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THE LOOP OF HENLE AS THE MILESTONE OF MAMMALIAN KIDNEY CONCENTRATING ABILITY: A HISTORICAL REVIEW

HENLEOVA PETLJA KAO PREKRETNICA SPOSOBNOSTI KONCENTRIRANJA BUBREGA SISAVACA: POVIJESNI PREGLED

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Summary

The first description of the renal tubules is attributed to Lorenzo Bellini in 1662 and four years later Marcello Malpighi described the glomerulus. In 1842 Sir William Bowman described the capsule that surrounds the Malpighian body and its connection with the renal tubule and introduced the "excretory" hypothesis of urine formation. In the same year, Carl Ludwig introduced the "filtration-reabsorption" hypothesis of urine formation. Bowman's hypothesis was accepted by the so-called "vitalists" and Ludwig's hypothesis by the so-called "mechanists". In the middle of this confliction, Jacob Henle described in 1862 the homonymous "U" shaped loop but his discovery has neglected. In 1942 Werner Kuhn, a physical chemist, proposed that the loop of Henle may be the natural analog of the hairpin countercurrent multiplication system which concentrates urine in mammalian kidneys. In 1951 Kuhn, Hargitay and Wirz showed experimentally that the loop of Henle was the most important part of the countercurrent multiplication system of urine-concentrating mechanism in mammalian kidneys. The new theory was accepted by English-speaking scientists later, in 1958, when Carl Gottschalk and Margaret Mylle published their experimental work and proved that Kuhn's theory was correct. Gottschalk summarized the evidence of the accumulated

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knowledge in 1962, three centuries after the first description of renal tubules and one century after description of Henle's loop.

Key words: *Loop of Henle, urine formation mechanism, vitalists, mechanists, countercurrent multiplication system.*

Introduction

At the end of the Mesozoic era, about 66 million years ago, mammals migrated from the water to terrestrial life. As a consequence, they were deprived from free access to water and sodium. In order to survive in the new environment, they had to develop an excretory organ with the capacity to independently conserve salt and water. This organ was no other than the kidney[1].

We know now that lower vertebrates are capable to produce isotonic or hypotonic urine. Nevertheless, only mammals and some birds are capable to produce hypertonic urine. This capability is essential to conserve water under conditions of environmental dryness and limited access to water [1].

The mechanism by which the mammalian kidney concentrates urine is complex and relies upon the specialized architecture and sophisticated function of certain nephron segments as well as accompanying blood vessels. The fundamental structure of urine concentration is the "U" shaped loop of Henle accompanied with the collecting duct and the vasa recta in an obligatory manner unique only among species with the capacity of urine concentration [2].

The loop of Henle was first described by the German pathologist Friedrich Gustav Jacob Henle in 1862, and presented with excellent accuracy in his monograph with the title: "Zur Anatomie der Niere" Von J. Henle. Gottingen. Verlag der Dieterichscen Buehhandlung. 1862. The monograph is accompanied with marvelous hand made illustrations showing the thin descending limb, the thick ascending limb and the transition from the thin ascending to the thick ascending limb. (Figure: 1). It took almost a century to recognize the importance of this structure in urine concentrating mechanism, for many years it was thought that loop of Henle has no functional significance and was considered only as an "incidence of organogenesis" [1,3].

The reason that Henle's discovery remained buried for a long time before physiologists recognize its importance in urine concentration is the lack of knowledge upon the structure and function of the elemental kidney unit,

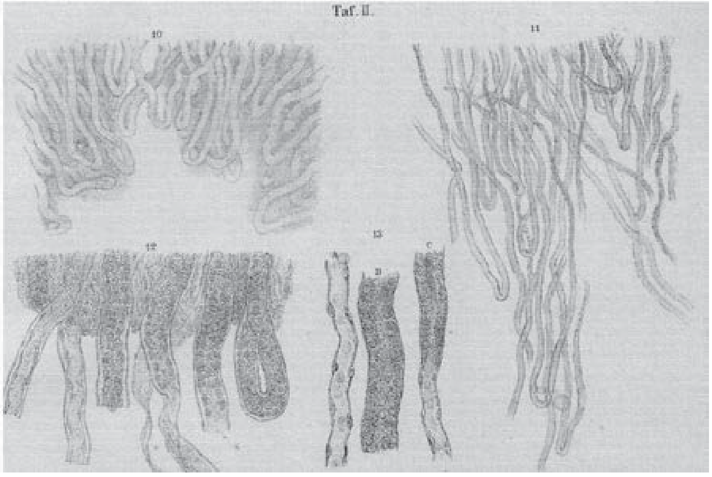


Figure 1: Left: Friedrich Gustav Jacob Henle (1809-1885). German physician, pathologist and anatomist. Right: Hand made drawings of the homonymous loop showing with accuracy the thin descending limb, the thick ascending limb and the transition from the thin ascending to the thick ascending limb. ("Zur Anatomie der Niere", 1862).

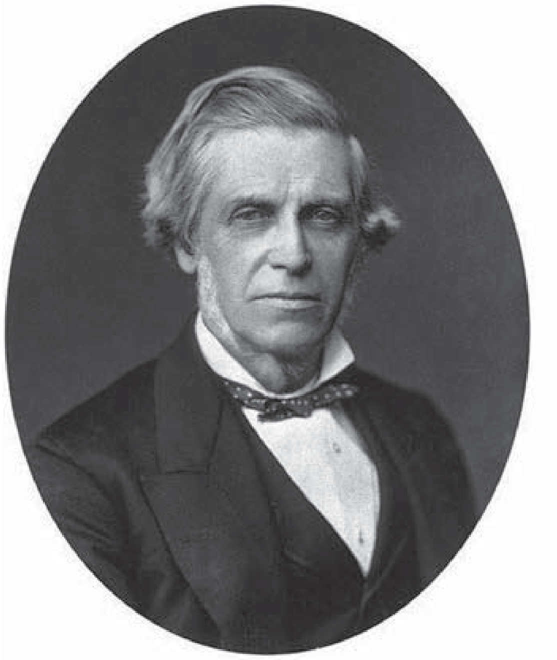
Slika 1. Lijevo: Friedrich Gustav Jacob Henle (1809.-1885.). Njemački liječnik, patolog i anatom. Desno: Ručno rađeni crteži homonimne petlje, koji točno pokazuju tanku silaznu granu, debelu uzlaznu granu i prelazak od tanke uzlazne ka debeloj uzlaznoj grani. ("Zur Anatomie der Niere", 1862.).

the nephron, as well as the lack of proper instruments for experimental work upon renal function and proper estimation of plasma and urine constituents.

The first description of renal tubule is attributed to the Italian anatomist Lorenzo Bellini who in 1662 described the papillary ducts which took his name. Four years later in 1666 the Italian physician and anatomist Marcello Malpighi described the glomerulus in the renal cortex, which took the name "Malpighian body", and its connection with the efferent and afferent arteries. He proposed also the possible connection with renal tubules but did not prove it [4].

The debate between "Vitalists" and "Mechanists".

After the above mentioned preliminary discoveries, it took about two centuries until the English surgeon, histologist and anatomist Sir William Bowman in 1842 described the capsule which surrounds the "Malpighian body" and its connection with the kidney tubules. (Figure: 2). Bowman investigated also the epithelium of uriniferous tubules and he was impressed with the similarities between this epithelium and the epithelium of excretory tubules of digestive glands and he arbitrarily inferred that tubular cells



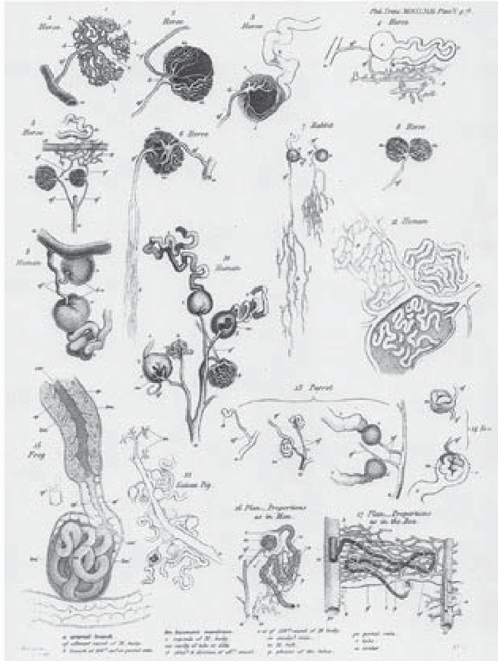


Figure 2: Left: Sir William Bowman, 1st Baronet, (1816 - 1892). English surgeon, histologist and anatomist. Right: Hand made drawings showing the glomerulus the surrounding capsule and the uriniferous tubules from many species including humans. ("On the structure and use of the Malpighian bodies of the kidney", 1842).

Slika 2. Lijevo: Sir William Bowman, I. Baron (1816. - 1892.). Engleski kirurg, histolog i anatom. Desno: Ručno rađeni crteži koji pokazuju glomerus, okružujuću kapsulu i urinoferusne tubule mnogih vrsta, uključujući i ljude. ("On the structure and use of the Malpighian bodies of the kidney", 1842.).

excrete the urine constituents and glomerulus produce only a stream of water which washes out the excreted solutes from tubules [4].

In his seminal paper "On the structure and use of the Malpighian bodies of the kidney, with observations on the circulation through that gland", presented to the Royal Society of London, 17 February 1842, http://www.jstor.org/stable/108143, he wrote:

"These tubes consists of an external tunic of transparent homogeneous tissue (which I have termed the basement membrane), lined by epithelium. The Malpighian bodies I saw to be rounded mass of minute vessels invested by a cyst or capsule of precisely similar appearance to the basement membrane of the tubes".

"... I injected some kidneys through the artery, by this method, in order to notice the nature of the vascular ramifications in the Malpighian bodies. I not only found what I sought, but the clearest evidence that the capsule which invest them is, in

truth, the basement membrane of the uriniferous tube expanded over the tuft of vessels".

"It occurred to me that as the tubes and their plexus of capillaries were probably, for reasons presently to be stated, the parts concerned in the secretion of that portion of the urine to which its characteristic properties are due (the urea, lithic acid &c.), the Malpighian bodies might be an apparatus destined to separate from the blood the watery portion".

"This abundance of water is apparently intended to serve chiefly as a menstruum for the proximate principles and salts which this secretion contains, and which, speaking generally, are far less soluble than those of any other animal product".

This arbitrary explanation was reinforced later, in 1874, when Rudolf Heidenhain of Breslau established the "excretory" hypothesis in urine formation which is known as the "Bowman-Heidenhain" hypothesis of "vitalists" [3,4].

In 1842, the same year that Bowman published his work, another brilliant mind in Germany, Carl Ludwig, a young physiologist in the University of Marburg, published his thesis in order to gain a senior degree. Carl Ludwing's thesis was a scientific work of 24 pages written in Latin "De viribus physics secretionem urinae adjuvantibus" (On the physical forces that promote the secretion of urine). Based upon his own experimental observations and literature available at that time, he introduced the hypothesis that glomerulus acts as a sieving filter which produces an ultra filtrate of blood free of cells and proteins and contains all the other constituents of the blood in the same concentration without any modification by the glomerulus itself. He continued that the volume of the filtrate is influenced by blood pressure variation in the renal artery and that, as it passes through the renal tubules, it undergoes reabsorption or secretion which alter the final concentration of certain substances in urine in relation to the blood [4,5]. (Figure: 3).

Somme details of his paper are as follow:

"... the membranes of the vessels in the glomeruli are subjected to high pressure, resulting in a copious secretion from the delicate glomeruli. When kidneys were injected with wax, I detected discharge of the wax from the glomeruli.

"The second physical process occurring in the kidney is an endosmotic action between the solution of salts secreted and the partly altered blood retained in the vessels. The first and best proof of endosmosis is the fact that, given the same composition of the blood, the concentration of the urine depends on the urine flow rate.

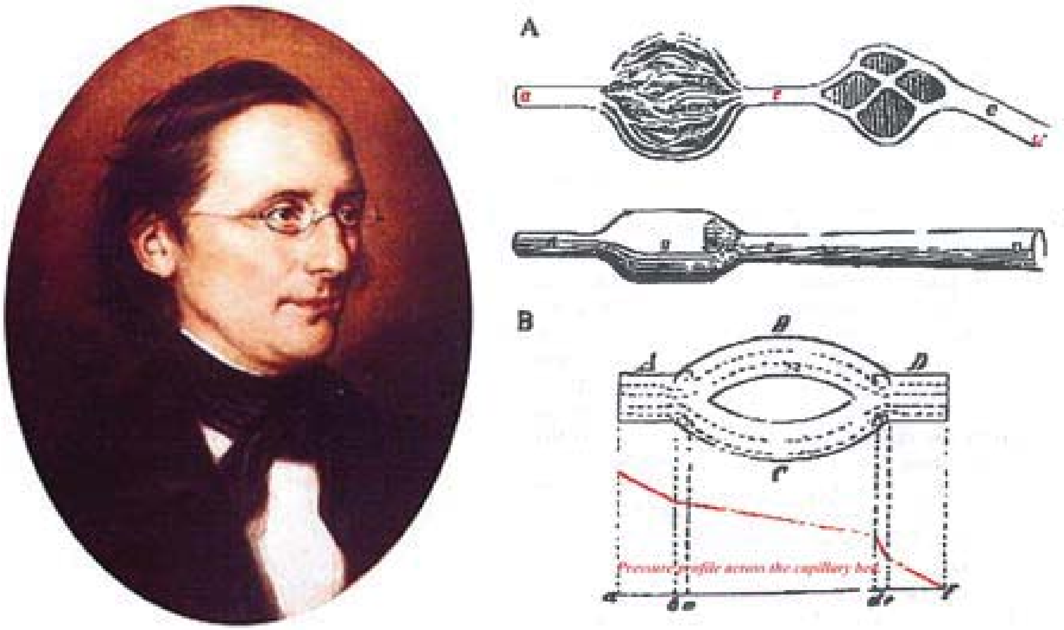


Figure 3: Left: Carl Friedrich Wilhelm Ludwig, (1816-1895). German physician and physiologist. Right: Hand made drawings showing in the upper panel a schematic representation of the glomerulus and the uriniferous tubule with its blood supply and in the lower panel blood hydrostatic pressure changes during its passage through the glomerular capillaries. ("De viribus physics secretionem urinae adjuvantibus", 1842).

Slika 3. Lijevo: Carl Friedrich Wilhelm Ludwig, (1816.-1895.). Njemački liječnik i fiziolog. Desno: Rukom rađeni crteži koji pokazuju u gornjem dijelu shematski prikaz glomerusa i urinoferusne tubule sa pripadajućim sustavom opskrbe krvlju te u donjem dijelu promjene hidrostatskog krvnog tlaka tijekom prolaska kroz glomerualne kapilare. ("De viribus physics secretionem urinae adjuvantibus", 1842.).

It is clear that the process of expulsion of the urine is as follows: "When the blood vascular system is filled with fluid, pressure is exerted against the walls of the glomeruli, and the water in the blood leaves the glomeruli and is taken up by the uriniferous ducts. It is here that endosmosis can occur as described above. The quantity of urine secretion is accelerated when the blood vascular system is filled with fluid, in which case the pressure against the walls of the glomeruli is increased".

In this work Ludwig introduced the hypothesis that the phenomena of living organisms are influenced from the laws of physics and chemistry and can be "*the consequence of simple attractions and repulsions between a limited numbers of chemical atoms*". With this revolutionary concept for his era, Ludwig introduced the hypothesis of "filtration-reabsorption" in urine formation which was accepted only from the so called "mechanists" and it would be a matter of controversy between them and "vitalists" for the following 80 years [3-5].

At the middle of this conflict, and with a lot of items of renal function unresolved, it was expected that Henle's discovery would be neglected. At the beginning of the 20th century, in 1922, Alfred Richards and his colleagues introduced a new method in the experimental investigation of renal function which is known as the "micropuncture technique" and proved that Ludwig was quite right in his pioneer concept regarding the mechanism of urine formation[4]. Somme details of his paper are as follow [4]:

"... it was possible to insert sharply pointed tubes into the space within Bowman's capsule and to abstract the fluid which issues from the blood of the glomerular capillaries ..".

"The results showed that the glomerular fluid is free from protein but contains chloride and glucose, both of these being absent from the bladder urine. It is alkaline, contains urea, and indeed every diffusible constituent of plasma for which we were able to make a test ..."

"These results seem to me to leave little room for doubt that, in amphibia, the glomerular urine actually has the same composition of a protein-free filtrate from plasma, precisely as Ludwig had imagined ninety-three years ago".

Thereafter the use of micropuncture technique and the measurement of Glomerular Filtration Rate (GFR) with the use of the polysaccharide inulin in animals as well as in man by Homer Smith and his colleagues in 1932 at the New York University Medical College provided the scientific community with a rapid increasing bulk of knowledge upon renal physiology and the interest of researchers turned mainly to the filtration, reabsorption and excretion of solutes along the nephron [6].

The countercurrent hypothesis

During 1940-1944 Europe was almost devastated by the 2nd World War but Switzerland's neutrality allowed some brilliant minds to continue their experimental work and produce knowledge, one of them was Werner Kuhn, Professor of Physical Chemistry in University of Basel, who worked upon the enrichment of sugar in water using semi-permeable membranes and phenol as an auxiliary liquid in a hairpin countercurrent system without any other external force. He showed that at each bend of the hairpin countercurrent system solute concentration increased by a factor n which equals to the length of the system divided by its width (n=L/W). Based upon these observations Kuhn and his colleague Kaspar Ryffel published, in 1942, a paper in German and proposed that the "U" shaped loop of Henle may be the natural analog of a countercurrent multiplication system capable to produce urine concentration in mammalian kidney but the paper overlooked by renal physiologists [7,8].

Although countercurrent exchangers and countercurrent multipliers were known among engineers and utilized in many applications in industry, mainly in heat exchange and solute concentration, the first description of the importance of heat exchange between arteries and veins in mammals is attributed to Claude Bernard in 1876. Many years later in 1940's Bazett and his colleagues showed experimentally the heat exchange between deep arteries and veins in the arms and the legs which prevents heat loss to the environment and achieves blood warming before entrance to the central circulation [1,9,10].

In 1950's extensive experimental work showed that Arctic mammals and birds utilizes a countercurrent heat exchange system between deep arteries and veins in their legs in order to prevent freezing while standing on icy ground or wading in icy water. It was also showed that some species utilizes a specialized network of arteries and veins bundles capable to exchange heat and gazes, known as "rete mirabile" which help them to regulate body temperature, to exchange oxygen in fish gills and in the case of deep ocean fishes to store oxygen in swim bladder in high pressures exceeding in some cases a hundred time the partial oxygen pressure of surrounding see water [1,9,10].

In early 1900's Karl Peter in his book "Untersuchungen uber Bau und Entwickelung der Niere" (Jena Fisher 1909, editor) first pointed to the relation between length of Henle's loop and urine concentrating ability among some species. Later in 1944 Sperber pointed again to the relation between length of Henle's loop and urine concentration because animals with long Henle's loops exhibited the greater urine concentrating ability[7].

Meanwhile in 1946 Bart Hargitay, a young graduate of chemistry at the University of Budapest, received a fellowship offered by the University of Basel where he joined Werner Kuhn. As the Iron Curtail closed this year he decided to stay in Switzerland and asked Professor Kuhn to accept him as a graduate student to work in a thesis. Kuhn assigned Hargitay to prove the hypothesis of countercurrent multiplication system of urine concentration in the kidney [8]. Hargitay contacted Dr Heinrich Wirz at the Physiology

Institute in order to obtain some knowledge about renal physiology and help him in experiments with animals. Wirz enthused with the idea and started promptly experiments with rat kidneys and later with Syrian hamster because the solitary papilla of this rodent protrudes in to the renal pelvis and it is easier for micropuncture and collect urine sample from renal tubules.

Soon thereafter the two researchers encountered a serious problem: the estimation of the chemical constituents had to be performed in a very scant sample of urine, about 10^{-7} ml, obtained by micropuncture. Hargitay decided to determine only the osmotic pressure of the samples by cryoscopic method according to the formula:

Relative freezing point depression = $100 \bullet \Delta_x - \Delta_{isot} / \Delta_{max} - \Delta_{isot}$

When Δ_x: the freezing point depression in x position in the kidney[8].

In order to perform their calculations they needed a cryo-chamber with temperature lower than -20° C. They used the cold room of the Burgerspital hospital in Basel. The procedure needed to be carried out in the room, under heavy clothes and furs in the middle of the summer, bringing with the micropipette urine samples and observing under polarized microscope the birefringence of ice formed at the melting point of each sample. They gathered multiple urine samples along the axis from the renal cortex to the papilla and found that in all samples the osmotic pressure was equal at the same level but it was gradually increasing at each deferent level from the renal cortex to the papilla. The lowest pressure was observed in the renal cortex and estimated to be 25 Atm while the greatest was observed in the papilla and was estimated to be 58 Atm [9]. (Figure: 4)

These findings as well as experimental findings from a mechanical hairpin model constructed by Hargitay and colleagues in his laboratory, prompted Hargitay and Wirz to consider that the "U" shaped loop of Henle is the natural analog of a hairpin countercurrent multiplication system in the kidney which by the antiparallel circulation of urine in the descending and ascending limb of the loop produces the maximum concentration of solutes at the bending point of the loop in the deep renal medulla.

Although they were ignorant of the specialized properties of the descending and ascending limb of Henle's loop concerning its water permeability and active sodium chloride transport, they realized that the single effect, by means of the leading process, in urine concentration mechanism could not be a component of hydrostatic pressure difference but an "electroosmosis" phenomenon which they described as follows: "*It seems much more likely that, in epithelial cells, energy from metabolism is used to establish a potential field and that in this potential field electroosmosis takes place*" [9].

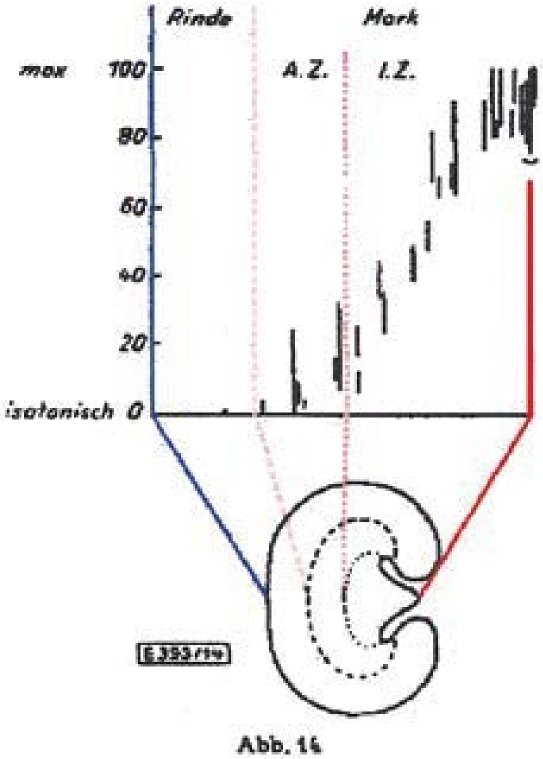


Figure 4: The original findings from experiments conducted by Bart Hargitay and Heinrich Wirz showing the increase of osmotic pressure from the renal cortex to the tip of the renal papilla.

Slika 4. Izvorni nalazi iz opita Barta Hargitaya i Heinricha Wirza koji pokazuju povećanje osmotskog tlaka od bubrežnog korteksa do vrha bubrežne papile.

In order to explain the sequence of events in urine concentration, they considered it mainly as a process of water absorption. They hypothesized that the electroosmosis phenomenon produces water transport from the lumen of descending limb to the interstitial space and then to the lumen of the ascending limb. They said that the latter delivers diluted urine to the distal convoluted tubule from which water is transferred to the blood. Hence, a final concentration of urine takes place in the collecting duct as it passes through the hypertonic medulla [9].

As we know now water permeability of the thin descending limb of Henle's loop is owing to the expression of aquaporin-1 (AQP-1) in its epithelium. Thorough investigation of this nephron segment showed that short looped nephrons do not express AQP-1 in their descending thin limb and they are practically impermeable to water. Conversely AQP-1 is expressed in the thin descending limb of long looped nephrons especially those extending deep in the medulla but AQP-1 expression is limited to the first 40% of their length and never beyond the last 2-2,5 mm before bending in the inner medulla. The remainder 60% of their length is devoid of AQPs and hence impermeable to water but it is permeable to urea and chloride ions because of the expression of urea transporters and chloride channels. The thick ascending limb of Henle's loop is impermeable to water but posses the capacity of active transport of sodium, potassium and chloride via the $Na^+:K^+:2Cl^-$ cotransporter which transfer sodium chloride to the interstitium and contributes significantly to the hypertonicity of the renal medulla [12,13].

The work was first presented in May 1951 by Hargitay to the Bunsen Gesellschaft at the meeting for physical chemists in Gottingen and a few weeks later by Wirz at the International Conference for Physiology in Copenhagen. The physical chemists accepted the findings by Hargitay and Wirz with enthusiasm but the physiologists expressed their skepticism and reluctance to accept the new theory. The work was published in German in the same year and thereafter it became a mater of investigation among German speaking scientists but not among English for at least the following 7 years. Wirz continued his experiments by micropuncture but he never managed to puncture with accuracy the lumen of Henle's loop especially at the tip of renal medulla [11,14,15].

During this period a considerable work upon urine concentration and dilution was conducted by Karl Julius Ullrich and was published mainly in German. Although during his contribution to Gottschalk's laboratory in Chapel Hill he published also some articles in English. Ullrich examined the composition of interstitial fluid in renal cortex and medulla and proved that except electrolyte accumulation other osmolytes especially urea contributes to the medullary hypertonicity of mammalian kidney [17]. He showed also that glycerophosphocholine and inositol accumulate in the renal medulla and act as osmolytes and that medullary collecting duct participates in urea recycling [17].

The reluctance of English speaking scientists to accept the new theory is in part attributed to the fact that the first half of 20th century was predominated by Homer Smith's proposals in renal physiology. In his book "The kidney: Structure and Function in Health and Disease", published in 1951, by drawing the nephron he omitted the loop of Henle and included only a part of the descending limp as short straight tubule. Smith believed that the urine concentration is accomplished at least by two mechanisms one attributed to passive reabsorption of water in the proximal tubule and another one attributed to active reabsorption of water in some parts of distal tubule although no evidence of active water reabsorption mechanism had been proved in any biological system. (Figure: 5).

He wrote exactly:

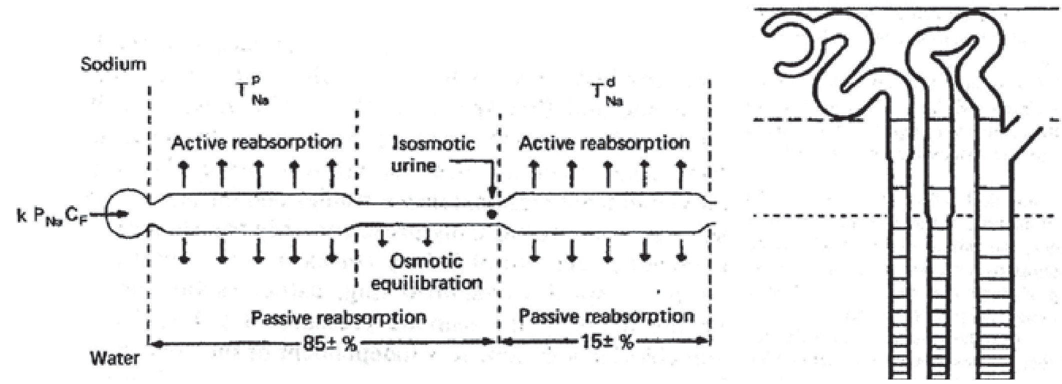


Figure 5: Left: The rectilinear model of the nephron omitting the loop of Henle proposed by Homer Smith in his book "The kidney: Structure and Function in Health and Disease". (London, Oxford University Press, 1951). Right: The countercurrent multiplication system with gradually increasing osmolality from the cortex to the renal medulla proposed by Kuhn, Hargitay and Wirz. (Das Multiplikationsprinzip als Grundlage der Harnkonzentrierung in der Niere, 1951).

Slika 5. Lijevo: Rektilinearni model nefrona bez Henleove petlje predložen od strane Homera Smitha u knjizi "The kidney: Structure and Function in Health and Disease". (London, Oxford University Press, 1951.). Desno: Protustrujni multiplikacijski sustav sa postupno povećavajućom osmolarnošću od korteksa do bubrežne medule po Kuhnu, Hargitayu i Wirzu. (Das Multiplikationsprinzip als Grundlage der Harnkonzentrierung in der Niere, 1951.).

"... the reabsorption of water by the renal tubules involves at least two more or less independent processes: 1. passive water reabsorption in the proximal tubule and thin segment (proximal system), and, under appropriate circumstances, in the distal tubule; and 2. active water reabsorption that is presumably confined to the distal system, i.e., in the distal tubule and possibly in the collecting ducts also".

When he asked by Carl Gottschalk what he believes about the countercurrent hypothesis he said: "*The smart boys don't believe in it*" [7,18].

Meanwhile USA entered the 2nd World War in 1941 and Alfred Richard's laboratory stopped the experiments with micropuncture for almost a decade. After the war Carl Gottschalk expressed the intention to revive renal micropuncture and asked Richard's advice upon restarting kidney micropuncture but for unknown reasons he discouraged him [18].

In 1952 Gottschalk joined the Department of Medicine at the University of North Carolina and established in Chapel Hill his "Micropuncture Laboratory" which was equipped with the Ramsey/Brown micro-osmometer built especially for the Chapel Hill laboratory [18]. Gottschalk recruited in his laboratory Margaret Mylle who was considered as "one of the most skilled micropuncturists in the word". Gottschalk's intention was to check the hypothesis proposed by Robert Berliner that the urine at the tip of the loop of Henle should be hypotonic [19].

After performing a series of brilliant experiments with Wistar rats, golden hamsters, one kangaroo rat and Psammomys obesus, they collected urine samples in nanoliter specimens from short looped nephrons, from the thin limb and the bend of loop of Henle, collecting ducts as well as vasa recta. They showed that fluid from the bend of loops of Henle, collecting ducts and vasa recta at the same level in the papilla were hyperosmotic and exhibited almost equal osmotic pressure [7,18].

After that Gottschalk published a brief report of his findings in an article less than one page in Science in September 1958 with the title: "Evidence that the mammalian nephron functions as a countercurrent multiplier system" establishing by this way the validity of "the new theory" proposed by Kuhn, Hargitay and Wirz [20]. According to Francois Morel's declaration, after personal communication with Gottschalk, he sent the data to Homer Smith before full publication. Homer Smith was so impressed by these findings that he asked from Gottschalk to postpone the full publication until he will make known his new opinion. After that Smith delivered a lecture with the title "The fate of sodium and water in the renal tubules", in October 17, 1958 at the Annual Postgraduate Week organized by the New York Academy of Medicine and he recognized the importance of the "new theory" with remarkable accuracy and humour [1,7]. In advance Gottschalk and Mylle published their findings in American Journal of Physiology the next year [21].

By extending their experiments they showed, by micropuncture in hamsters, that the water permeability of thin descending limb of Henle's loop greatly exceeded that of the thin ascending limb. Experiments in hamsters with diabetes insipidus showed that the fluid collected from the loop of Henle and blood from the vasa recta, at the tip of the papilla, were hyperosmotic in contrast to the fluid of the adjacent collecting ducts which was hypo-osmotic. These experiments showed that water permeability and urine concentration in the thin descending limb of Henle's loop is independent of the presence of ADH and that the final concentration of urine takes place in the medullary collecting duct [7].

Gottschalk summarized the evidence of the accumulated knowledge upon the countercurrent hypothesis in a lecture with the title "Renal tubular function: lessons from micropuncture" presented in "The Harvey Lectures" (series 58) in 1962 three centuries after the firs description of renal tubules and a century after Jacob Henle's description of the homonymous loop in mammalian kidney.

The above mentioned fundamental work was simply the beginning followed by an enormous experimental investigation of renal physiology based first upon micropuncture and later upon microperfusion and patch clamp technique which expanded our knowledge upon ion channels properties. Genetic analysis of specific ion and solute transporters upon molecular level as well as the use of specific gene knockout animals enabled researchers to unravel step by step the mysteries of renal function [13,22,23].

Any further detailed analysis of the ongoing research upon this very interesting topic is beyond the scope of this historical review but the adventure is still in progress because "we have to go miles before sleep".

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Sažetak

Prvi se opis bubrežnih tubula iz 1662. pripisuje Lorenzu Belliniju, a četiri je godine kasnije Marcello Malpighi opisao glomerul. Godine 1842. je Sir William Bowman opisao kapsulu koja okružuje malpigijevo tjelešce i njegovu vezu s bubrežnim tubulima te uveo "ekskretornu" hipotezu o stvaranju urina. Iste je godine Carl Ludwig uveo je "filtracijsko-reasorpcijsku" hipotezu stvaranja urina. Bowmanova je hipoteza bila prihvaćena od strane tzv. "vitalista", a Ludwigova hipoteza od strane tzv. "mehanicista". U jeku tog sukoba Jakob Henle opisao je 1862. homonimne petlje u obliku slova "U", ali njegovo je otkriće zanemareno. Godine 1942. je Werner Kuhn, fizikalni kemičar, predložio ideju da je Henleova petlja možda prirodni analogon kopče protustrujnog multiplikacijskog sustava koji koncentrira urin u bubrezima sisavaca. Godine su 1951. Kuhn, Hargitay i Wirz eksperimentalno pokazali da da je Henleova petlja najvažniji dio protustrujnog multiplikacijskog sustava mehanizma za koncentriranje urina u bubrezima sisavaca. Nova je teorija prihvaćena od strane anglofonih znanstvenika kasnije, 1958. godine, kada su Carl Gottschalk i Margaret Mylle objavili svoj eksperimentalni rad i dokazali da je Kuhnova teorija bila točna. Gottschalk je sažeo dokaze sakupljenog znanja 1962., tri stoljeća nakon prvog opisa bubrežnih tubula i jednog stoljeća nakon opisa Henleove petlje.

Ključne riječi: *Henleova petlja, mehanizam formiranja urina, vitalisti, mehanicisti, protustrujni multiplikacijski sustav.*