves at the junctions of the great veins and arteries with the heart, as well as those between the atria and ventricles, served to guide the flow of blood. Although not the first to do so, Harvey stated conclusively that venous blood flows from the right auricle (atrium) into the right ventricle, thence through the pulmonary artery to the lungs, and back through the pulmonary vein to the left auricle. His conclusion was based in part on careful observation of the hearts of "cold-blooded animals."

> Since the close contact of the heart and lungs in man has probably been a source of error, as I have said, the common practice of anatomists in dogmatizing on the general make-up of the animal body, from the dissections of dead human subjects alone, is objectionable. It is like devising a general system of politics, from the study of a single state, or deigning to know all agriculture from an examination of a single field. It is fallacious to attempt to draw general conclusions from one particular proposition.
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> If only anatomists were as familiar with the dissection of lower animals as with that of the human body, all these perplexing difficulties would, in my opinion, be cleared up.
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> If a live snake be cut open, the heart may be seen quietly and distinctly beating for more than an hour, moving like a worm and propelling blood when it contracts longitudinally, for it is oblong. It becomes pale in systole, the reverse in diastole. . . . The vena cava enters at the lower part of the heart, which plainly transmits it by a tube analogous to an artery. This may be confirmed by inspection, or section of the artery, the blood spurting with each beat of the heart.
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> It is not hard to see the same thing in other animals with but a single ventricle, as toads, frogs, serpents and lizzards. . . . It is obvious in opening these animals that the blood is transferred from the veins to the arteries by the heart beat. . . . I have perceived that the same thing is very apparent in the embryos of animals possessing lungs . . . (pp. 53–54, 82–83).

According to tradition, the right ventricle pumped blood to the lungs for their nourishment, while air returned to the left heart. Here, mixed with refined blood seeping from the right ventricle through the pores in the ventricular septum, air took part in the formation of the vital spirits. Harvey wrote:

> The blood is supposed to ooze through tiny pores in the septum of the heart from the right to the left ventricle, while the air is drawn from the lungs by the large pulmonary vein. According to this many little openings exist in the septum of the heart suited to the passage of blood. But, damn it, no such pores exist, nor can they be demonstrated! (pp. 21–22)

With no direct passage from the right to the left side of the heart, blood could only make this transit by flowing through the lungs.

Having established the path of blood through the heart and lungs (*de Motu Cordis,* Ch. I–VII) Harvey undertook to demonstrate the next and truly revolutionary proposition—that blood moves in a circle in the body as a whole, as well as through the lungs, from heart to arteries, through pores in the tissues to veins, and thence to the heart again (*de Motu Cordis,* Ch. VIII–XIV).

> So far we have considered the transfer of blood from the veins to the arteries, and ways by which it is transmitted and distributed by the heart beat. There may be some who will agree with me on these points because of the authority of Galen or Columbus or the reasons of others. What remains to be said on the quantity and source of this transferred blood, is, even if carefully reflected upon, so strange and undreamed of, that not only do I fear danger to myself from the malice of a few, but I dread lest I have all men as enemies, so much does habit or doctrine once absorbed, driving deeply

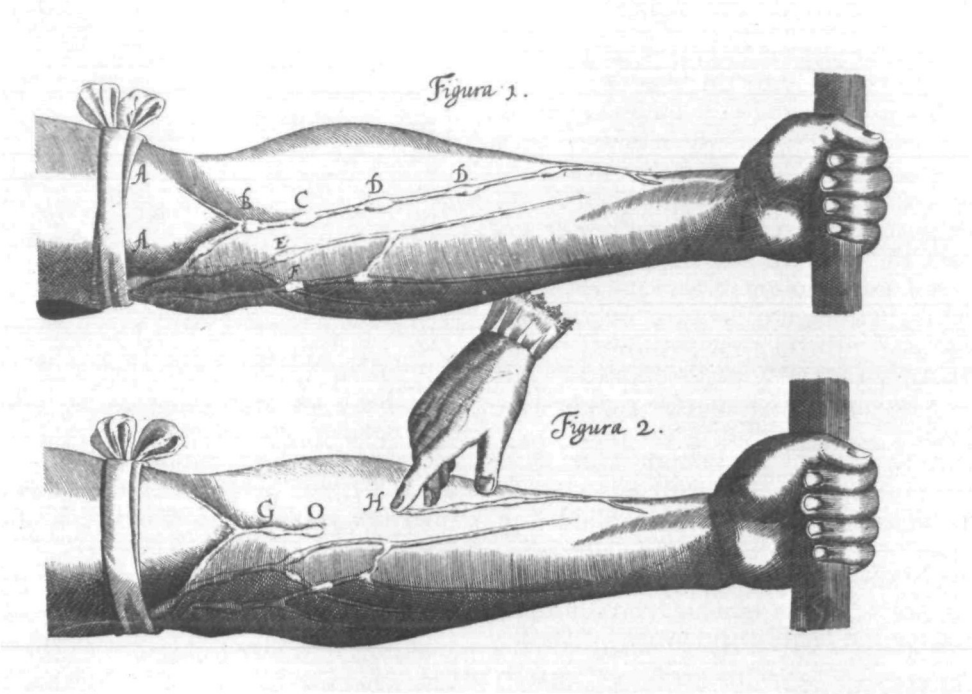

FIG. 2. Two panels of a series of drawings in *de Motu Cordis* illustrating the function of the valves of veins. In *Figura 1*, a ligature had been placed around the arm of the subject at A–A, to block the return of venous blood. Swellings of the distended veins, at B–F, indicate the location of valves. In *Figura 2*, a vein has been blocked by light pressure at H, and its contained blood pressed centrally towards G. The valve at O prevents blood from flowing back, so the stretch from H to O remains empty (invisible in the drawing). (From *de Motu Cordis*, translation by C. D. Leake.)

> its roots, become second nature, and so does reverence for antiquity influence all men. But now the die is cast; my hope is in the love of truth and in the integrity of intelligence (p. 69).

Briefly summarized, Harvey's observations and conclusions were as follows: (1) The amount of blood continuously returning to the heart by the veins was so great that it could not be supplied by continuous production from the food consumed. Were the blood supplied to the arteries not recirculated, the veins would soon be drained. Harvey drew these conclusions from a fascinating quantitative analysis. He estimated the volume of the left ventricle as about 2 to 3 ounces, and guessed that it might empty out into the aorta, on each beat, a fourth to an eighth part of its content—perhaps a dram (1 dram = ⅛ ounce, or $\frac{1}{64}$ pound) on a single beat.

> The heart makes more than a thousand beats in a half hour, in some two, three, or even four thousand. Multiplying by the drams there will be in half an hour either 3,000 drams, 2,000 drams, five hundred ounces, or some other such proportionate amount of blood forced into the arteries by the heart, but always a greater quantity than is present in the whole body . . . (p. 74).

(2) The amount of blood driven from the heart into the arteries is much greater than is needed for nutrition of the tissues. So great is this amount that the arteries would be flooded if they did not deliver their contents to the veins.

(3) Blood can flow in veins in one direction only—towards the heart—because of the presence within them of the valves, beautifully illustrated by Harvey's great teacher Fabricius (Fig. 2).

Harvey drew this conclusion:

> It has been shown by reason and experiment that blood by the beat of the ventricles flows through the lungs and heart and is pumped to the whole body. There it passes through pores in the flesh into the veins through which it returns from the periphery everywhere to the center, from the smaller veins into the larger ones, finally coming to the vena cava and right auricle. This occurs in such an amount, with such an outflow through the arteries, and such a reflux through the veins, that it cannot be supplied by the food consumed. It is also much more than is needed for nutrition. It must therefore be concluded that the blood in the animal body moves around in a circle continuously, and that the function of the heart is to accomplish this by pumping. This is the only reason for the motion and beat of the heart (p. 104).

The treatise concluded (Ch. XV–XVII) with a discussion which Harvey felt to be supportive of his contention that blood circulates. This section is of great interest because of some remarkable observations that he made, and also as recalling that William Harvey based his work, as must all scientists, on the contemporary state of human knowledge. It is moving to read his statements clinching, as he thought, the argument for the role of the heart in circulation:

> It will not be irrelevant to point out further that even according to common ideas, the circulation is both convenient and necessary. In the first place, since death is a dissolution resulting from lack of heat, all living things being warm, all dying things cold (Aristotle . . .), there must be a place of origin of this heat. On this hearth, as it were, the original native fire, the warming power of nature, is preserved. From this heat and life may flow everywhere in the body, nourishment may come from it, and on it all vegetative energy may depend.
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> That the heart is this place and source of life, in the manner just described, I hope no one will deny.
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> The blood, then, must move, and in such a way that it is brought back to the heart, for otherwise it would become thick and immobile, as Aristotle says. . . , in the periphery of the body, far from its source. We note that motion always generates and preserves heat and spirit, while in quietness they disappear. So the blood, in the extremities, thickens from the cold and loses its spirit, as in death. Thus it must come back to its source and origin to take up heat or spirit or whatever else it needs to be refreshed (pp. 105–106).

For all his amazing capacity for independent analysis, for ingenious experiments and careful observation of the relationships of structure and function, William Harvey was, indeed, a man of his time.

A perspective on Harvey's discovery of the circulation of blood

Modern scholarship indicates that Harvey recognized as early as 1616 that blood passes from the right to the left side of the heart through the lungs, in opposition to prevailing doctrine. Nevertheless, *de Motu Cordis* was not published until 1628. The delay is understandable, for Harvey was an eminent physician, busy in practice, research, and the activities of the royal court. Besides, he must have known that his theory of blood circulation, rejecting as it did the firmly entrenched Galenical doctrines, would meet opposition in the scholarly and medical communities.

In fact there was a prompt hostile reaction, with detailed refutations on the grounds of traditional teachings published by James Primrose in England (1630) and the Danish professor Oluff Worm (1632) among others. But younger scholars, for example Jan de Wale (1604–1649), professor at Leyden, accepted Harvey's ideas, and his work was followed, in due course, by a cascade of new discoveries. One of the apparent weaknesses in Harvey's argument was his assumption that there were "pores" in the flesh, through which blood passed from the arteries to the veins. Within a few years, however, Marcello Malpighi had observed the tiny vessels (now termed capillaries) in the lungs of frogs (1661). Antony

van Leeuwenhoek, too, recorded the existence of capillaries in the tissues of fishes and amphibians.

Soon connections were discovered between the lymph vessels of the gut and the great veins, leading to the link between the lymphatic system and circulation overall. Physiology, firmly based on anatomical knowledge, was underway.

Thus Harvey's discovery of the circulation—the flow of blood from the heart through arteries and tissues, thence back by the veins to the heart—was of fundamental importance in providing an experimental basis for further study of the circulatory system. Perhaps more important than the specific discoveries, however, were the methods he used, for these became guides for further work in physiology and are central to functional morphology as well. Careful description of structure is essential but is not enough—structures must be observed in the living condition while actually carrying out their functions. Harvey stressed the value of comparing similar structures in different animals—for example, his understanding of heart function resulted largely from observations on the slowly beating hearts of fish, amphibians, and invertebrate animals. He introduced quantitative measurement as critical to the analysis of the motions of the heart and blood, estimating the volume of blood flowing through the heart as compared with the total amount of blood in the body. And, basic to all his work, he followed the Baconian method of observation leading to hypothesis, the hypothesis to be confirmed or rejected on the basis of further observations (SAAWOK—III, pp. 591–596). Perhaps as amazing as any aspect of Harvey's work was his ability to free himself from traditional and established concepts. For example, he did not try to account for the function or distribution of vital and animal spirits, but restricted himself to observations and hypotheses within his ability to make and to test.

Many elementary textbooks of physiology, and some general biology texts, name Harvey and identify him as the "discoverer of the circulation." Such brief reference, however, hardly does justice to the opportunity to reflect on Harvey's remarkable work. For today's student, this can serve as a prime example of the methods of scientific investigation—of science as a way of knowing. Beyond his careful application of scientific procedures, Harvey was a scholar, well aware of the ideas of other workers. He made use of the valid findings, especially in anatomy, while rejecting the rich growth of erroneous concepts of function. And Harvey shared his ideas with others, in lectures and conversation and in his admirably clear writing. In Harvey's own day his seemingly radical conclusions could have resulted in personal danger—he was a younger contemporary of Galileo (1564–1642), who was forced to recant his revolutionary teachings; and followed by just a generation Michael Servetus (1511–1553), burned at the stake by Calvin for his avowed unorthodoxy in views of religion and the natural world. Harvey recorded his puzzlement and wonder at his findings, and today's reader can sense the tension, even fear and triumph at the confrontation of his experimental results with accepted dogma. This, once again, is a reminder that science is an exciting, sometimes dangerous, and always challenging pursuit—not at all the dull process of amassing solid facts that many non-scientists suppose it to be.

References

Entralgo (1957), Foster (1901), Frank (1980), Franklin (1949), Gotfredsen (1957), Harvey (1628—translation 1941), Weil (1957).

CLAUDE BERNARD: DEVELOPMENTS IN PHYSIOLOGY, AND THE REGULATION OF THE INTERNAL ENVIRONMENT

In the years following Harvey's discovery of the circulation, biological science progressed relatively fast in some areas, but slowly in others. The growth of understanding of anatomy at the whole organism level proceeded apace, and the development of the microscope and histological methods gave access to the fine structure of tissues. Progress in physiology continued at what may be called the organ level—the discovery of the lymphatic system, further advances in understanding the

mechanics of breathing, and so forth—but analysis of function was handicapped by lack of background in physics and chemistry. But these latter fields advanced rapidly with the work of van Helmont (1577–1644), Evangelista Toricelli (1608–1647), Robert Boyle (1627–1691), Joseph Priestley (1733–1804), Antoine Lavoisier (1743–1794), and others. By the mid-19th century there was a great acceleration in physiology, as in other aspects of biological science. This was a time when major advances occurred—it was the time of Charles Darwin and Alfred Russel Wallace, in England, Wilhelm Roux and Karl Ludwig in Germany, and Claude Bernard and his younger contemporary Louis Pasteur in France. It is startling, in retrospect, to realize how rapidly new ideas evolved at this time, setting the stage for many of the developments of our own time.

Claude Bernard (1813–1878) was born in the tiny village of Saint-Julien in Beaujolais country. He came to Paris, after a brief period as a pharmacist's assistant in Lyons, and studied medicine. After receiving his medical degree he was appointed assistant to François Magendie (1783–1855), Professor of Medicine at the Collège de France. Magendie, famous in his own day as an avid experimentalist, is remembered in our's chiefly because he formulated, more or less simultaneously with the English physician Charles Bell, the concept of different motor and sensory functions of the spinal nerve roots in mammals ("Law of Bell and Magendie"). Magendie performed experiments on living animals in his laboratory at the Collège de France, though this laboratory was only a single, ill-equipped room. Claude Bernard, too, experimented, but at first he had to carry out his work in meager "private laboratories"—rented rooms in the vicinity of the Collège de France. Generally our students are unaware of the great improvements that have occurred in the conditions for physiological and other biological work in the past hundred years or so, with the establishment of permanent laboratories with equipment, more or less in working order, at hand.

Although Claude Bernard achieved great recognition among scientists and prestige among intellectual leaders and politicians as well, today's physiologist can sense the frustrations and discomforts that he must have suffered from lack of equipment, techniques, and financial support. He believed that true understanding of function required experimentation on living mammals. At a time when surgery on people was carried out without or with only primitive anesthesia and antisepsis, animal surgery must have been as stressful for physiologists as it was traumatic to their subjects. There was a small but vocal movement against vivisection, and Bernard's life was made more difficult because members of his family were antivivisectionists. There can be no real disagreement as to the crucial contributions of experiments on living animals to our understanding of physiology. Today the conditions for these experiments are far more controlled and humane than they were in Claude Bernard's time, reflecting in part the great advances made in this period in techniques for surgery on humans. Nevertheless, the ethical questions about use of animals to elucidate basic physiological questions remain, and the tide of antivivisectionism is far stronger today than it was in Claude Bernard's time.

In spite of difficulties Claude Bernard pursued his investigations, and within a relatively short time came to be recognized as the leading physiologist in France, on a par with the most outstanding scientists of Europe. He was a skilful and meticulous experimenter, and was the first to discover the role of the liver in maintaining the level of glucose in the blood. Bernard discovered and isolated glycogen and showed that the liver forms this compound by a process involving "ferments" (an early term for enzymes). Fragments of liver removed from the body could produce glucose, but this capacity was destroyed by heating the tissue. These findings were quite unexpected and of special significance in Bernard's day, for at that time most people believed that plants alone could produce organic compounds. It was thought that animals were only able to destroy these compounds. The synthesis of the large, complex molecules of glycogen and glucose by the liver dis-

proved this contention. Further, Bernard formulated the concept of internal secretion—the liver, acting as a gland, formed and secreted its product, glucose, into the blood rather than onto the body surface, as in the case of other glands (*e.g.*, salivary and sweat glands) recognized at the time (Fig. 3).

Claude Bernard was deeply interested in nutrition, and discovered the role of the pancreas as a major source of enzymes (ferments) in digestion. He carried out studies on nerves and found evidence of their regulation of the caliber of blood vessels (vasomotor control). This neural action determined blood supply to the tissues and Bernard convinced himself, incorrectly, that nerves were also crucially and directly involved in regulating tissue metabolism. He investigated pharmacological agents and poisons, seeing these as tools, as it were, for dissecting living processes. His work with curare, a plant product used by certain Brazilian natives as an arrowhead poison, gave a start to the investigation of the special sensitivity of nerves to this poison. Carried on by later investigators, this work led to identification of the junction of nerves with skeletal muscles (motor end plates) as the site of attack by curare, and contributed in significant ways to our present knowledge of neuromuscular transmission.

An important and persistent problem in animal biology, from the earliest times to the first decades of the 20th century, was whether living processes were truly unique, or rather were merely manifestations, though admittedly extremely complex, of the common processes of the non-living world. Although opinions grouped under a single heading were in fact extremely varied, the general concept of the uniqueness of biological processes—that is, that they are separate and special, unlike those of the inanimate world—is generally termed *vitalism*. Probably most early workers assumed that vital processes were involved in living things. By the early 18th century, however, Herman Boerhaave (1668–1738), a Dutch chemist and professor of medicine, challenged this idea in his important work *Institutiones Medicae* (1708). He taught that

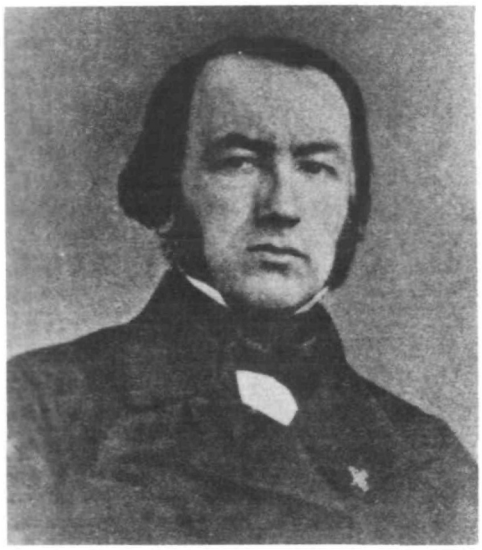

FIG. 3. Claude Bernard at age 36 (1849). At this time he was engaged in studies of the control of the blood sugar, and the effects of curare on neuromuscular function. (Slightly modified from Olmsted, 1938.)

physical and chemical laws determine biological function. The state of biological science at the time did not allow for an unequivocal answer to this question, and the great French anatomists Georges Cuvier (1769–1832) and Marie François Xavier Bichat (1771–1802), among others, held to vitalism as necessary to explain the great complexity and unusual properties of living beings. Claude Bernard argued against a rigid application of vitalism, in part because it placed some aspects of function beyond the scientist's tools and ideas of inquiry. For example, in his important book *Introduction à l'Étude de la Médicine Expérimentale* (1865) (*An Introduction to the Study of Experimental Medicine*) he wrote (p. 185):

> In fact, we are often duped by such words as life, death, health, disease, idiosyncrasy. We think we have explained when we say that a phenomenon is due to a vital influence, a morbid influence, or an individual idiosyncrasy. We must really learn, however, that vital phenomenon means only a phenomenon peculiar to living beings, whose cause we do not yet know; for I think that every phenome-

> non, called vital today, must sooner or later be reduced to definite properties of organized or organic matter. We may, of course, use the expression vitality as chemists use the word affinity, but knowing that fundamentally there are only phenomena and conditions of phenomena which we must learn; when the conditions necessary to phenomena are known, then occult, vital and mineral forces will disappear.

He was not free of the influence of vitalism, however, for he belived that the synthesis of organic compounds could only be carried out by living cells. This was a widely held concept, generally discounted only after Friedrich Wöhler (1800–1882) synthesized, in the laboratory without the intervention of living cells, the organic compound urea from (chiefly) inorganic starting materials.

As his study of animal function progressed, Claude Bernard's concepts widened and became more synthetic. He recognized and was deeply interested in the commonalities of function of plants and animals—indeed, of all forms of life. Partly for this reason, he de-emphasized specific aspects of structure in animals, although he recognized clearly that

> Anatomy is the necessary foundation of physiology, and never can we become good physiologists if we are not first deeply versed in anatomical studies and trained in delicate dissections, so as to be able to make the preparations which are often required for physiological experiments. . . (Bernard, 1865, p. 117).

Nevertheless, he objected to the idea that anatomy can reveal physiological processes directly. He argued against the dominance of anatomy over physiology in academic organization. Thus, Claude Bernard's influence contributed to a growing trend in the 19th century which had some negative results—the weakening of the intimate relationship between anatomy and physiology, both in academic institutions and in scientists' conceptual schemes. Bernard himself, as an excellent experimentalist, did not divorce structural from functional approaches, but his successors sometimes addressed biological systems as "black boxes," to be analyzed by chemical and physical means with little attention to structure (see, for example, Schiller, 1968).

In 1878, the last year of Bernard's life, *Leçons sur les Phénomènes de la Vie Communs aux Animaux et aux Végétaux* (*Lectures on the Phenomena of Life Common to Animals and Plants*) was published. In this book, as in previous lectures, he developed an idea of great significance for biological scientists of his day, as for our own. Impressed by discoveries about the role of the circulation in regulating the supply of glucose and other substances to the tissues, he perceived blood and other body fluids as an internal environment surrounding all the body cells. In higher animals this environment is precisely regulated by processes of intake and excretion:

> The life of the organism takes place, not in the external environment, atmospheric air for organisms breathing air, fresh or salt water for aquatic animals, but in the *liquid milieu intérieur* (internal environment) formed by the circulating fluid which surrounds and bathes all the anatomic elements of the tissues; this is the lymph or plasma, the liquid part of the blood which, among higher animals, penetrates the tissues and constitutes the totality of the interstitial fluids, basic to all local nutrition, and source and center of all elementary exchanges.
>
> The stability of the *milieu intérieur* is the primary condition for a free and independent life; the mechanism which ensures in the internal environment the maintenance of all the conditions necessary to the life of the elements (translated from *Leçons sur les Phénomènes*, pp. 112–113).

The stability of the internal environment in perspective

As more and more experiments have been carried out and information accumulated, Claude Bernard's great and illuminating concept has endured although it has been greatly amplified, modulated, and extended. Walter B. Cannon (1871–1945),

a leading American physiologist of the first half of the 20th century, carried out many experiments on the autonomic nervous system as the agent implementing this stability or steady state. He proposed the term *homeostasis* to describe it. Homeostasis is the maintenance of relative constancy—within limits allowing considerable fluctuation—of the animal's internal environment in spite of continuing metabolism, activity, and the stresses imposed by changes in the external environment. Joseph Barcroft, subjecting the idea of fixity (stability) to an exhaustive critical review (1932), analyzed the measurable variability occurring within the domain of "fixity." In addition he pointed out that precise regulation is of particular importance to the more complex functions of the central nervous system.

We know now that many factors besides neural control participate in homeostasis, and that these operate at every level ranging from the synthesis and regulation of molecules (enzymes, receptors, etc.), the cell membranes and subcellular structures (mitochondria, the Golgi apparatus, etc.) to control of extracellular fluids. Hormones, autacoids, and second messengers with precisely regulated formation and function are involved in intricate interactions, and there is continuous feedback and "cross-talk" between many controlling systems. Recently great emphasis has been placed on behavior as a key factor in homeostasis. For example, ectothermic vertebrates such as reptiles may regulate body temperature very precisely by behavior selecting appropriate micro-climates (basking in the sun, seeking shade) reinforced by rapid and precise adjustments of the circulation.

A further important modification of the concept of homeostasis has come from comparative physiology, for many animals, especially invertebrates, are capable of living effectively under drastically changing conditions by adjusting their rates of cellular activities, rather than by modulating the immediate environments of their cells. Claude Bernard conceived of the internal environment chiefly in terms relevant to the human and other mammals, and many of his ideas are still valid in this narrow context. The later modifications are typical of the continuing building and expanding character of science. For in this field in general the great contributions of early workers cannot be evaluated narrowly in terms of their continuing accuracy in the face of later research. As an example, Darwin's concept of evolution, though of fundamental importance in development of all later biological science, is not valid in every detail. Similarly, Claude Bernard's idea of constancy of the internal environment has been modified by later work. Yet its crucial significance stands, because the internal environment and its regulation provided a major central concept to stimulate further experiments and hypotheses, and to systematize the rapidly accumulating masses of information during the years following Claude Bernard's work (Robin, 1979). And it is notable that this concept has elements of form—not form of cells, tissues or organs, but rather organization expressed through living processes. The organism must be considered as a whole and not in terms of the compartmentalized functioning of its systems. Only as these operate to preserve the relationship of the cells to the internal environment can animal life endure.

In my own experience as a young physiologist I sometimes felt daunted by the vast mass of knowledge about animal structure and function, a seeming mountain range of established facts riddled with unanswered and seemingly unanswerable questions. My despair at this vastness and uncertainty was mitigated—sometimes even turned to elation—by such illuminating concepts as evolution, complementarity of structure and function, and homeostatic regulation. Today's students enter a world of biological science far more immense and complex than I did, a generation ago. I feel convinced that our students can be helped, as they move into this world, by learning something of the origin of our great organizing concepts. All of these emerged from matrices of observations, questions, hypotheses, and experiments as ideas of individual scientists, then were modified, reshaped, and refined by

later workers. The development of such a valid scheme of organizing concepts, based on a vast background of research, is a unique power of science as a way of knowing.

References

Barcroft (1932), Bernard (1865—translated 1949), Bernard (1878—reprinted 1966), Cannon (1929, 1932), Foster (1899), Grmek (1968), Olmsted and Olmsted (1952), Robin (1979), Schiller (1967, 1968).

ADJUSTMENTS TO OSMOTIC STRESS—THE COUNTERCURRENT SYSTEM OF THE KIDNEYS OF MAMMALS

The evolution of the mammal's way of life, including a high and regulated body temperature allowing continuing activity in a relatively wide range of environmental temperatures, entails great cost in metabolic terms. Not only must the mammal procure and devour far more food than reptiles, amphibians and fish of comparable size, but also mammals have high rates of production of metabolic wastes. Correlated with these characteristics are, of course, many others including the remarkable complexity and effectiveness of mammalian kidneys. As a group, mammals have an important capability to form urines of widely varying osmotic pressure, effectively conserving water and excreting wastes and other excess solutes, or excreting excess water, depending on moment by moment metabolic demands. For example, human urine can range in osmotic concentration from one-fifth to fivefold the concentration of blood plasma. Other mammals, particularly those adapted to life under conditions of water deprivation (*e.g.*, desert or marine environments) can achieve even greater ranges of urine concentration. This capacity has contributed significantly to the success of mammals in adaptation to life on earth. The countercurrent system of flow of fluids through the kidney nephrons plays a major part in these osmotic adjustments. And this system is a good illustration of the central role of form–function interactions in animal biology.

Discovering the ways of operation of vertebrate kidneys

For centuries it has been recgnized that the kidneys are involved in removal of excess materials and wastes from the body. As discussed in the previous essay (Moore, p. 488), Aristotle taught that blood percolated through the kidneys, straining off wastes. Other observers elaborated ideas about urine production, relating the quality and volume directly to many of the body's ills. This early awareness of the role of kidneys in the body's economy is not surprising in view of the obvious anatomical connections of the kidneys and ureters with the urinary bladder and urethra. Large arteries and veins are seen in conspicuous relation to the kidneys. Moreover, certain aspects of renal function are easily—almost automatically—observed. For example, everyone knows that excess drinking is followed by increase in the volume of urine, while strongly or characteristically scented food materials (garlic, asparagus, and so forth) give rise to similarly scented urines. In the days before modern plumbing and the free use of running water for sanitation, urine was collected in containers and its characteristics could hardly go unnoticed. Indeed, human urine was not only observed, but was valued and used in various ways—as a reagent for healing or magic, and as fertilizer, for example. Patriotic ladies in the American Civil War (1861–1865) were urged to harvest the contents of chamber pots for use in production of gunpowder. Physicians since before the days of Hippocrates and up to the present time have studied urine as offering clues to the individual's state of health.

Advance in the understanding of kidney function required more, of course, than knowledge of the kidney's gross structure, rich blood supply, and production of urine. Definitive investigation of the fine structure of these organs began with Marcello Malpighi (1628–1694) who described the minute, globular tufts of blood vessels, in the outer layers of kidney tissues, that bear his name today as Malpighian corpuscles. What was the function of these odd struc-

tures, that seemed to be continuous with the tubules making up most of the kidney tissue? A reasonable guess was that they might slow the flow of blood through the organs, facilitating the sieving of wastes from the blood into the urine. More than a hundred years after Malpighi's discovery the English physician William Bowman (1816–1892) carried out painstaking studies of kidneys of various mammals and some non-mammalian vertebrates (1842—Fig. 4). He injected suspensions (*e.g.*, "vermilion and size"—dyed starch) into the afferent blood vessels of kidneys to define the course of blood flow, observing the preparations with the microscope at magnifications up to 320×. In this way Bowman discovered that the capillaries of the Malpighian corpuscles did not empty directly into the uriniferous tubules as some earlier observers had affirmed. Instead, each tuft of capillaries was closely invested with a delicate and transparent structure, the blind end of a uriniferous tubule expanded into a chamber or capsule. The tubule running from this capsule was surrounded by a capillary meshwork fed by the arteriole draining the Malpighian corpuscle, and leading further into venules.

The discovery of this relationship between the tubules and their associated blood vessels led Bowman to the idea that urine is formed by two processes: (1) flow of water and salts from the capillaries into the capsules, and (2) subsequent secretion of waste products and other solutes into this fluid by glandular activity of the cells of the tubules. The eminent German physiologist Karl Friedrich Wilhelm Ludwig (1816–1895), however, challenged the idea of a secretory function of the kidneys (1861, pp. 373–428). He proposed instead a strictly mechanical hypothesis. According to this idea, fluid similar in composition to plasma was filtered passively through the walls of the capillaries into Bowman's capsule, and thence flowed down the tubule. Reabsorption of some of the filtered materials then occurred, by simple back diffusion, into the blood in the rich network of vessels surrounding the tubules. Ludwig based this hypothesis in part on experiments showing that the rate of urine formation was directly related to the pressure of arterial blood perfusing the kidney, and in part on the demonstrated structure of the kidney. Yet another basis for this contention was his opposition to the idea that kidney cells could select "actively" among valuable materials and wastes in the blood, conserving some and rejecting others by secretion into the tubules. Such activity seemed to demand explanation in terms of vitalism, an approach to which Ludwig was deeply opposed. Arguments continued for half a century between Ludwig and his successors, on the one hand, and on the other physiologists who supported the concept that renal tubules could indeed carry out active and selective secretory processes.

In the 1920s Alfred N. Richards (1876–1966), an American physiologist, and his co-workers undertook experiments which eventually led to the end of the controversy. The critical experiments of these workers involved an approach from both structural and functional angles. Extremely fine pipettes were inserted into different regions of the nephron—Bowman's capsule or sections of the tubules—allowing aspiration of minute volumes of fluid. The fluids were then subjected to ultramicro analysis and compared in composition with blood. The exact locations from which the samples were obtained were determined by observation with the microscope and histological techniques. In summary of the work of Richards and many others, the fluids in Bowman's capsule have been found to be essentially identical with plasma (except devoid of the huge molecules of the plasma proteins) thus implicating passive filtration in their origin. Fluids sampled from different regions of the tubule diverge in composition from the filtrate in the capsule, indicating that, as the pre-urine flows along the tubule, some substances are reabsorbed from it (*e.g.*, glucose) and some are secreted into it (*e.g.*, certain organic acids and bases including oxalate, penicillin, choline and histamine). Such micropuncture studies have been developed, refined and extended and are in use today in continuing, sophisticated analysis of tubular function.

Starting in the 1930s and also continuing

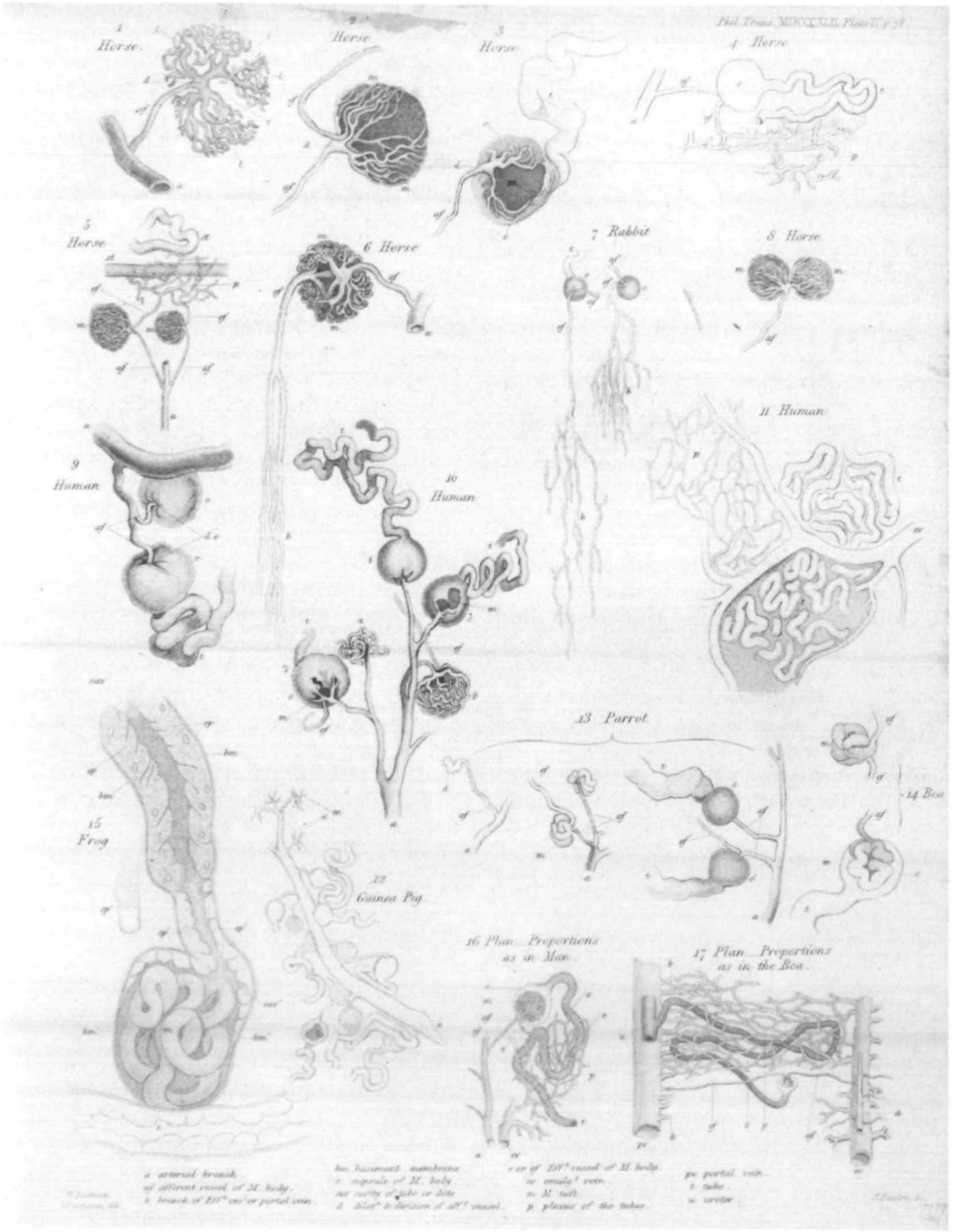

FIG. 4. Bowman's illustration of Malpighian bodies, tubules, and connections with blood vessels in horse, rabbit, human, guinea pig, parrot, snake (boa), and frog. When the contrast material injected into the renal artery filled but did not rupture the capillaries, these appeared clearly defined (as in the upper left-hand corner, drawing #1). But if the capillaries were ruptured, the delicate blind end of the renal tubule was filled, forming a spherical mass to delineate the structure now termed Bowman's capsule. This is shown well in the central drawing, #10. (Bowman, 1942.)

vigorously today are new, often indirect and fairly non-invasive studies with techniques such as the clearance method developed extensively by Homer Smith (1895–1962) and many other workers. The picture of renal function in the mammal, as it stands today, may be briefly summarized as follows: Each of the glomerular capillary tufts (up to 2 million in a young adult human) filters fluid into the corresponding nephric tubule. As this fluid traverses the tubule it is modified by processes of the tubular cells involving diffusion, active and coupled transport so that a tiny fraction of the original filtrate emerges, at last, greatly modified in composition, into the ureters. From these it passes to the urinary bladder and is shed from there more or less unchanged in composition.

The patterns of function of the excretory systems in vertebrates other than mammals show considerable variation as might be predicted from their evolutionary diversity, and the mammalian pattern resembles as well as diverges from these. For example, in other vertebrates glomeruli may be present, large and numerous (as in some fish and amphibians) or fewer and even absent altogether (as in some marine teleost fish, and desert amphibians and reptiles). Correspondingly, glomerular filtration may be relatively significant, or reduced or suppressed altogether (in aglomerular species, tubular secretion rather than glomerular filtration initiates urine formation). Nephrostomes—ciliated funnels drawing fluid from the body cavities directly into the renal tubules or veins—are found in some fish and amphibians. Tubular functions, and the arrangements of blood vessels supplying the kidneys, vary in kind and significance depending on the particular species concerned. After emerging from the ureters, the urine of non-mammalian vertebrates is generally further modified, with conservation of water and/or ions as the urine traverses or is stored in such organs as the cloaca, rectum, or urinary bladder. In mammals the urine entering the ureters is in essentially completed form, with little modification occurring in its further course to the outside. This explains, in part, the nearly unique role of the countercurrent system of the mammalian kidneys in osmotic regulation.

The countercurrent system

The picture of the kidney tubules of mammals, as known to Bowman and his contemporaries, was incomplete. There were many elaborately coiled tubules in the outer region of the kidney (cortex, outer medulla), with Malpighian corpuscles nestling among them. Innumerable straight tubules and blood vessels coursed through the more central (medullary) region of the organ, running towards the outflow at the renal pelvis and origin of the ureter. Today we understand the structure of the mammalian nephron—it is clearly figured in virtually every elementary textbook of biology or physiology—so it may be hard for the student to imagine the great difficulty of figuring out the immensely complex intermeshing of the myriads of delicate, tortuous nephrons making up a real kidney.

Several decades after Bowman's work, and late in his own life, a careful and industrious German morphologist, Jacob Henle (1809–1885) used the injection method to reveal a hitherto unsuspected link in the structure of the mammalian nephron—the presence of a long section of tubule of very narrow diameter, bent back upon itself to form a hairpin loop, and inserted between two stretches of coiled tubule (proximal and distal convoluted tubules). The slender lengths of Henle's loops, packed in orderly arrays within the kidney medulla, are now known to be essential for the mammalian kidney to form hypertonic urine. Such structures are absent in most other vertebrates (some birds have similar thin loops, but these are in general less elaborated than those of mammals). This is not to say that nonmammalian vertebrates are unable to regulate the osmotic pressure of the final excretory product—urine, or urine after modification in the cloaca, urinary bladder, and/or the rectum. Such regulation is crucial to the maintenance of the stability of the internal environment. The completed excretory fluid is dilute as compared with the extracellular fluids

when water is in excess (as is generally the case for some amphibians), and more concentrated when there is an excess of solute as compared with water (as in tetrapods in dry habitats).

Beginning with the pioneering work of the English pharmacologist E. B. Verney (1894–1967; see, for example, Verney, 1947) it gradually became clear that a peptide hormone (actually, several related hormones) formed in the hypothalamus and stored in, then released from the posterior pituitary, is central to osmotic regulation in all tetrapod vertebrates. When the level of this hormone, antidiuretic hormone or ADH, in the blood is high, water is conserved. When the circulating ADH level falls, this conservation is reversed. The site of action of ADH differs among different animals. Thus it acts to different degrees on the skin, glomeruli, tubules, and urinary bladders of amphibians, and on glomeruli and tubules, bladder, cloaca, and rectum in reptiles and birds. In contrast, in mammals ADH has long been known to have no significant effect on glomerular filtration, but rather to alter the volume and concentration of urine before it enters the bladder. When ADH levels are high, the urine can achieve concentrations far above that of blood, whereas relatively dilute urine is formed when ADH levels are low. The delivery of ADH to the blood is under the control of the hypothalamus, and dependent on sensory input from the brain itself (osmoreceptors) and from the cardiac atria and associated great veins (mechanoreceptors). (Discussions of ADH and osmoregulation are given in LaPointe, 1977; Gorbman *et al.*, 1983.)

Observations from comparative anatomy and physiology suggested that the site of action of ADH on the mammalian kidney might be the loops of Henle, since these structures are found chiefly in mammal kidneys, just as the direct action of ADH on kidneys is also characteristic of mammals. At the time it was also thought that the delicate looped sections of tubules might function by having the capability to secrete water "actively" (*i.e.*, by harnessing cell energy for the process). This was not a particularly attractive hypothesis since then, as now, there was no evidence that any cell or tissue could secrete water in this manner, and the thin, delicate cells making up Henle's loop seemed particularly unlikely as candidates for such a special role.

In 1942 the Swiss scientists W. Kuhn and K. Ryffel made the suggestion, based on structure alone, that the loops of Henle might represent a countercurrent system, an organization familiar to engineers. In a system where two fluids of different composition (or temperature) flow adjacent to one another but in opposite directions and are able to exchange solutes (or heat), marked gradients can be built up along the axis of flow. The gradients are magnified (multiplied) in a countercurrent multiplier system in which fluid flows in a U-shaped tube first in one direction, then in the opposite direction while exchange takes place between the contents of the two arms of the tube. Could such a build-up of concentration of salts in Henle's loops, where pre-urine clearly flows away from, then backward towards the cortex, account for the production of urine more concentrated than blood? Such concentration has a cost in energy, of course, and Kuhn and Ryffel suggested that the energy could be supplied mechanically, through hydrostatic pressure of fluid in the tube.

The paper by Kuhn and Ryffel had little impact for a decade—it was, after all, the period of World War II, with near total disruption of research not committed to military activities. Then at the start of the 1950s Hargitay, Kuhn and Wirz reintroduced the idea, and showed that fluids in the medulla of the kidney are, indeed, strongly hypertonic to blood (Hargitay and Kuhn, 1951; Wirz *et al.*, 1951). Their procedure was beautifully simple. They froze excised rat kidneys, cut them into thin sections, and then watched with the microscope the melting of the ice crystals within the tissues as the temperature was raised gradually. It is of course familiar that the higher the osmotic pressure of a fluid, the lower its freezing point and, correspondingly, the lower the temperature at which, when frozen, it melts. The Swiss investigators saw that ice crystals melted at a sig-

nificantly lower temperature in the medulla than did those in the cortex (Fig. 5). They pointed out that somewhat similar observations on frozen kidney tissue had been made as early as 1902, but the data were uninterpretable then, and forgotten. Now these data gave clear evidence that a standing gradient of osmotic pressure must exist in the kidney, with high concentrations localized in the renal medulla, hence in and around the loops of Henle. Wirz and co-workers replaced the idea of hydrostatic pressure as the source of energy for concentration, postulating instead a series of active secretory and passive diffusion processes to account for the build-up of osmotic gradients.

These suggestions were received cooly at first, but within a few years it came to be generally accepted that the demonstrated gradient was the result of such processes operating in the context of countercurrent flow along and around the hairpin loops (Lotspeich, 1958; Wirz and Dirix, 1973). Micropuncture experiments showed that fluid emerging from the loops into the distal convoluted tubules (en route out of the nephrons, towards the collecting ducts) was less concentrated than blood. These results confirmed that solute must have been accumulated in the environs of the thin loops. This would account for the high osmotic pressure of this region, demonstrated by Wirz and co-workers.

Many additional data have been collected, leading to the current view (still in process of development). This may be outlined as follows: Active transport of sodium ions, with coupled transport of chloride ions in the ascending limb of Henle's loop, where the cells are impermeable to water, provides the driving force for build-up of the osmotic gradient in the intratubular fluid in its counter flow. Urea, diffusing in from the collecting ducts, participates in the organization of the medullary gradient, at least in some mammals. Structural differences (in permeability, transport capabilities) in different regions of Henle's loop underlie the actual operation of this countercurrent multiplier system. Recent descriptions of some aspects of these processes are given, for example, in Bulger

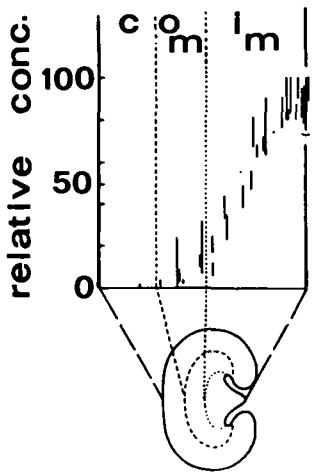

FIG. 5. Data presented by Wirz and co-workers showing that the relative osmotic pressure of kidney tissue, indicated by melting of ice crystals in the tissue, varies from the cortical to the inner medullary region. The relative position in the kidney is indicated by the abscissa and the diagram of the kidney, divided into regions as follows: C = cortex; OM = outer medulla; IM = inner medulla. The ordinate gives *relative osmotic pressure* in any region of the kidney, defined as (*osmotic pressure of tissue* − *osmotic pressure of plasma*) divided by (*maximum osmotic pressure of tissue* − *osmotic pressure of plasma*). (Slightly modified from Wirz *et al.*, 1951.)

and Dobyan (1982) and Greger (1985); also in textbooks such as Gorbman *et al.* (1983), Eckert and Randall (1982), Gordon *et al.* (1982), Schmidt-Nielsen (1983), Vander *et al.* (1985).

An important clue is still missing from this account of how mammals can form urine of varying volume and osmotic pressure. What is the role in this process of the controlling hormone, ADH? Wirz suggested that it might affect the permeability of the collecting duct cells to water. If present in high concentration in blood (under conditions of water deficiency), ADH would increase the permeability of the cells of the collecting ducts to water. Water could then flow down the osmotic gradient into the fluids surrounding Henle's loops, and be swept back by the blood flow into the general circulation. In this way, water would be conserved for the body's uses. When ADH levels in blood were low, however (*i.e.*, under conditions of water excess), the collecting ducts would remain imperme-

able to water and the hypotonic pre-urine delivered by the distal convoluted tubules to the collecting ducts would flow out of the body. Hence, excess water would be eliminated.

Experiments using micropuncture techniques confirmed that ADH increases the permeability of the collecting ducts. Thus the hypothesis stands today, although it has been elaborated and refined by the results of many ingenious experiments. For example, it is now known that ADH does not enter duct cells to activate the changes in permeability, but instead works through the intracellular second messenger, cyclic AMP.

Studies chiefly dependent on techniques of electron microscopy have filled in certain details as to how the permeability characteristics of the cell membranes of the collecting ducts may be altered. Many globular protein masses appear within these membranes when they are highly permeable to water (under conditions of high ADH levels in the blood, or when duct tissue is exposed to ADH). From these observations, and studies of the urinary bladders of toads which respond to ADH much as do the collecting ducts, it is inferred that the globular masses are indeed "water channels"—protein complexes which facilitate passage of water through the otherwise water-impermeable, lipid-protein organization of the cell membrane. The incorporation of these water channels into cell membranes occurs rapidly—within minutes—of exposure of the tissue to ADH (Wirz and Dirix, 1973; LaPointe, 1977; Wade, 1978; Morel and Doucet, 1986; Gluck and Al-Awqati, 1980).

Today there remain many unanswered questions about the details of this sophisticated osmotic regulation by mammalian kidneys, but the outlines of the processes appear to be well established. In summary, the blood ultrafiltrate formed in the Malpighian corpuscle is modified drastically as it passes along the complicated passageway within the nephron, and most of the fluid is reabsorbed here into the capillaries surrounding it. As it traverses the ascending arms of Henle's loops, the pre-urine is depleted of solutes so that a steep gradient builds up in and around these structures. The hypotonic pre-urine discharged from the distal tubule into the collecting duct may pass out of the body unchanged (excreting excess water). Or, under the influence of circulating ADH, much of the water may diffuse back from the collecting ducts into the medulla and be returned to the body (conserving water). These processes are crucially dependent on details of kidney structure which may be listed as follows: (1) The filters of the Malpighian body that form the pre-urine. (2) The array of active tubular cells that this fluid must traverse. (3) The long hair-pin loops of Henle, allowing osmotic build-up. (4) Parallel loops of blood vessels, which conduct reabsorbed fluid away from the medulla without disrupting the osmotic gradient. And, finally, (5) the labile cell membrane structure on the collecting ducts, with readily adjustable permeability depending on the presence or absence of water channels within them.

Perspective—kidney function and the internal environment

In the middle of the present century, Homer Smith summarized much of the information then available on kidney function, relating it explicitly to the major concept of homeostatic regulation. He stated:

> The principles formulated by Bernard and Cannon are especially pertinent to the problem of renal function. In all the higher animals the plasma has indeed a remarkably constant composition, not only from individual to individual but among distantly related groups. This constancy is in large part a consequence of the activity of the kidneys, which under all conditions excrete a urine of such composition as to offset any tendency toward deviation in the composition of the plasma. In the last analysis, composition of the plasma is determined not by what the body ingests but by what the kidneys retain and what they excrete (Smith, 1951, p. VI).

In the decades since Smith wrote this, our views on the regulation of the internal environment have become both wider and more specific. On the one hand, it is clear

that processes controlling intake of water and nutrients, and the behaviors on which they depend, are regulated by sophisticated neuroendocrine mechanisms. These functions have roles as important and various as those of renal excretion.

On the other hand, the participation of the kidneys in homeostasis is more pervasive and subtle than Smith and other physiologists knew at mid-century. The kidneys are endocrine glands as well as excretory organs. Renal receptors sense the oxygen content of blood and, if this falls significantly, the kidneys release a hormone (erythropoietin) that stimulates the blood-forming organs to increase the formation and release of red blood cells. The kidneys are also the origin of the enzyme renin which acts on blood proteins (angiotensinogens) to start formation of a cascade of powerful hormones, angiotensins. Renin is released by special renal cells in response to lowered flow of blood to these tissues (monitored by receptors that sense blood pressure and delivery of sodium ions to the kidneys). Angiotensins drive homeostatic responses—increase in blood pressure, increase in adrenal cortical steroids stimulating sodium absorption by the intestine and kidney tubules, release of ADH by the posterior pituitary, and specific behaviors in search of water. Other local hormones and autacoids participate in the intricate functioning of the kidneys as prime agents of regulation of the internal environment.

Many students in introductory biology and physiology courses meet the countercurrent concept, particularly in connection with excretion and temperature regulation. The idea is not particularly easy to grasp, as may be seen by reviewing its relatively slow penetration into the thinking of the community of physiologists. We suggest that the task of understanding countercurrent mechanisms may be made easier by sketching the background of questions, observations, and hypotheses from which these concepts emerged.

The gradual development of our present understanding of kidney function offers a rich field of illustration of the methods of science as a way of knowing. Morphological observations leading to hypotheses of function were basic to the study of the kidneys. Contradictory hypotheses led to vigorous controversy and experimentation, and some of this was fruitful in suggesting new hypotheses, although, in reading the early literature, one senses much wastage of effort in defense of data that proved unsound, and positions which proved to be untenable. This statement is not unique to this area, of course, because science advances in stop-and-go fashion, in spite of false starts as well as through leads which prove fruitful. In this as in other studies of form and function, the comparative approach has been of great value. For example, Bowman came to understand the relationship between kidney tubules and their blood supply through study of several diverse vertebrate species. The pioneering work of A. N. Richards and his co-workers, in applying the techniques of micropuncture, were carried out on the kidneys of amphibians because of the relative simplicity and large size of their nephrons. And, as in all other aspects of our field, the understanding of renal function remains tentative. The exact mode of operation of the countercurrrent system is still unknown, and every year sees the publication of a vast amount of research on aspects of kidney biology, normal and pathological, bearing on basic questions of morphology, physiology, development, genetics, and evolution.

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OXYGEN, CELL ENERGY, AND CELL STRUCTURE

From earliest times it must have been recognized that breathing—the flux of air in and out of the body—is necessary for life. A common phrase, used even today

on occasion, is "the breath of life." For generations and until recent times, a test of death was the failure of breathing. And one of the commonest ways to kill is strangulation. Both casual observations and detailed study of the mechanics of breathing lead to a question which may have been asked since the earliest times of coherent speculation about human and animal biology—what is the function of the air taken in, then expelled from the lungs? Answers to this question had to be highly speculative, of course, when both the nature of air and the physiology of the living body were unknown. Some suggestions, encoded in Galenical tradition, included the cooling of the blood (overheated by the innate heat of the heart), and a role in the synthesis of vital spirits.

Early stages in identifying the processes of metabolism

Progress in gaining knowledge about the function of breathing followed fairly quickly on the discoveries that air is made up of a mixture of gases, and that only one of these—later named oxygen—is necessary for life. The great French scientist Antoine Lavoisier, working in collaboration with Armand Séguin, concluded that respiration is a process of living organisms essentially identical with combustion as it occurs in the inanimate world. As such, respiration produces heat and requires oxygen. This animal combustion takes place, according to Lavoisier, in the lungs. But this idea was questioned by several workers. It was disproved when Claude Bernard measured the temperature of blood in the left heart, and found it cooler, after its flow through the lungs, than the blood on the right side of the heart. Gradually it came to be recognized that respiration is different from combustion of non-living systems, and is a necessary energy-yielding activity of all cells.

By 1875, after great effort, hemoglobin had been discovered as the "vehicle" carrying oxygen in the blood to these cells. During the late 19th and early 20th centuries a distinction was established between anaerobic (oxygen independent) respiration, as seen for example in yeast, and the aerobic (oxygen demanding) respiration of most animals. Later it was found that animal cells have a dual pattern of respiration, at least as far as carbohydrates and some other important metabolites are concerned. The first steps involve degradation of organic compounds independently of a source of oxygen, leading to the formation of pyruvic acid (and lactic acid under anaerobic conditions, in many animals). Subsequently, these products can be fully metabolized to carbon dioxide and water, but only in the presence of oxygen.

As more and more powerful techniques of biochemistry were developed, the individual steps in glucose breakdown were identified. By the end of the fourth decade of the present century, biochemists had established the now familiar sequence of reactions in glucose breakdown to 3-carbon product (pyruvic acid, lactic acid), and had identified the ubiquitous and absolutely essential organic co-enzyme for this process, NAD (nicotine adenine dinucleotide—built in part from nicotinic acid and accounting for the fact that this substance is a B vitamin, a dietary essential for the life of many animals including ourselves). The experimental routes to the establishment of these concepts were extraordinarily complicated and confusing. A good account of the work is given in Fruton (1972).

The finding, deeply puzzling at first, that the metabolism of glucose required the presence of phosphate ions, was explained after the discovery (in the mid-1930s) of the highly unstable compound ATP (adenosine triphosphate). The entire complicated sequence of events, from the entry of glucose (or other carbohydrates) into cell metabolism till its dissolution into lactic acid or other products, was of use to the cell only because of the simultaneous production of ATP from the cellular pools of ADP (adenosine diphosphate) and inorganic phosphate ions. Before the 20th century reached mid-point, it became clear that ATP is a common energy transfer molecule, carrying chemical energy, as it were, from metabolic processes to such energy-demanding activities of cells as movement (as in muscle cells), transport of ions or

molecules across cell membranes (as in secretory cells), and generation of electrical potential differences (as in nerve and muscle cells).

The role of oxygen in cell metabolism

Quantitative studies showed that the anaerobic processes of cells, though effective in starting the breakdown of metabolites for ATP synthesis, were far from completing this process. Aerobic metabolism, continuing the processes with molecules partially degraded anaerobically, yielded many more molecules of ATP than did anaerobic metabolism. Thus, the function of oxygen in respiration has proved to be of great significance, even though cells can extract some energy from metabolites in its absence (an example is a sudden burst of contraction of skeletal muscles in vertebrates, which is powered by anaerobic processes but leads quickly to the adverse effect of accumulation of lactic acid in and about the muscle cells).

The information about cell metabolism sketched so far was based on a rich variety of biochemical techniques. Their power grew ever greater as biochemists developed ways of isolating individual enzymes, co-enzymes, and substrates from cells, destroying cell structure, of course, in the process. Hypotheses about biochemical pathways could be checked by assembling putative enzymes and substrates—the latter often synthesized by ingenious methods of organic chemistry—under conditions optimized with respect to the presence of co-enzymes, necessary ions, pH, and so forth. From the 1940s on, the growing use of radioactive or rare stable isotopes of hydrogen, carbon, oxygen, nitrogen and other atoms made possible the tracking of compounds, groups of atoms, and individual atoms through metabolic processes, and opened wholly new ways of attack on the cell's activities.

There resulted, then, the establishment of such well known schemes as the anaerobic glycolytic pathway (glucose to pyruvic acid) and the tricarboxylic acid or Krebs cycle, the essentially common route by which animal cells, utilizing oxygen, complete the metabolism of pyruvic acid to carbon dioxide and water. These biochemical schemes are the amazing outcome of the imagination, resourcefulness, and technical skills of hundreds of individual biochemists working over the course of decades. It is truly unfortunate that the bare skeletons of the processes are often thrust at students in elementary biology classes, without explanation or background development, and have thus become classic examples of material to memorize for examinations, then forget as fast as possible.

Some mechanisms for forming ATP

As noted above, in anaerobic metabolism a relatively small amount of ATP is formed—about 2 molecules for every glucose molecule entering the metabolic mill. The way in which this occurs is well established. A phosphate ion and a glucose molecule (6 carbon atom substrate) combine in a process catalyzed by a specific enzyme. The resulting molecule is altered and cleaved in a series of closely linked steps. Eventually a 3-carbon atom product is formed which we will call, for simplicity, A-P (it is actually D-glyceraldehyde-3-phosphate). This is then oxidized in a complicated, enzyme-catalyzed reaction in which two electrons are transferred (with accompanying protons) to NAD, and a second phosphate group is linked to the 3-carbon substrate. The result is a new molecule with one of the two phosphate groups in highly unstable linkage—in the terminology of the eminent biochemist Fritz Lipmann, this is a "high energy" phosphate bond. This product compound may now be designated B~P (it is D-1,3-diphosphoglyceric acid). Yet another specific enzyme effects a shift of the unstably linked phosphate group from B~P to ADP, forming ATP. This process is termed *substrate-linked phosphorylation*—substrate-linked, because A and B are actual participants or substrates in the metabolic processes; and phosphorylation, the addition of a phosphate group to another molecule (in this case, ADP).

But metabolism does not stop at this point if oxygen is available to the cell. The carbon chain of pyruvic acid is broken down

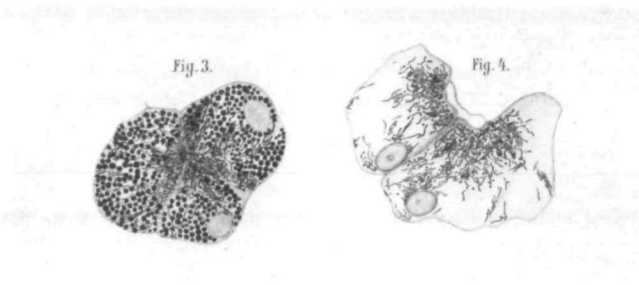

FIG. 6. Part of an illustration of cells in sections of the liver of a frog, *Rana esculenta.* In the left-hand drawing (Fig. 3) fat droplets colored with osmium fill the cell. These are absent in Figure 4, after lipid extraction, and the threadlike structures, later recognized as mitochondria, are clearly visible. (From Altmann, 1890, Plate III.)

completely to form carbon dioxide. The many steps in the process are designated as the familiar Krebs cycle (or tricarboxylic acid cycle). The protons and electrons removed from the substrate are eventually combined with oxygen, with water as end product. Many molecules of ATP are formed as well—about 38 for every glucose molecule that originally entered the metabolic sequence. This formation of ATP, dependent on the presence of oxygen, is termed *oxidative phosphorylation.* At a relatively early stage in the analysis of this process it was established that oxidative phosphorylation is quite different from substrate-linked phosphorylation, since phosphate is not found linked to the many intermediate products of the breakdown of the carbon chain beyond pyruvic acid. Then what is the mechanism of oxidative phosphorylation?

For several decades biochemists attempted to solve this problem using methods similar to those that had proved so successful in other phases of biochemical work—the unravelling of metabolic pathways including anaerobic glycolysis. Intensive searches were launched for enzymes and co-enzymes and other components involved in phosphorylation, and many indeed were found. But the crucial step—the link between oxidation (removal of electrons) and formation of ATP—proved elusive. Was there a substance A—a very reactive, hence very unstable intermediate, let us say—combining with phosphate and then converted to another fleeting intermediate, B~P, that could account for phosphorylation? Actually, some experimenters found evidence for a number of such systems, but none of these stood up to critical testing. Nevertheless, very important discoveries were made in the late 1950s by David Green, Albert Lehninger and others who showed that the processes of the Krebs cycle and oxidative phosphorylation do not take place in the cell cytoplasm, but only in mitochondria. In this respect they differ from the reactions of glycolysis which are localized in the cytoplasm.

Mitochondria and oxidative phosphorylation

Mitochondria, highly distinctive subcellular structures, are found in all eukaryotic cells. Their existence was first noted by microscopists at the end of the 19th century. At that time microscopes did not have enough magnifying power to give much information about these structures, but they were intriguing and stimulated controversy. The German anatomist Richard Altmann described peculiar particles, "Elementarorganismen" (elementary living organisms), and thought that they were of great significance, sharing life properties with bacteria (Altmann, 1890) (Fig. 6). These particles were later renamed mitochondria. At the time of Altmann's work his methods and observations were criticized as subject to error and artifact. But this is one of the many examples of early prediction of biological phenomena confirmed by later work. New histological methods and the development of electron

microscopy made possible clear observations on mitochondria, found to be actual and universal cell structures. Moreover, biologists generally agree now that mitochondria are indeed related to bacteria—derived through long evolutionary passage from symbiotic bacteria that invaded eukaryotic forms and became permanent and necessary components of the cells of modern eukaryotic organisms.

A word about the size of mitochondria may be in order here, because we now know so much about them that, in imagination, they tend to take on grossly enlarged dimensions. Choosing, somewhat arbitrarily, a rod-shaped mitochondrion 2.5 μm long and 0.2 μm across, one can estimate its volume and calculate how many similar mitochondria could be packed within a cube 1 mm on the side. The number is about 3 billion. Individual living cells contain scores to thousands of mitochondria, varying from cell to cell with type, size, age, and state of activity.

Mitochondria can be separated more or less intact from living cells in a number of ways. For example, cells suspended in fluid (adjusted carefully for ion and substrate composition, osmotic pressure and pH) are ground up, gently and at low temperature, to break the cell membranes and release their mitochondria into the surrounding fluid. The mitochondria can then be separated by centrifugation from other cell fragments, and subjected to varying conditions to control the chemical reactions occurring within them. Once it was established that mitochondria are the sites of oxidative phosphorylation, intense experimental energy was focused upon them. The amount of ATP formed in defined conditions, relative to oxygen used, offered clues to the fundamental processes involved. And mitochondria can themselves be broken down, yielding membrane fragments that seal up to form tiny, membrane-bounded sacks or vesicles. These, too, under rigidly defined conditions, can form ATP from ADP and inorganic phosphate ions.

Electron microsopy as well as biochemical and physical methods have shown mitochondria to have highly distinctive structure. They can be ovoid, rodlike, or threadlike, and may branch in complicated configurations. Their configuration can change with changing states of activity. They are bounded by two membranes—an outer membrane contiguous with the general cytoplasm; and an inner membrane which generally shows deep infoldings (cristae). Held within the inner membrane, as in a highly complex sack, is the matrix of the mitochondrion (Fig. 7). All of the enzymes, co-enzymes and other factors needed to carry out the reactions of the Krebs cycle are located in the matrix. Most of the elements of the machinery—enzymes, co-enzymes and other reactants—needed for oxidative phosphorylation are built into the structure of the inner membrane itself. And although this membrane is very impermeable, it contains special proteins (carriers) which can shuttle through its barrier, into or out of the matrix, such crucial reactants as substrates, ATP, and phosphate ions. In contrast, the outer membrane of the mitochondrion appears to be rather simple and inert with relation to function, for it is quite permeable to the molecules and ions that are involved in the various functions of mitochondria.

Mitchell's chemiosmotic hypothesis to explain oxidative phosphorylation

Perhaps a short digression would be appropriate here, to fill in some background behind this quite complex field of study. It may be hard for the student in an elementary biology course to imagine the intensity of heat and fervor that is generated by the search for the mechanism of oxidative phosphorylation. There are organisms that can live without oxygen, of course—blue green and other anaerobic bacteria, protozoans parasitic in vertebrate intestines, and so forth—but the great preponderance of life as we know it depends directly on oxidative phosphorylation. A dramatic illustration of this is the death within seconds which results if a person is poisoned with cyanide—a specific blocker of one link in the chain of processes needed for oxidative phosphorylation—or with carbon monoxide, which simply prevents

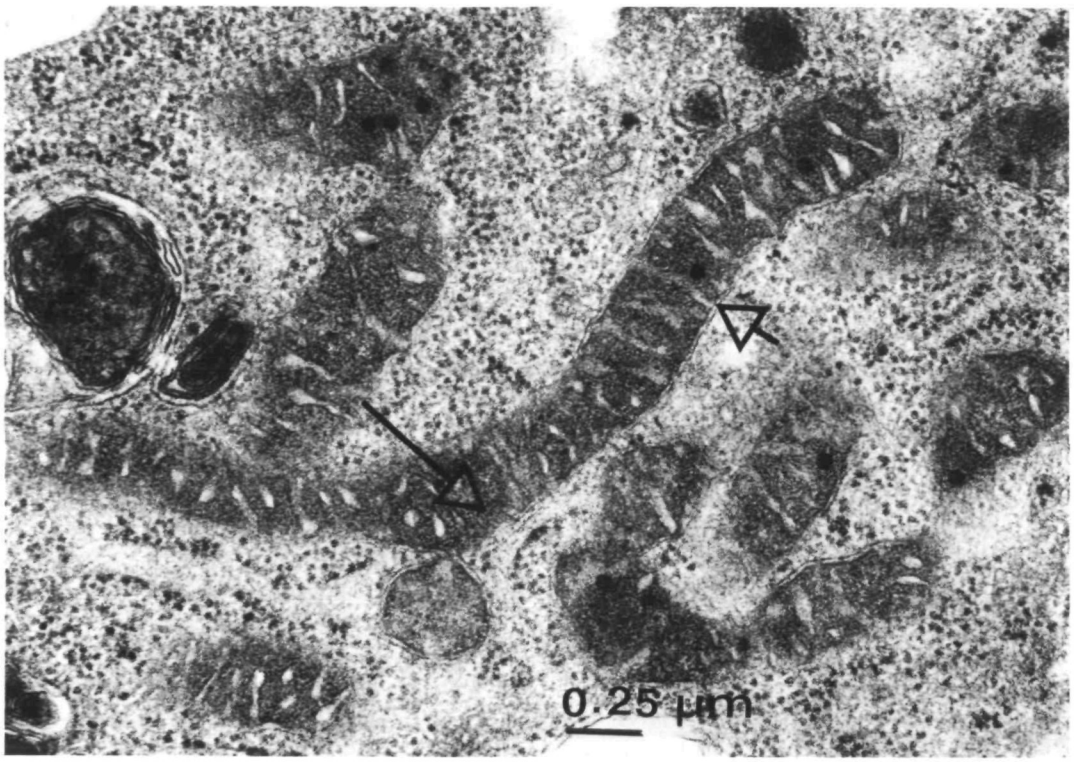

FIG. 7. Electron micrograph of mitochondria in the cytoplasm of a kidney cell derived from an infant hamster, and grown in tissue culture. The head of the short arrow points to the inner membrane close to an infolding forming a crista. The head of the long arrow is embedded in the matrix. Magnification indicated by the scale marker. (Micrograph by D. L. Luchtel.)

the hemoglobin of red blood cells from delivering oxygen to the tissues.

Because of the great importance of the fundamental problem, and its amazing resistance to attack and analysis, emotions run high in the study of oxidative phosphorylation. In a less lofty plane, moreover, the field is one of high status, offering to successful workers rewards in terms of recognition and support for research. Into this heated intellectual atmosphere, in 1961 Peter Mitchell, an English biochemist working more or less alone except for collaboration with Jennifer Moyle and a few others, introduced a wholly new approach. He questioned the very existence of high energy intermediates in oxidative phosphorylation, and proposed instead that the structure of the mitochondrion is central to the process. Moreover, he formulated a mechanism involving this structure. Hydrogen ions (protons) removed from substrates are pumped out of the matrix across the inner mitochondrial membrane. Within the matrix, oxygen, which has entered it by diffusion from the outside (*i.e.*, the cytoplasm), takes up electrons which previously have been transferred from substrates to NAD and thence to a complex series of reactants in the inner mitochondrial membrane (electron transport chain). Thus across the inner membrane there accumulate hydrogen ions, on the outer side, and on the inside reduced oxygen atoms (O^-, hydroxyl ions). This highly asymmetrical distribution of ions functions as an electrochemical battery, storing energy. In the utilization of this energy, hydrogen ions move back into the matrix across the membrane, according to

the electrical and concentration gradient through a complex enzyme system that has the capacity to bind ADP and inorganic phosphate, as hydrogen and hydroxyl ions unite to form water. In sum, the end results of metabolism are achieved—synthesis of ATP as a product of substrate usage, with water as waste product.

Peter Mitchell not only proposed this idea, but also pointed out that related mechanisms could account for the production of ATP by the complex membrane systems of bacteria, and by chloroplasts of plant cells. He designated this mode of phosphorylation as chemiosmotic—*chemi-*, because of the chemical bonds formed, and *osmotic* indicating that physical movement of protons was involved directly in the process of energy transfer (Mitchell, 1961, 1966).

Mitchell did not have experimental data on which to ground the chemiosmotic hypothesis solidly, and it was rejected by most other biochemists at the time. Nevertheless, this hypothesis proved to be exceedingly fruitful. Experiments designed to test it showed that there is, indeed, a gradient of hydrogen ions and an electrical potential difference across the mitochondrial inner membrane. Moreover, pharmacological agents which act by disrupting these gradients also prevent phosphorylation. The simple exposure of isolated chloroplasts to steep proton (pH) gradients without light or other external energy source, resulted in phosphorylation. Many other lines of evidence converged to confirm Mitchell's hypothesis, and by the late 1970s it was, in effect, accepted by the biochemical profession (Boyer *et al.*, 1977; Skulachev and Hinkle, 1981). In 1978 Mitchell received the Nobel Prize for this most significant contribution.

But this is not at all the end of the story. The chemiosmotic hypothesis is undergoing many tests and changes. Work continues intensively on problems of oxidative and photosynthetic phosphorylation, since the physicochemical details of the mechanisms of these processes, are still unknown except in barest outline. (Many further details are given in current textbooks of biochemistry and cell biology, *e.g.*, Alberts *et al.*, 1983; Darnell *et al.*, 1986; Lehninger, 1982.)

A perspective on oxygen, cell energy, and cell structure

This account began with seemingly simple questions—What is the function of the air taken in and expelled by an animal's breathing movements? Why is oxygen necessary for its life? The account then sketched 200 years or so of speculation and investigation, leading to the present concept of membrane, or chemiosmotic phosphorylation. Like many other aspects of biological science, the inquiry began with observations in which form and function were seen together, then followed a path of closer and closer biochemical analysis in which observations of structure came to seem more or less irrelevant. For years a criterion of success, in description of biochemical reactions, was the demonstration that they could be carried on *in vitro*, separated from the living system. A process was surely established if it could be reconstructed completely from purified enzymes, substrates, and other chemically identified materials. The validity and importance of this approach has been proved over and over again, as biochemists have discovered organic compounds and synthetic or degradative pathways for great numbers of biological processes. Among these, of course, is the glycolytic pathway for production of ATP from glucose in anaerobic metabolism. As this (as well as many other processes) is carried out by soluble enzymes of the cytoplasm (*i.e.*, enzymes not built into subcellular structures), the *in vitro* or isolation methods were fully appropriate for its analysis. As biological investigation advances, however, the study of function cannot be isolated from structure, even at the subcellular level. This is amply proved by the discovery of the crucial role of membrane organization in oxidative phosphorylation. Many of today's developments in the understanding of cell biology depend on coordinated approaches of biochemists, electron microscopists, and others in which process is seen as inextricably related to

structure. This statement holds true not only for mitochondria but also for the study of chromosomes, Golgi apparatus, lysosomes and other subcellular machinery carrying out the fundamental processes of life.

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Structures Built by Living Cells—The Shell of the Bird's Egg

The significance of form–function interactions does not end, of course, at the outer boundaries of living cells. An immense variety of non-living structures are formed at the interface of plasma membranes and the non-living surround—for example, capsules of bacteria, plant cell walls, mucous films, and shells, scales, feathers and fur. At greater distances from cell membranes are other characteristic constructs of organisms—examples, cited almost at random, include worm tubes and rodent burrows, wasp and bird nests, insect cocoons and spider webs, ant hills and termite mounds, and the clothing and buildings, simple or as cómplex as space vehicles, of human beings.

In each of these examples, highly specific structure can be interpreted directly in terms of the functions to be served. This is a richly diverse and intriguing area to investigate, offering students the chance to ask many questions that are still unanswered, and that yet relate directly to a variety of basic biological phenomena. The shell of the bird's egg can serve as an example. What is the structure, in physical and chemical terms, of the eggshell, and how is it built up by cooperation of the cells of the bird's reproductive organs? What functions does it serve? Does it vary under varying environmental conditions? How do eggshells differ from species to species or among members of different genera? What is the evolutionary history of this structure? In the following paragraphs some aspects of bird eggshell form and function will be outlined as background for this topic. References at the end of the section can serve as points of departure into this and other aspects of the fascinating area of construction by animals.

Among the 9,000 or so highly diverse species of birds, there is a common phenomenon of the greatest significance to the biology of animals of this Class. This is their reproductive pattern, with formation of fertilized eggs encased in calcareous shells. The structure of the shells, and the protection which they provide to the developing embryos, is of interest both for theoretical reasons and for practical applications in culture of birds as food sources and in conservation of wild species. Investigations of eggshell structure began in the 19th century, but the most useful results have only been obtained in the last few decades. The lack of early progress is understandable. Methods with which to analyze the construction of these hard and very thin shells (*e.g.*, 0.3 mm, in the case of the domestic fowl, *Gallus domesticus*) were not available until quite recently. Such methods include electron microscopy, precise physical techniques for measuring shell composition and exchange of heat and gases (water, oxygen, carbon dioxide) across the shell, as well as detailed observations in the field to describe the behaviors of parent birds necessary for successful development of the embryos.

The richly yolk-filled egg of the bird enters the (usually single) oviduct through the infundibulum, a funnel-shaped opening closely applied to the ovary's surface. The egg is fertilized by sperm which have penetrated this far. As the egg moves along the oviduct, it is layered with nutritive and protective coats of albumin, then with shell membranes built up of intermeshing networks of protein molecules. These membranes form a basis on which the shell is formed as the egg is carried along through the shell gland. The shell is made up chiefly of calcium carbonate in orderly crystalline arrays, embedded in a protein matrix and separated, at intervals, by spaces, or pores. The pores traverse the thickness of the shell, and may open freely to the outside, or be more or less occluded at the surface. In many eggs a delicate layer of inorganic

or organic materials (cuticle) covers the outermost surface (Fig. 8).

The eggshell protects the embryo from attack by bacteria and other small parasites. Its mechanical strength is important in preventing damage when the brooding parent sits on the eggs, or jostles them in the periodic turning procedure. As a whole, the egg is a partially closed system. It contains all the water and nutrients needed for development of the embryo, and the shell itself is a store of calcium and some other minerals needed for growth. But the embryo must carry on exchanges with the environment through the shell. Its development requires a continuing supply of oxygen, whereas carbon dioxide and water—products of metabolism—must be removed (non-gaseous metabolic wastes are stored in the allantois).

The shell has high conductivity to heat, a necessary feature since the temperature of the egg must be controlled from the outside. The embryo can develop successfully at only a very narrow range of temperatures, maintained as a result of activities of the parent(s) through prior structuring of the nest, and by brooding behavior. Treating the eggs like extensions of its own body, the brooding parent warms the eggs in a relatively cold environment, but draws off external and metabolic heat if the environment is hot.

Finally, it is through the barrier of the shell that the embryo receives information, in the form of sounds or mechanical movements, from the parents and adjacent eggs. This information may be used to synchronize hatching of the clutch of eggs, and in defense against predators (reviewed by Drent, 1975).

Students may find it interesting to compare the bird's egg with the vehicles designed by humans for space flight. The egg is less self-contained than the space vehicle, in that exchange of materials is required by the embryo but not by the space traveller. Both bird and human are critically dependent, however, on controlled exchanges of heat and information with their surroundings.

Obviously, many specific aspects of the structure of the egg require detailed functional analysis. The aspect which has been subjected to the most intensive investigation is the organization of the pores in the shell—their shape, distribution, number, and conductivity to gases. Herman Rahn, Charles Paganelli and co-workers, as well as many other scientists, have carried out important studies showing that these variables are closely linked with development and survival of the bird embryo. There are numerous excellent reviews of this and related work (Carey 1980; Metcalfe *et al.*, 1987). Briefly, several important generalizations have been established. In the range of eggs from the tiny (less than 1 g) eggs of humming birds to the massive (more than 1,400 g) eggs of ostriches, and in spite of considerable variation in rates of development, all eggs lose water at rates such that, from laying to hatching, the total water loss averages about 15% (mean; range 10 to 23%) of initial egg mass. Moreover, this rate of loss appears to be optimal, since hatching and survival of the young birds is reduced if the water loss is increased (*e.g.*, by exposure to a dry environment, when the embryo desiccates) or decreased (in a water saturated environment, when the embryo "drowns"). Oxygen intake into the

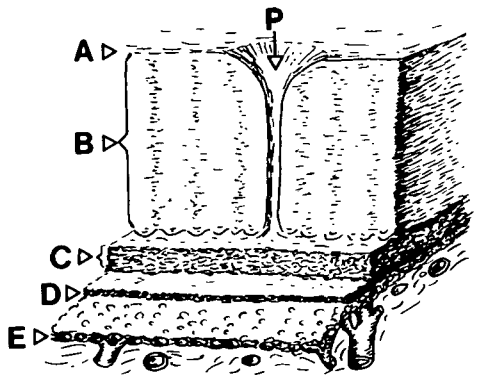

FIG. 8. Diagram of a single pore in a hen's eggshell, as if cut to show the structure of the shell. P = pore, with funnel-shaped opening to the outside. A = outer surface where the extremely thin cuticle is located. B = calcareous layer, *ca.* 300 μm. C = outer shell membrane, *ca.* 50 μm. D = inner shell membrane, *ca.* 15 μm. E = chorioallantoic capillary mesh, underlying the inner shell membrane at later stages of development. A hen's egg has about 10,000 pores such as this. (Redrawn from Wangensteen *et al.*, 1970.)

egg, and carbon dioxide output, are remarkably uniform also, over the spectrum of bird species and egg sizes. Rahn and co-workers have measured the conductances of these gases and found them to be predictable in terms of total pore area (estimated from numbers and sizes of pores). The rate of growth of the embryo is correlated with the gas conductances of the shell (Ar and Rahn, 1985).

Within this general framework of similarity, variations occur among eggs in relation to ecological and other factors. For example, the studies of Cynthia Carey and co-workers (summarized in Carey, 1980) illustrate the importance of control of pore structure in the case of birds that reproduce at high altitude. The rate of diffusion of gases is inversely proportional to atmospheric pressure, so the egg laid at high altitude would be expected to exchange gases faster than a similar egg at sea level. Since the partial pressure of oxygen decreases with altitude, the availability of external oxygen to the high altitude egg is reduced. The two factors—diffusion rate and availability—act in opposite directions from the egg's point of view, and may tend to cancel one another as far as oxygen exchange is concerned. Moreover the mountain-dwelling embryo, at least at later stages of development, can compensate for decrease in oxygen delivery through the shell by adjusting the oxygen carrying capacity of its blood (reviewed by Black in Carey 1980).

The situation with water is quite different from that with oxygen. The partial pressure of water vapor within the egg depends on the egg's temperature. This is regulated at a relatively constant level by the parent bird, whether at low or high altitude. Since water diffusion is accelerated at high altitude, there is a tendency for the egg to lose water relatively rapidly in these conditions. This could result in dangerous dehydration of the embryo. Carey has found, however, that female birds, when laying at high altitude, produce eggs with significantly reduced rates of water loss. Possible explanations for the decrease in percentage water loss of such eggs include, for example, increased thickness of the shell, increase in water content, decrease in temperature of the egg, or decrease in pore surface area. The results indicate that the last possibility—a change in the actual structure of the eggshell—accounts for the observation. Astonishingly, it appears that the bird lays eggs with pores appropriate to the atmospheric pressure of its immediate environment, and that an individual bird can change egg pore area in response to a move from a particular altitude (*e.g.*, high) to another (*e.g.*, lower). Such results have been obtained with domesticated hens, and they are corroborated by suggestive though incomplete data from wild birds as well. So far, it is not known how birds can recognize the need for, and effect the physiological responses of the oviduct and shell gland which must occur to produce such changes in pore area.

Besides variations in altitude, other conditions, both normal and pathological, affect the structure of the eggshell. For example, birds that habitually nest over water (*e.g.*, the Pied-billed Grebe, *Podilymbus podiceps*) have eggs with exceptionally large combined pore area, facilitating loss of water to the relatively moist air surrounding them. Megapode birds of Southern Pacific areas (*e.g.*, the Australian Mallee Fowl, *Leipoa ocellata*) brood their eggs by burying them in mounds heated by rotting vegetation (or, rarely, with geothermal heat). In the case of these birds the shell is exceptionally thin, permitting faster outward diffusion of metabolic water (reviewed by Drent, 1975). Pathology of bird eggshells is well known, too. In flocks of domestic hens deprived of adequate dietary calcium, the eggshells are poorly formed and fragile. A relatively recent environmental problem is the poisoning of birds that have fed in areas contaminated with pesticides (DDT and others). In these cases the shell glands do not function normally. Excess phosphate and magnesium ions are deposited, displacing and disorganizing some of the calcium in the crystalline arrays of the shell. The shell is weakened so the egg is easily crushed during brooding or with other mechanical stresses (Fox, 1976; Cooke, 1979; Board and Scott in Carey 1980; Risebrough, 1986).

In this, as in all other cases of non-living structures built by animals, it is very obvious that form and function are inextricably interrelated. Perhaps a less obvious conclusion is that each structure subserves many interrelated functions. As the diverse systems are studied in detail, more and more aspects of form–function are revealed. For example, the bird's eggshell obviously affords the embryo mechanical support and protection. It is less obvious that, somewhat like the walls of the traditional gingerbread house, the shell also provides calcium (and other materials) needed for the embryo's development. The beautiful integration of form and function resulting from control of gas exchange by the number and character of pores and the thickness of the shell was not discovered until thorough studies had been made on both the structure and the gas conductances of eggshells of many species of birds. Explorations into other systems, such as those in the reference list below, offer many ways of extending these generalizations.

References

Bird eggshell. Ar and Rahn (1985), Carey (1980), Cooke (1979), Drent (1975), Fox (1976), Metcalfe *et al.* (1987), Paganelli *et al.* (1987), Rahn and Paganelli (1979), Rahn *et al.* (1987), Risebrough (1986), Wangensteen *et al.* (1970–1971).

References in the broad area of structures built by animals are vast in number and extremely diverse in content. The selection given here is meant only to serve as an introduction:

General aspects. Collias and Collias (1976), Hansell (1984).

Feathers and hair. Chapman (1984), Sawyer *et al.* (1984), Stettenheim (1972).

Insect hives, nests. Dumpert and Johnson (1978), Seeley (1985), Spradbery (1973).

Mollusc shells and egg cases. Pechenik (1986), Simkiss and Wilbur (1977), Vermeij and Dudley (1985), Watabe (1983), Wind and Wise (1976).

Mucus. Chantler *et al.* (1982), Elstein and Parke (1977), Prezant (1985).

Nests—bird, mammal. Aeschbacher and Pilleri (1983), Collias and Collias (1984), Montgomery and Gurnell (1985).

Spider webs. Foelix (1982), Nentwig (1987).

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