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A 2000-year history of nephrology: 10 enduring scientific landmarks

Leon G. Fine

Department of Biomedical Sciences Cedars-Sinai Medical Center and University of California, Los Angeles, California - USA

INTRODUCTION

As interesting as a continuous and complete coverage of an area of evolving scientific thought may be, it is equally intriguing to attempt to pluck out of this history, a small number of contributions which have held up over time and which have allowed a field to lurch forward in a way which appears to be understandable. This analysis of 2 thousand years in the history of nephrology attempts to do just that. By *nephrology* is meant the field which encompasses the structure and function of the kidney, its role in homeostasis, its diseases and the attempts by the medical profession to prevent, cure and manage such diseases.

Events which are recent tend to assume an exaggerated importance which could wane substantially over the passage of time. So, in looking back over 2 millennia to decide what constitutes an "enduring contribution," it is surprisingly easier to judge older contributions than newer ones. Accordingly, less coverage is devoted to the more recent contributions.

Ten seminal contributions are presented, which have moved the field of nephrology forward in a substantial way. A few intervening events are included where appropriate. What emerges, somewhat disturbingly, is that nothing of real substance may occur over large spans of time, despite much industry and activity and many publications. Each step is characterized in this history as an "understanding" so that the evolution of the ideas has continuity.

CONTRIBUTION 1: THE UNDERSTANDING THAT THE KIDNEYS ARE THE SOURCE OF URINE

Galen of Pergamum (131-201 AD), *On the Natural Faculties and the Usefulness of the Parts of the Body*, ca. 150-200 AD

Against the backdrop of Aristotle who held that diseases can be understood through an understanding of different humors, there arose the remarkable Galen of Pergamum (1). Galen was a Greek physician who spent time in Alexandria and moved to Pergamum to become physician to the gladiators, a fact which must have advanced his understanding of human anatomy and surgery considerably! Galen's ideas held sway in Western medicine for a millennium and a half. He was the very first "experimentalist" in the modern sense of the word, and he devised the first notion of a system of human physiology (2).

Although Galen's system of physiology was fundamentally flawed, it persisted as a dogma because no other experimentalist arose to refute it on the basis of hard observations. Galen pointed out that every butcher is aware of the fact that the kidneys are connected to the bladder by the ureters. He further pointed out that people who suffer from dysuria or retention of urine experience pain in the loins, which points to a connection between bladder and kidneys. This simple fact, says Galen, clearly refutes the "ingenuity" of Asclepiades (1st century BC), who believed that the bladder is a sponge or a piece of wool that absorbs vapors formed from ingested fluid and converts it

into fluid. Galen was incredulous that the "Compact and impervious nature of the bladder" with "two strong coats" was not apparent to seemingly observant people: "Why do vapors not pass through the peritoneum and the diaphragm by this analogy and fill the whole abdominal cavity and thorax with water?" "How does one explain the fact," he asked, "that when the bladder is filled with water or tied at its neck and squeezed all round, it does not let anything out but retains its contents? Does this not refute the idea of inlets via the ureters?" (2).

To answer this question, Galen experimented on a living animal, in perhaps the first well-devised renal physiology experiment described. In Book I, chapter 13, of *On the Natural Faculties* (1), he wrote:

> Now the method of demonstration is as follows. One has to divide the peritoneum in front of the ureters, then secure these with ligatures, and next, having bandaged up the animal, let him go (for he will not continue to urinate). After this, one loosens the external bandages and shows the bladder empty and the ureters quite full and distended – in fact almost on the point of rupturing; on removing the ligature from them, one then plainly sees the bladder becoming filled with urine.
>
> When this has been made quite clear, then, before the animal urinates, one has to tie a ligature round his penis and then to squeeze the bladder all over; still nothing goes back through the ureters to the kidneys. Here, then, it becomes obvious that not only in a dead animal, but in one which is still living, the ureters are prevented from receiving back the urine from the bladder. These observations having been made, one now loosens the ligature from the animal's penis and allows him to urinate, then again one ligatures one of the ureters and leaves the other to discharge into the bladder. Allowing, then, some time to elapse, one now demonstrates that the ureter which was ligatured is obviously full and distended on the side next to the kidneys, while the other one – that from which the ligature has been taken – is itself flaccid, but has filled the bladder with urine (1).

In these simple experiments, Galen showed that fluids cannot pass through the wall of the bladder, and that urine enters via the ureters but cannot reflux back into them.

To the question "How is the urine formed in the kidneys and how is it propelled to the bladder?" Galen responded with 2 alternatives: either the kidneys "attract" urine, or the veins exert a propulsive action on the kidneys. He pointed out that if the latter alternative were true, not only urine but also the whole of the blood would be squeezed into the kidneys. Because this does not occur, he concluded that the kidneys exert their own attraction on urine. Furthermore, he says:

> If the kidneys are like sieves and readily let the thinner serous portion [i.e., urine] through and keep out the thicker portion, then the whole of the blood contained in the vena cava must go to them just as the whole of the urine is thrown into the filters. Thus it is that, if the blood-serum has to percolate through the kidneys, the whole of the blood must come to them and not merely one part of it (1).

Remarkably, here we hear an idea raised 2 thousand years ago which suggests that the blood may be filtered into a thick and a thin component.

Before leaving Galen, this insight of his is worthy of consideration:

> The amount of urine passed every day shows clearly that it is the whole of the fluid drunk which becomes urine, except that which comes away with the dejections or passes off as sweat or insensible perspiration. This is most easily recognized in winter in those who are doing no work but are carousing, especially if the urine be thin and diffusible; these people rapidly pass almost the same quantity as they drink (1).

Galen concluded that function of the kidneys is to "reduce the blood of its watery portion."

Nothing much was added to the ideas of Galen over the ensuing millennium. The Middle Ages saw physicians assigning diagnoses and prognoses according to the color and appearance of the urine, a practice called uroscopy. Elaborate schemes and diagrams supported such practices in the literature.

CONTRIBUTION 2: THE UNDERSTANDING THAT BLOOD CIRCULATES IN A CONTINUOUS CYCLE THROUGH THE ORGANS AND TISSUES

William Harvey (1578-1657), *An Anatomical Disquisition on the Motion of the Heart and the Blood in Animals*, 1628

One thousand five hundred years elapsed between the writings of Galen and those of William Harvey. Harvey did not write about the kidneys, nor did he write about

the body fluids. So why include him here? What is the process whereby a single individual establishes a new paradigm in science? How did Harvey arrive at a realization which changed the fundamental understanding of how the human body works?

The simple answer is that the revolution in thought which Harvey led was so profound that no history of medicine, in any field, can be written without including the understanding that blood circulates through the organs and delivers its constituents to such organs (3). Harvey's magnum opus, the *De Motu Cordis* was published in 1628 (4). One has to remember that physiology in the 1600s was still derived from Galen. Overturning this was no mean feat, which is why it took Harvey a decade from the time of his understanding of the circulation, to willingly publish his "new concept of the heart's movement and function of the blood's passage around the body." It took a further 25 years for his peers to accept his conclusions!

Perhaps part of his genius lay in understanding how to establish the facts. For, first and foremost, Harvey was an experimentalist. His knowledge of parts of the body came only by comparing animals of different species, whether winged, terrestrial or aquatic, viviparous or oviparous. He almost certainly engaged in the dissection of many species. But not only did he dissect dead animals, he vivisected them too. It was his vivisections that lay at the heart of his ability to understand function. But there were other unique attributes which Harvey undoubtedly possessed. Not only did he learn from all species, but he understood that much could be learned from studying development. His experimental method, furthermore, employed interventions including ligatures, inflation techniques and injections, many of which were entirely novel in concept. Finally, he was able to think quantitatively, the oft-quoted example being his calculation of cardiac output from the product of stroke volume and pulse rate. This led to his conclusion that the only way this large volume of blood could be accommodated was not by formation and destruction but by constant recirculation.

Let it not be thought that Harvey was an iconoclast who brushed aside everything from the past. He subscribed to the conventional idea that the main function of the heart was the distribution of heat. Only later did he see the blood as the vehicle for this process. He also accepted that the function of the lungs was to deliver blood to the heart in order to relieve it of its excess heat. In this scheme, the heart acted to distribute heat, and the lungs acted to cool the instrument of the heart – i.e., the blood. Furthermore, he never denied the notion that the lungs concoct blood and "spirit," as do the heart and liver, and following Aristotle, he equated warmth with perfection and related this to the nobility of animal species. He also believed in the attractive power of parts of the body.

What did Harvey discover? The dogma at the time was that blood ebbs and flows in the great veins connected to the right side of the heart, with some blood percolating through the septum. There it combined with "spirit" (pneuma) and was turned into arterial blood, which was then distributed to all parts. While the passage of blood from the right ventricle through the lungs to the left ventricle may have been feasible due to the porous nature of the lungs, this same concept was not intuitively applied to the greater circulation – blood was also thought to ebb and flow in the arteries.

By direct observation in living animals, Harvey observed that, upon contraction (systole), the heart lifts upward to strike the chest, becoming harder and paler. He concluded that the size of the ventricle decreases and that "blood flows out as the heart erects." He proved this by making an incision in the ventricle from which blood spurted. What had previously been inferred, Harvey confirmed by experimentation, including observations on cold-blooded animals with slower heart rates which allowed for more precise measurement.

Essentially Harvey established with certainty (i) that ventricular systole (and not diastole) causes the apex beat, (ii) that ventricular systole corresponds to arterial diastole (expansion) and (iii) that blood flows from right ventricles to left ventricles through the lungs.

Blood delivered from the heart enters the kidneys via its arteries and leaves via its veins. (Harvey did not explicitly address the kidneys.) What its functions are in this context was left for others to discover. Harvey's disciples and followers in England gradually came to realize that blood contains a constituent which is essential for life and that this constituent enters it via the lungs. It took another century for Joseph Priestley to discover oxygen. No attention was paid to the kidneys!

In addition to these fundamental discoveries, Harvey created a method for conducting biological experiments which endured for centuries. The essential points of his method were (i) phrase a pertinent question, (ii) make continuous observations over time, (iii) perturb the system to expose hidden properties, (iv) seek additional examples in biology, and (v) design an experiment which could yield a contrary result. This approach is as relevant today as it was at the time of Harvey.

CONTRIBUTION 3: THE UNDERSTANDING THAT THERE IS A FINE STRUCTURE TO THE KIDNEYS AND THAT THIS PROVIDES AN INSIGHT INTO HOW IT MAY FUNCTION

Marcello Malpighi (1628-1694), *De Viscerum Structura*, 1666

The landmark anatomical treatise of Andreas Vesalius (1514-1574) paid little attention to the kidneys (2). Eustachius, in 1564, in his *Opuscula Anatomica,* illustrated the multipapillated human kidney with a pelvicaliceal system (he was the first to observe the adrenal glands). In describing the substance of the kidney, he said: "For my part, I think that there are certain furrows and small canals in the substance of the kidney which are caused only for flowing liquids and fluids. It is through these that urine is filtered into the renal cavity" (2). Lorenzo Bellini (1643-1704), an Italian anatomist, also introduced the concept that the kidneys are made of a hard, fleshy substance, and also spoke of "fibers that are continuous from the outermost surface to the bellow of the pelvis" (2). When these filaments (tubules) were compressed, Bellini observed water welling up everywhere. With the bravado of the true investigator, he showed that "if you are not afraid to present this to your tongue you will discover a certain saltiness and, in some, the taste of urine." He observed that the same "juice" arises from a kidney that had been sectioned. With the use of a magnifying lens, he observed that when tubules are compressed, "the urine is very clearly seen welling out as if pushing forth from so many little water pipes.... From these things one can confidently infer that the substance of the kidney ... is nothing else than ... a mass of canalicular and capillary spaces through which urine flows into the pelvis."

Only 4 years after Bellini's description of the kidney, Marcello Malpighi (1628-1694) published his classic description of human anatomy in *De Viscerum Structura* in 1666 (5), in which there was a section titled "De Renibus" (On the kidneys) (Fig. 1).

One of the most important contributions made by Malpighi was to demonstrate the existence of capillaries, using a frog's lung preparation. This was the missing link in Harvey's description of the circulation.

Malpighi devoted a whole section of his anatomy to the kidneys. This work was a landmark in understanding renal function because of its great detail and clarity, and was unsurpassed for nearly 2 centuries afterward. So precisely were the kidneys described by Malpighi, that he apparently did not feel the need to use illustrations. This is indeed surprising in light of the magnificent plates that accompany his embryological and botanical works.

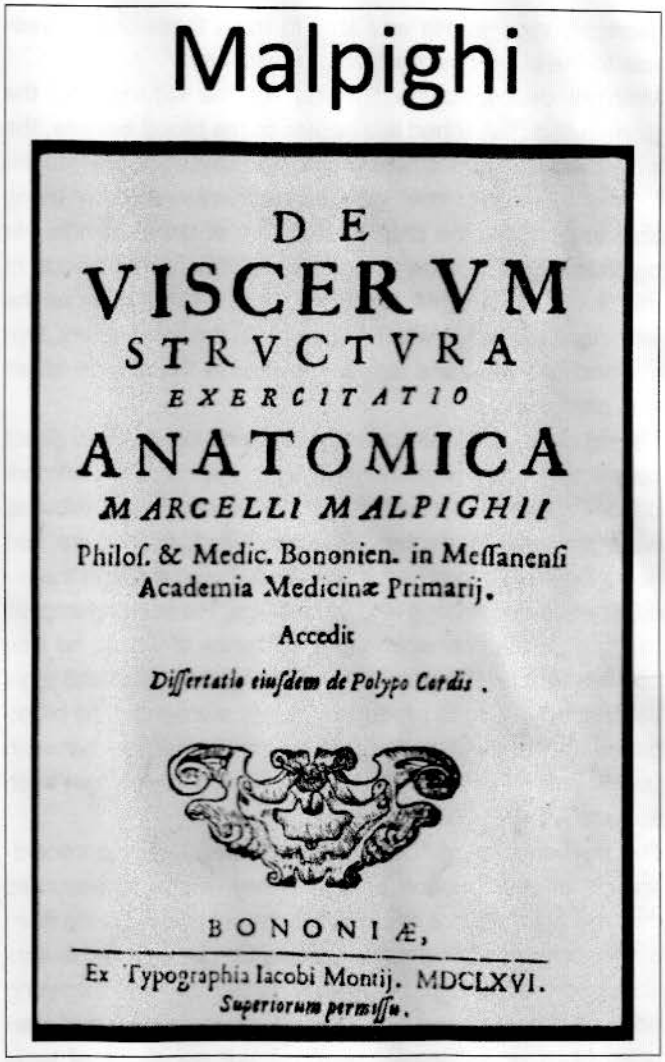

Fig. 1 - Title page to Marcello Malpighi's *De Viscerum Structura* (1666), on human anatomy, in which a section on the kidney ("De Renibus") appeared.

Malpighi first described the lobular appearance of the external surface of the kidney and correctly concluded that this is the vestige of the lobular structure of the fetal kidney. The interior of the kidney is also clearly divided into many-sided pyramids, each with a discrete blood supply. He then dramatically described the "very small round bodies, like a coil of small worms" on the surface of the kidney. These were seen to be attached to the tortuous vessels (i.e., tubules) that, "after short, sharp convolutions close to the outer surface, run in a straight course toward the pelvis" (5). In examining the substance of the renal cortex, Malpighi noted

excretory vessels. He was able to trace these urinary vessels to the outermost surface of the kidney.

Malpighi described the "glands" of the kidney (i.e., the glomeruli) as "attached like apples to the blood vessels, the latter swollen with the black liquid and stretched out into the form of a beautiful tree" (5). He was frustrated at not being able to perceive the precise structure of these glands. He considered them to be surrounded by the terminal buds of the blood vessels, thus being stained the same color as the vessels when a dye was perfused into the renal artery. Dye injected into the veins similarly arrived at the glands, albeit less predictably.

Having concluded that both arteries and veins are in direct connection with the glomeruli, Malpighi addressed the more difficult question of whether the urinary vessels (i.e., tubules) were similarly connected. He acknowledged that he had never been able to observe liquids perfused through the renal arteries penetrating into the tubules. He even attempted to highlight the connection (the existence of which, he was convinced, must be present) by ligating the ureter and renal veins of a live dog to produce a kidney swollen by the blood driven into it. Despite the fact that a connection between glands and urinary vessels was suggested, it was "not such as satisfied the senses in all particulars."

The final chapter of "De Renibus" is devoted to a consideration of the function of the kidney. Malpighi assumed that the glands were responsible for separating urine from blood; however, the precise mechanism by which this was achieved eluded him. He clearly stated that the excreting bodies probably have little pores through which small particles (salty or sulfurous) can pass but not those of large size or different shape. All particles of use to the organism are retained, whereas those of no use are eliminated. He supported this contention by observing that when blood is thinned, urine may become bloody; when it grows thicker bleeding subsides.

Contribution 4: The realization that there are different forms of kidney disease, which can lead to disorders of multiple organ systems

Richard Bright (1789-1858), *Reports of Medical Cases: Selected With a View of Illustrating the Symptoms and Cure of Diseases by a Reference to Morbid Anatomy*, 1827

Richard Bright worked as a physician in Guy's Hospital, London. His contribution was seminal in that he organized and elaborated information and presented it in a way that persuaded the medical community that expertise in diseases of the kidney was necessary and that clinical observations linked to pathological correlations exposed different patterns of disease. If the disease were better understood, the appropriate treatment would be more likely to be selected.

Bright employed detailed clinical histories and physical examinations of his patients coupled to an analysis of the urine for the presence of albumen (6). He confirmed in a large series of patients that the existence of albumen in the urine is a sign of kidney disease. His publications described individual patients in terms of the onset and progression of their symptoms and, since death was a common outcome, the autopsy findings, which revealed multisystem disease.

The following description of a patient typifies Bright's style of observation:

> CASE VII
>
> Elizabeth Stewart, aged about 40. This woman, who appeared to have been exposed to the difficulties and temptations of the lower classes, had for eight years been subject to slight attacks of dropsy; during which time she had twice been in the London Hospital laboring under this disease, and had received relief. She ascribed her present attack to great exposure about a year ago, having walked in the rain from Deal to Gravesend without afterwards putting off her wet clothes. She was admitted Guy's in October 1826, greatly swollen with anasarca, the serum running from her legs: she passed but little urine, and her breathing was greatly oppressed. She first particularly attracted my attention November 25. At the time she had been taking the Pil. Scillae cum Hydrargyro till her mouth was very sore, combined with other diuretics: all her symptoms were greatly improved; the swelling had nearly subsided. Urine increased to nearly three pints in the twenty-four hours; pretty clear and natural in appearance: but from the history she gave of herself, her pallid cachectic appearance, and the soft unnatural feel of her flesh, I was led to suspect that this might be one of those cases in which the urine would coagulate, and probably the kidneys proved diseased. Accordingly, on the application of heat to the urine I found that it *coagulated very considerably*: and she stated that for the last six months she had experienced a good deal of pain and uneasiness in her loins.
>
> The improvement she had experienced was but temporary. In about a week the urine again become most exceedingly scanty; the quality varied much. On the 10th of December I found it to be scanty and clear, but *coagulating by heat, becoming first milky and then loaded with a great num-*

ber of flakes. She spoke very decidedly as to feeling at all times a *pain, weight, and weakness across her loins*. There was after this time frequent evidence of inflammatory action going on within the chest, and of effusion into the cavities, which led to several changes in the medicine, and to the application of blisters. – Jan. 2nd. She did not pass above an ounce of urine in the night. On the 3rd there were about four ounces, coagulating freely; and on this Dr. Bostock was so kind as to make some experiments.

Jan. 18th. She has been growing decidedly worse for the last three days: before that time she had been so much better as to be sitting up the greater part of the day. She is now confined to her bed, can scarcely lie on either side: her abdomen begins to swell, and her hands are oedematous; she has a frequent dry cough; her face is puffy. Urine scanty, and she complains of pain all around the lower part of the body.

27th. Evidently sinking, complaining much of pain passing through from the chest to the back; sits nearly erect; coughs, and expectorates a tough mucus slightly tinged with blood. – She died on the following morning.

SECTIO CADAVERIS

We were not permitted to examine the chest. In the abdomen three or four pints of clear serum were effused. The liver was slightly lobulated in its appearance, and the acute margin rounded; the peritoneal coat a little thickened. The substance of the liver rather increased; the acini light-coloured, not projecting the least; the intervening substance of a brighter red than natural. Gall-bladder rather small, but containing well colored bile. Kidneys small, rather lobulated, of a semi-cartilaginous hardness, completely granulated; the small whitish or yellow granules projecting with red intervening spaces, so as to form a scabrous surface, both appearing and feeling rough. On making a longitudinal section, the kidney cut with the resistance of a schirrous gland; the tubular part was drawn much nearer to the surface than is natural; the cortical part indistinctly granulated throughout, of a grayish drab about mixed with purple (6).

Bright himself conducted the autopsies with an artist present to record the appearances of the organs (Fig. 2).

Bright was to conclude that there are more than 1 form of chronic kidney disease. He thought that there were at least 3. His approach was to be enduring. It has been modified by new diagnostic technologies including biopsy, chemical tests and imaging, but the basic approach will remain fundamental to the practice of nephrology, if it ultimately survives as an independent discipline.

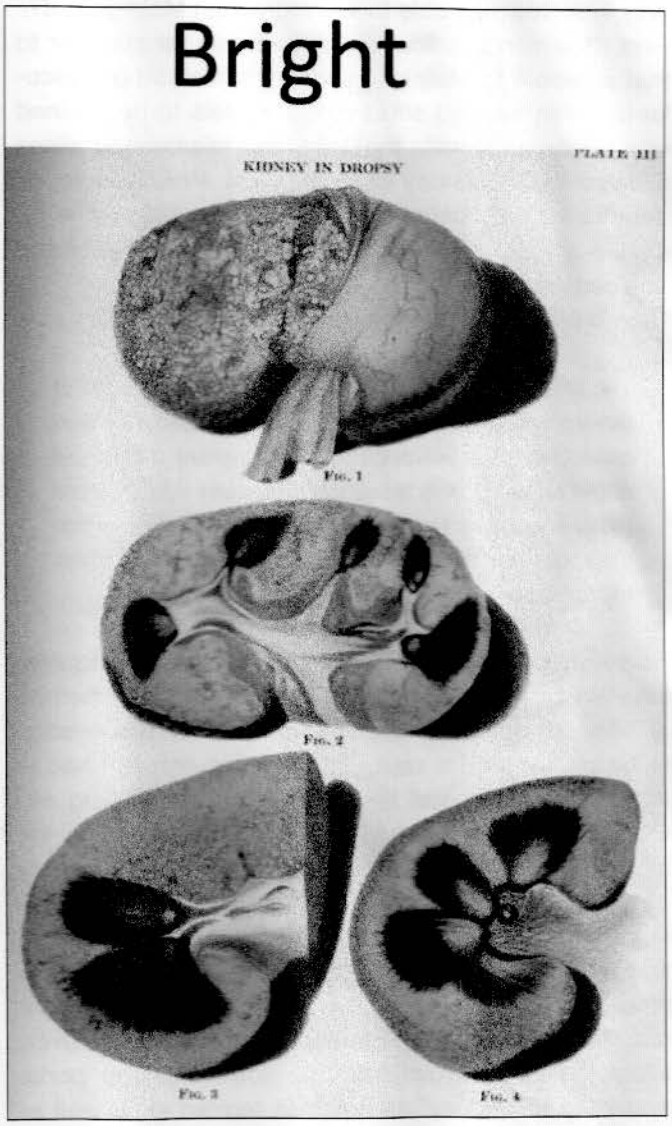

Fig. 2 - Richard Bright's depiction of the kidneys in dropsy, 1827 (6).

CONTRIBUTION 5: THE REALIZATION THAT THE GLOMERULAR CAPILLARY TUFT ACTS AS A FILTER WHICH IS PART OF A DISCRETE ANATOMICAL UNIT

William Bowman (1816-1892), "On the Structure and Use of the Malpighian Bodies of the Kidney, With Observations on the Circulation Through That Gland," 1842

Bowman's description of the nephron (as we now call it) was the most definitive contribution to the microscopic anatomy of the kidney and remains accurate in all of its details (7).

Why was Bowman able to see more than Malpighi did? First, Bowman's optical equipment was far superior to that available to Malpighi; second, a method of vascular injection allowed small blood vessels to be outlined with clarity; and finally, the use of thin microscopic slices allowed the continuity of anatomical structures to be determined with certainty. Bowman's investigations included a wide variety of species, including parrots and boa constrictors!
Here is his classic depiction of the human nephron:

> The Malpighian bodies I saw to be a rounded mass of minute vessels invested by a cyst or capsule of precisely similar appearance to the basement membrane of the tubes. Seeing these similar tissues in such close proximity, it was not easy to resist the conviction that the capsule was the basement membrane of the tubes expanded over their vessels (7).

However, Bowman was unable to gain an unequivocal view of this continuity. It was here that the method of vascular injection allowed the critical observation to be made, for, he says, "the injected material had in many instances burst through the tuft and, being extravasated into the capsule, had passed off along the tube" (7). He was now able to construct the anatomy of the nephron with great clarity, and the single plate in his paper (7), containing 17 separate figures, illustrates more effectively than many lines of text how precisely he appreciated the structure of the nephron. The afferent and efferent arterioles, respectively supplying and emerging from the glomerulus (the "portal system of the kidney" as Bowman termed it), as well as the peritubular capillary plexuses are shown with clarity and precision. His graphic depiction of the human nephron is unsurpassed (Fig. 3).
Did Bowman's revealing anatomical insights lead him to an appreciation of the function of the glomerulus? His own words could not be more explicit:

> It would indeed be difficult to conceive a disposition of parts more calculated to favor the escape of water from the blood than of the Malpighian body. A large artery breaks up in a very direct manner into a number of minute branches each of which suddenly opens into an assemblage of vessels of far greater aggregate capacity than itself and from which there is but one narrow exit. Hence must arise a very abrupt retardation in the velocity of the current of blood. The vessels in which this delay occurs are uncovered by any structure. They lie bare in a cell from which there is but one outlet, the orifice of the tube.... Why is so wondrous an apparatus placed at the extremity of each uriniferous tube if not furnish water to aid in the separation and solution of the urinous products from the epithelium of the tube? The secretion is brought from the tubules of the gland in a fluid state and only becomes solid by the reabsorption of its aqueous portion after it has traversed the tortuous canals where it was formed (7).

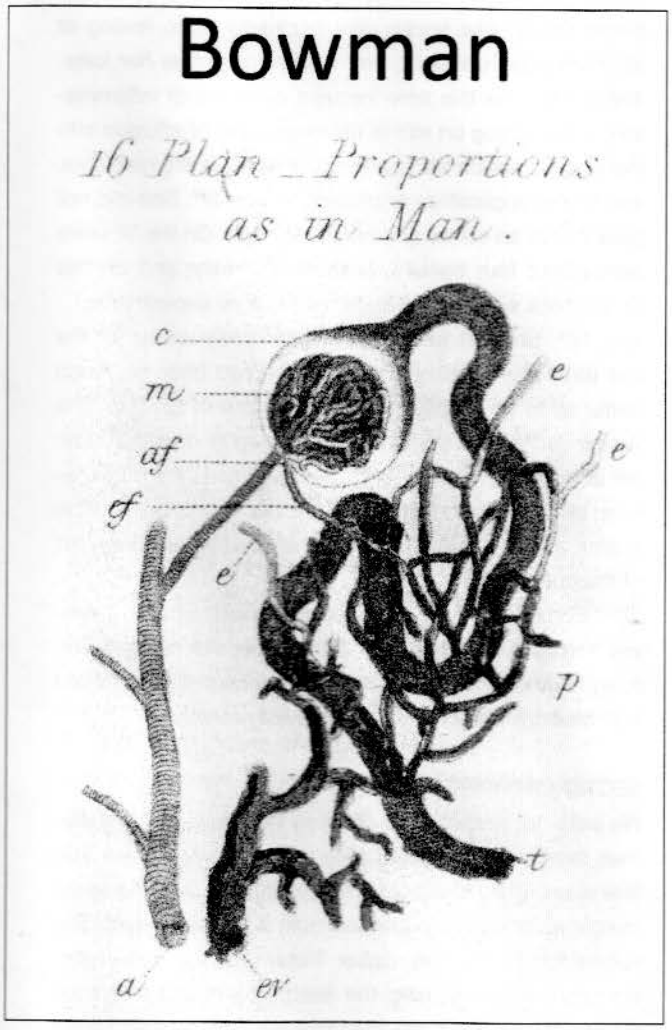

Fig. 3 - William Bowman's depiction of the anatomy of the human nephron, 1842 (7).

Although Bowman did not articulate the concept of filtration in physiochemical terms, his clear understanding of the existence of 2 separate processes now recognized as glomerular filtration and tubular reabsorption, and his precise description of the anatomy of the nephron, became the basis for all future studies on the physiology of the kidney.

CONTRIBUTION 6: THE REALIZATION THAT GLOMERULAR FILTRATION CAN BE EXPLAINED ON PHYSICAL PRINCIPLES: A BIOPHYSICAL PROCESS OF ULTRAFILTRATION

Carl Friedrich Wilhelm Ludwig (1816-1895), *Lehrbuch de Physiologie des Menschen*, 1852 and 1856

A new vision of physiology was emerging in the mid-19th century. In the words of Carl Ludwig (8):

> In accordance with this experience, it is concluded that all the phenomena of the animal body are the consequence of simple attractions and repulsions (between a limited number of chemical atoms) such as can be observed when these elementary components collide. This conclusion will be irrefutable, when it is proven, with mathematical precision, that the above mentioned elementary conditions are so ordered, with respect to direction, time and mass, in the animal body that all the accomplishments of the living or dead organism must, of necessity, follow from their interactions.

Ludwig's experiments had revealed to him that the fluid component of blood can cross a semipermeable membrane in both directions and that this occurred through minute openings. However, for protein-containing solutions, this process falls off, because, he presumed, the pores are becoming clogged up. But the rate of filtration was found to increase as the pressure increased. Since the laws of hydraulics were well known, Ludwig was able to predict the hydrostatic pressures and flows in the glomerular and peritubular vascular components of the kidney which he depicted with clarity (Fig. 4).

Since the urine was known to be derived from blood, and the concentrations of its constituents are not altered during filtration, any change in concentration in the final urine must be due to entry or exit of filtration from the filtrate. Ludwig's rigid application of physiochemical principles put to an end any other "vitalistic" theories which were extant. He was able to speculate that glomerular filtration might be regulated by contractions or dilations of the afferent and efferent arterioles.

Ludwig was also able to propose that reabsorptive and secretary processes must take place in the renal tubules. It subsequently became clear that these latter processes could not be explained solely according to the laws of hydrodynamics, as Ludwig postulated at the time. He

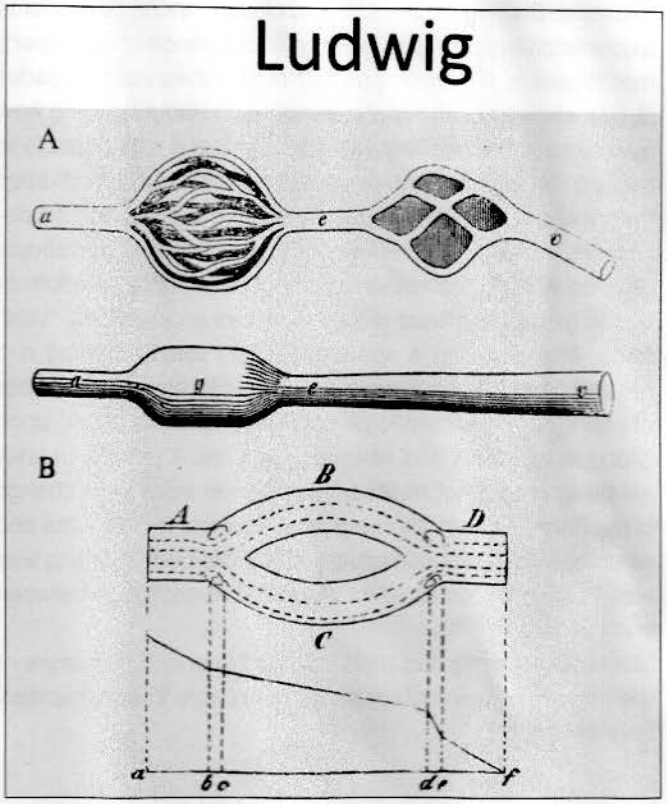

Fig. 4 - Karl Ludwig's depiction of the forces determining ultrafiltration across glomerular capillaries, 1852 (8).

gradually changed his view to include the idea that "some special component of the tubular wall reduces the rate of diffusion or that sodium chloride is reabsorbed back into the blood stream by some force analogous to a chemical force" (8). This was in line with contemporaneous views that cells could selectively transport solutes across their membranes. How they achieved that was not clear.

CONTRIBUTION 7: THE REALIZATION THAT THE RENAL EXCRETION SERVES THE FUNCTION OF MAINTAINING THE CONSTANCY OF BODY FLUID COMPOSITION AND VOLUME: INTRODUCTION OF QUANTITATIVE PHYSIOLOGY, PATHOPHYSIOLOGY AND MEDICINE

Ernest Henry Starling (1866-1927), *On the absorption of fluids from the connective tissue spaces, 1899* and *The Fluids of the Body*, 1909

Ernest Henry Starling was professor of physiology at University College London and was medically trained, which explains his ability to cross the border between physiology and

medicine. Starling's quantitative approach opened the door to a quantitative clinical approach to fluid balance and compartmentalization, which blossomed over subsequent decades (9, 10). He was centrally concerned with understanding how fluid crosses the capillary wall and whether it was possible to deduce the forces which governed this process. His filtration theory was a milestone in quantitative physiology. His predecessors, Ludwig and Heidenhain, had concluded that simple physiochemical processes could not fully explain the formation of lymph, and were drawn to invoke an additional "vital" force. After entering a number of blind alleys, Starling and his colleague William Bayless were able to provide estimates of changes in hydrostatic pressure of capillaries based upon changes in arterial and venous pressures. A change in arterial pressure did not necessarily mean an equivalent change in capillary pressure. By measuring changes in pressures and volumes in different compartments of the body, Starling was able to infer the forces which govern fluid exchange between such compartments.

Moreover, Starling had realized that the oncotic pressure of the plasma proteins plays an essential role in transcapillary fluid dynamics:

> Whereas enormous pressures of the salts and crystalloids in the various fluids of the body are of very little importance for the function of absorption by the blood vessels, the comparatively insignificant osmotic pressure of the albumin is, I believe, of great importance....
> The importance of these measurements lies in the fact that, although the osmotic pressure of the proteids of the plasma is so insignificant, it is of an order of magnitude comparable to that of the capillary pressures; and whereas capillary pressure determines transudation, the osmotic pressure of the protein of serum determines absorption ... so that, at any given time, there must be a balance between the hydrostatic pressure of the blood in the capillaries and the osmotic attraction of the blood for the surrounding fluids. With increased capillary pressure there must be increased transudation, until equilibrium is established at a somewhat higher point, where there is a more dilute fluid in the tissue-spaces and therefore a higher absorption forced to balance increased capillary pressure. With diminished capillary pressure there will be an osmotic absorption of salt solution from the extravascular fluid, until this becomes richer in proteids; Here then we have the balance of forces necessary to explain the accurate and speedy regulation of the quantity of circulating fluid (9).

In his book *The Fluids of the Body* (10), Starling encapsulates the function of the kidneys as follows:

> The function of the kidney is to keep the composition of the circulating fluids constant, and we can therefore alter the urine in any direction according to the nature of the change which we bring about in the composition of the body.
> The kidney therefore presents in the highest degree the phenomenon of "sensibility," the power of reacting to various stimuli in a direction which is appropriate for the survival of the organism: a power of adaptation which almost gives one the idea that its component parts must be endowed with intelligence (10).

It was in the footsteps of Starling that mid-20th century "giants" in renal physiology later walked. Homer Smith, James Shannon, James Gamble, Dexter Van Slyke, John P. Peters and Robert Pitts were to introduce quantitative analysis into measurement of kidney function and homeostatic mechanisms and to propose how the nephron is regulated in health and disease.

Contribution 8: The realization that kidney function could be understood at a cellular level, leading to the creation of therapeutic agents which regulate kidney functions

Hans H. Ussing (1911-2000): Koefoed-Johnsen and Ussing, "The Nature of the Frog Skin Potential," 1958

It was none other than a marine biologist who opened the door to understanding how a previously described "vital force" moved ions, solution and fluids across the walls of the nephron. Hans Ussing was a scientist studying ion transport, and the introduction of radioactive tracers had made it possible to study unidirectional ion transport. Ussing's seminal observations were made on the isolated frog skin throughout his career. Together with his colleague Koefoed-Johnsen, he proposed a model of a polarized cell which transports ions and water from one side to the other (11). This model was derived from the ability to distinguish active from passive transport and the coupling of water movement to ion transport (Fig. 5).

The model opened the door to the understanding of nephron function. All that was needed was to find methods of gaining access to the renal tubular cells which constitute the nephron. Thus were born the methodologies of micropuncture, isolated renal tubular perfusion and isolated membrane studies and ultimately, single transporter and ion channel analysis. Eventually from

such approaches, the abilities to confirm mechanisms of tubular reabsorption and secretion, urine concentration and dilution and acidification hormone responsiveness became possible. It became possible to test earlier hypotheses such as that of Hargitay, Wirz and Kuhn who proposed a counter current model of urine concentration, and ultimately led to the search for genetic defects which explained tubular transport diseases.

Equally important, this very basic model which Ussing proposed led to the search for agents which could alter tubular transport and affect excretory rates. Although diuretic substances had been discovered and used in the treatment of dropsy, an appreciation of how they worked at the nephron level led to the more detailed understanding of these agents and to the development of more potent and more site-specific agents.

CONTRIBUTION 9: THE REALIZATION THAT SOME HUMAN RENAL DISEASES MAY HAVE AN IMMUNOLOGICAL BASIS AND THAT THESE COULD BE MODIFIED BY PHARMACOLOGICAL AGENTS

R. Schwarrz, A. Eisner and W. Dameshek (1900-1969), "The Effect of 6-Mercaptopurine on Primary and Secondary Immune Responses," 1959, and R.A. Lerner and F.J. Dixon, "Transfer of Ovine Experimental Allergic Glomerulonephritis (EAG) With Serum," 1966

In 1902, Charles Richet, a Nobel Laureate and the grandfather of a modern-day leader in nephrology, Gabriel Richet, described the effects of repeated injections of a biological extract into animals. Even in very dilute amounts, these led to death, and the phenomenon was named "anaphylaxis." This was the first revelation that there could be a dark side to immunity. The mechanisms of anaphylaxis were not known, but subsequent studies by Donath and Landsteiner and by Von Pirquet confirmed this.

There was little progress made with any relevance to kidney disease until the seminal paper of Schwartz, Eisner and Dameshek in 1959 (12), in which they demonstrated that an immune response to a foreign antigen could be modified by a chemical agent and that appropriately timed 6-mercaptopurine administration could induce acquired tolerance to a foreign antigen.

The first convincing application of this principle to kidney disease came from Lerner and Dixon in the United States (13). They described the fact that serum globulin

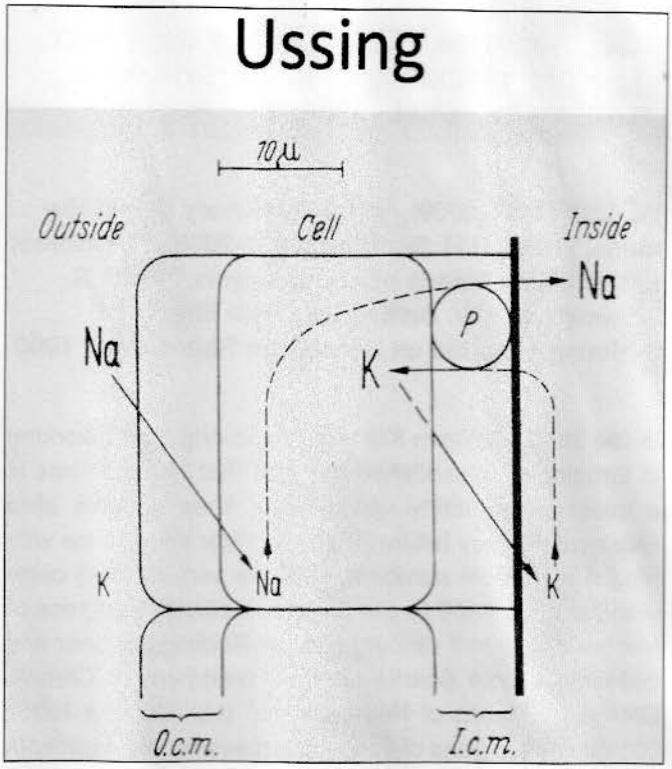

Fig. 5 - Koefoed-Johnson and Ussing's diagram of a polarized cell, illustrating the processes which determine transcellular sodium transport, 1958 (11).

derived from sheep made nephritic by immunization with glomerular basement membrane, contained a specific kidney-fixing antibody. This antibody was found to be capable of inducing an immediate, transient glomerulonephritis when injected into unilaterally nephrectomized lambs. The disease was characterized by proteinuria, polymorphonuclear neutrophil (PMN) infiltration into the glomerulus, and localization of globulin in a linear fashion along the glomerular capillary walls. They could absorb this out with isolated sheep basement membranes. A subsequent demonstration by them, that human anti–glomerular basement membrane antibodies could be transferred into monkeys and that these antibodies could be localized to the site of injury in man and in monkeys, strongly suggested a causal role in glomerular injury.

Since those understandings became widespread, the role of specific immunoglobulins in causing human kidney disease, and the ability to detect these and to reduce their effects using immunosuppressive treatment, have become central to the modern understanding of "primary" kidney diseases and their treatments, and novel molecular targets for therapies are starting to emerge.

Contribution 10: the realization that life can be extended in patients with end-stage renal disease

W. Kolff (1911-2009), "Artificial Kidney Use in Humans," 1945; B.H. Scribner (1921-2003), "Treatment of Uremia by Means of Hemodialysis," 1960; R. Schwartz and W. Dameshek, "The Effects of 6-Mercaptopurine on Homograft Rejections," 1960

In the 1940s, William Kolff, of Dutch origin and working in Groningen, established the fact that hemodialysis is a treatment modality which could keep patients alive with acute kidney failure (14). Of greater importance with regard to patient numbers, was the very modest communication in 1960 to the first International Congress of Nephrology, a half century ago, by Belding Scribner and colleagues from Seattle on the "Treatment of Chronic Uremia by Means of Hemodialysis" (15). Using a Teflon cannula as a means of vascular access in only 4 patients with chronic uremia, they showed, over a total of 29 patient-years, that chronic hemodialysis was a feasible modality for maintaining human life in the absence of renal function, over long periods of time.

The era of renal replacement therapy had arrived. A direct extension of the demonstration of clinical immunosuppression was the emergence of the field of renal transplantation. Once again it was Schwartz and Dameshek who extended their original observations into the field of organ rejection and the effects of an immunosuppressive agent on this phenomenon in animals (16). This work was extended into renal homograft rejection in the same year by Roy Calne. (Schwartz and Dameshek had assessed their findings at a meeting in London the previous year.) This line of work was continued in the research laboratory of Joseph Murray (Nobel Laureate) and Frances Moore and John Merrill at the Peter Bent Brigham Hospital in Boston and led to the first human renal transplantation. Similar work was going on in Paris at the Necker Hospital under Jean Hamburger. Chemical immunosuppression spread from bench to bedside and became the cornerstone of modern renal transplantation.

Conclusions

I have attempted to identify the seminal contributions which have led the field of nephrology to where it stands today. There is little doubt that some of the choices made here are open to dispute. What is not in dispute is that all of the contributions discussed are landmarks in the field.

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Address for correspondence:
Leon G. Fine, MD
Department of Biomedical Sciences
Cedars-Sinai Medical Center
8700 Beverly Blvd
Los Angeles, CA, 90048 USA
leon.fine@cshs.org

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