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# Principles of Evolution of the Excretory Organs and the System of Homeostasis

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**Abstract**—The function of the excretory organs in metazoan invertebrates and vertebrates is aimed at maintaining homeostasis. Two patterns of the morpho-functional organization of these organs can be distinguished: I—a combination of ultrafiltration and subsequent reabsorption with partial secretion of substances, or II—only a secretion of molecules of a certain type. Here we substantiate the principle according to which in the type I organs the key role of the proximal tubule is to reabsorb fluid, which is ideal in terms of its solute composition and concentration, and to secrete a number of organic acids and bases, while the distal tubule is responsible for adjusting the composition of inorganic substances. Everything what does not meet these criteria is to be excreted. The type II organs (salt glands, aglomerular kidneys) are inherent to those animals whose kidneys do not ensure osmoregulation. During evolution, the mammalian kidney developed the mechanism which regulates the redistribution of fluid within the nephron: altered reabsorption of ions and water in the proximal segment can cause an influx of some additional fluid to the distal segment where the regulatory systems adjust the amount of reabsorbed substances. The central tendency in the evolution of the kidney in vertebrates, including humans, is to increase the rates of glomerular filtration and proximal reabsorption. A similarity between molecular mechanisms of transmembrane and transepithelial transport of substances has been revealed in the evolution of excretory organs, with distinctions concerning mainly the structure of the regulatory molecules and the intensity of urine formation.

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## INTRODUCTION

The problem of the origin of life and the evolution of living beings is permanently on the agenda. Yet in 1921, V.I. Vernadsky, when delivering a lecture at the Petrograd House of

Writers, said that “there are no questions more important to us than the question of the riddle of life, of that eternal riddle mankind has been facing for millennia” [1]. The great Darwin’s work “On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the

Struggle for Life” (1859) elucidates the ways and means for the emergence of novel biological species but casts no light on the origin of the very first “living thing”. The core of his work was to attempt to understand how the highest result, which the mind is able to imagine, the formation of higher animals, ensues directly “from the struggle in nature, from hunger and death” [2]. Following the emergence of the first cell and single-celled organisms, after a huge period of time, the first multicellular plants and animals came into existence. Integration of individual cells into a multicellular organism required the emergence of the system of fluids of the internal environment, as well as the respiratory, circulatory, digestive and excretory systems. The multicellular organism needed its functions to be regulated and any biologically relevant information to be transmitted. The demand arose for the stabilization of physicochemical parameters of fluids of the internal environment, i.e. for homeostasis [3–6].

Multicellular plants and metazoan animals began to continuously exchange substances with the external environment, to consume and excrete salts and water. During the long evolutionary history of life on Earth, nature had repeatedly to choose the way of further development, as it was in forming the internal environment in metazoans. One of the key distinctions between the animal and plant kingdoms is that animals developed a single internal environment, stabilization of which peaks in mammals, particularly in humans. In contrast, transport of fluids in plants includes three distinct types: short-range intracellular flows, radial intercellular flows in parenchymal tissues, and axial transport along the specialized conducting system in vascular plants [7]. Animals form their special, circulatory, system of fluids of the internal environment. Homeostasis is an extremely costly process in terms of the energy needed to maintain physicochemical stability of fluids of the internal environment, which is essential for any cell of the organism but especially for the brain. In the evolution of homeostasis, the excretory organs, particularly the kidneys, played a pivotal role [6]. Each moment, a quarter of the minute blood volume comes into the kidney, and only a bit smaller volume reaches the human brain [8–10]. The goal of this paper is to present current views on the evo-

lution of the excretory system as being crucial to physicochemical stabilization of fluids of the internal environment and to attempt to answer the central questions of fundamental science: what are the principles of the morphofunctional organization of this system in metazoan organisms and why did it evolve this way and not otherwise? [11].

Such an approach underlies classical evolutionary physiology [12] which integrates data of evolutionary morphology and evolutionary biochemistry, thus offering a clue to how an individual creates its adaptive capacities [13–15]. Proceeding from this method, an attempt will be made here to unravel the logic behind the evolution of the system of fluids of the internal environment and specifically of the renal excretory system as a major organ of this system in metazoans, particularly in their higher representatives (mammals, including human beings). In this context, a focus will be on which conditions of the internal environment are needed to realize the higher forms of nervous system’s activity, such as mind, consciousness, high creativity. In this respect, it is of prime importance to consider both sides of the process that attracts now great interest of researchers: (1) issues of brain functions and (2) issues of the physicochemical properties of the internal environment which provide the very possibility of brain performance. In his time, Joseph Barcroft spoke out in the sense that suggesting high intellectual development in an environment whose properties are not stabilized is the same as seeking music in the noise of broadcast interference or tracing the ripple left by a boat on the surface of the Atlantic Ocean [5]. Therefore, when dealing with physiology of mental activity, one should consider the integrity of an individual and realize that higher brain functions, intellect, require a high development not only of the brain itself but homeostasis either, as it is an indispensable prerequisite for successful brain performance. A key role in the system of homeostasis is played by the kidneys and the system of their regulation.

## EXCRETORY ORGANS OF INVERTEBRATES

Most single-celled and metazoan organisms have one or another excretory system [16–18]. Metazoans may have emerged following migration

of protocells from the “potassium” reservoirs to the sea where sodium ions were dominant, so that they had to adapt to a novel environment while remaining at the same time isosmotic to their habitats. Many groups of contemporary marine organisms have no specialized osmoregulatory organs. They permanently live in seawater, providing water–salt metabolism independently in each individual cell. Due to this, these organisms manage to maintain the constancy of their volume and ion concentrations. Metazoans of different taxa are distinguished by a diversity of their excretory organs. The representatives of those metazoan groups whose excretory organs are investigated quite thoroughly will be considered below. The excretory function involves the body surface, accumulating cells, protonephridia, metanephridia, kidneys, antennal glands, gills, Malpighian tubules, coxal glands etc. [16, 19, 20, 21]. The protonephridia derive from the ectoderm and are present in nemerteans, turbellarians, cestodes and nematodes. The metanephridia are described in polychaetes and oligochaetes; their coelomoducts (green glands in crustaceans, head nephridia in insects, kidneys in mollusks) represent epithelial tubules that are mesodermal in origin [22]. In insect, the Malpighian tubules also participate in excretion, as well as the fat body performing the role of “kidneys of accumulation” [20].

In the evolution of metazoans, a specialized excretory organ, the protonephridium, may have emerged first in turbellarians [20]. Inside its tubule, blind from one side, there is a ciliary bundle beating of which provides constant influx of the liquid from the parenchyma into the tubule. In addition to protonephridia, amoebocytes are also involved in excretion: after accumulating some metabolic waste products, they are removed from the organism. The excretory system of trematodes is represented by protonephridia that are made of so-called flame cells. The two main protonephridial tubules are connected to the bladder through the orifice of which liquid wastes are excreted outwards. In insects, the main excretory organs are Malpighian tubules: total wastes are secreted into the tubular lumen and then effuse into the gut at the border between its middle and hind segments, while solid waste products are

removed through the anus. Insects also develop a complex in which an important role in water and solute reabsorption belongs to the gut. This allows insects to adjust excretion of substances from the organism and to save water. In metanephridial coelomoducts, the excretory function is based on secretion of fluids of the internal environment into the tubular lumen, their subsequent reabsorption in the proximal tubule, additional secretion of some substances [24], and excretion of liquid wastes into the bladder. This process resembles that occurring in the kidney of vertebrates.

### RENAL FUNCTION IN THE EVOLUTION OF VERTEBRATES

The kidneys perform in vertebrate a number of homeostatic functions, including excretion of substances. Two notions should be discriminated: the renal functions as such and the processes that ensure their implementation [25]. The realization of homeostatic functions comprises four processes: fluid ultrafiltration, reabsorption, secretion, and synthesis of substances. Let us consider the excretory organ functions as exemplified by the mammalian kidneys in which this subject matter is studied best [25–27]. The kidneys play a key role in providing the constancy of the following physicochemical parameters of fluids of the internal environment: (1) blood osmolality (osmoregulation), (2) stabilization of the fluid volume (volumoregulation), (3) individual ion concentrations in blood serum, (4) acid-base status (serum pH), (5) blood coagulability due to secretion of the thrombopoietic growth factor, (6) the number of red blood cells (RBC), (7) arterial tension regulation (secretion of renin and other substances), (8) normalization of serum concentrations of proteins, amino acids carbohydrates and lipids. Such a wide spectrum of renal functions shows that the mammalian (human) kidneys are the main effector organ of the homeostasis system. The implementation of these functions requires abundant blood supply, and actually up to a quarter of the entire blood volume ejected into the aorta flows every minute through the human renal vasculature [8].

The major task of the excretory organs is maintaining the composition constancy of the

**Table 1.** Excretory organs with a dominance of ultrafiltration and reabsorption

Excretory organ	Animal	PT	DT	UB	Reference
Protonephridium	Rotifer <i>Asplanchna</i>	+	+	+	17
Metanephridium	Annelid <i>Lumbricus terrestris</i> L.	+	+	+	17
Antennal gland	Crayfish, <i>Astacus</i>	+	+	+	17
Bojanus organ	Mussel, <i>Anodonta</i>	+	+		17
Archinephron	Hagfish, <i>Myxine glutinosa</i>	+	+		30
Kidney	Lamprey, <i>Lampetra fluviatilis</i>	+	+		47
	Pike, <i>Esox lucius</i>	+	+	+	
	Frog, <i>Rana temporaria</i>	+	+	+	47
	Lizard, <i>Agama caucasica</i>	+	+		47
	Chicken, <i>Gallus domesticus</i>	+	+	–	47
	Rat, <i>Rattus norvegicus</i>	+	+	+	47

PT—Proximal tubule, DT—distal tubule, UB—urinary bladder.

extracellular environment, thus creating optimal conditions for high performance of all cells in an organism. To provide extra high stabilization of the extracellular fluid in some specialized organs (e.g., in the brain), there are some additional means, such as the blood–brain barrier.

## TWO TYPES OF EXCRETORY ORGANS

Having outlined most general information on the metazoan excretory organs, let us try to formulate the basic principles of their morphofunctional organization. The functional classification proposed below is based on discriminating whether functional activity of the excretory organ ensures the maintenance of the “ideal” blood (or hemolymph) composition with regard to a totality of all substances, or this organ excretes in behalf of homeostasis only a certain chemical substance (or group of substances), e.g., some inorganic ions or organic substances.

This classification principle allows the metazoan excretory organs to be divided into two groups. One of them is arranged in such a way that enables continuous maintenance of an “ideal composition” of the internal environment and, thereby, excretion of any substances that are unnecessary to the organism. This function is performed by the organs differing by their morphology and embryogenesis: nephridia, coelomoducts, kidneys (Table 1). Another group

of excretory organs secretes a narrow range of certain substances, e.g., some ions ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ). These organs were called salt glands and are presented by the rectal gland in elasmobranchs, the avian and reptilian nasal glands [16]. They provide secretion of the solutions that are more concentrated than blood, and this is the main mechanisms for osmoregulation in fish, reptiles and birds [16, 28] (Table 2). Excretion of the certain group of substances in marine teleosts is implemented by cells of the branchial apparatus and the aglomerular nephrons [29]. In the latter, cells are able to secrete some organic acids and bases, divalent cations (e.g.,  $\text{Mg}^{2+}$ ) and sulfates that are subsequently excreted. These fish drink seawater and then desalinate it by secreting sodium ions and chlorides through the gills and divalent cations through the kidneys.

The functional organization of the type 1 excretory organs is based on a combination of different variants of ultrafiltration with subsequent reabsorption of the useful substances. Such a functional design of the excretory organs refers to the most widespread pattern of the excretory system’s morphofunctional organization in metazoans, including humans. Ultrafiltration takes place in the Malpighian body of the vertebrate kidney. In some groups of animals, fluid comes into the tubular lumen from the coelomic cavity. In the nephrostome, protonephridium and metanephridium, fluid that is similar in its

**Table 2.** Organs of selective excretion

Object	Organ	Substance	Reference
Shark, ray	rectal gland	Na, Cl	54
Marine teleosts	gills	Na, Cl	54
Marine teleosts	aglomerular nephron	Mg, SO <sub>4</sub>	40
<i>Iguana iguana</i>	salt gland	Na, K, Cl	54
Lizard, <i>Sauromalus</i>	nasal salt gland	K, Cl, HCO <sub>3</sub>	55
Turtle, <i>Caretta caretta</i>	lacrimal salt gland	Na, Cl	28
Gull, <i>Larus argentatus</i>	nasal salt gland	Na, Cl	28
Human	sweat gland	H <sub>2</sub> O, Na, Cl	8

composition to the internal environment comes through the different mechanisms into the tubular lumen. This fluid is isosmotic to the internal environment, and during reabsorption all the necessary substances return to blood, while the unneeded are excreted.

A study of the renal nephrons in vertebrates showed that there are two groups of animals. In one of them, represented by fish, amphibians, reptiles, birds and mammals, all the nephrons have the glomeruli which accommodate glomerular filtration of varied intensity. The other group is represented by more than 20 species of marine teleosts referring to 12 families which have completely of partially aglomerular nephrons [29]. The authors of these studies supposed that the development of the aglomerular nephrons is a kind of adaptation to decreased excretion of water, since the blood osmolality in marine teleosts is close to that in other vertebrates, i.e. several times lower than that of seawater. As a result, marine teleosts have to continuously desalinate seawater they drink in order to survive in the oceanic environment [16].

However, another explanation is equally possible. The initial forms of vertebrates emerged in seawater. The hagfish (*Myxini*) inhabit the ocean for more than 480 million years but even now remain stenohalinic. They have the paired kidneys, and the urine formation process (uropoiesis) begins in the glomus [30]. Both in the archinephron of the hagfish *Myxine glutinosa* L. and the kidney of the lamprey *Lampetra fluviatilis* L., the glomus is structured into nephrons that include the glomeruli and the tubule consisting of several segments (Table 1) [31, 32]. In the glomerulus of

the hagfish archinephron, like in the lamprey and rat, the wall of the glomerular filter comprises the endothelium, basal membrane and podocytes. The initial process of urine formation in hagfish and lampreys includes fluid ultrafiltration into the nephron lumen. In the stenohalinic hagfish *Myxine glutinosa* L., plasma osmolality is  $980 \pm 104$  mOsm/kg H<sub>2</sub>O, which is close to that of seawater ( $898 \pm 65$  mOsm/kg H<sub>2</sub>O) [30], with the concentration index U/P for Na<sup>+</sup> being  $1.07 \pm 0.1$ . Epithelial cells of the archinephron's duct have a brush border composed of microvilli [30], while urine has a higher concentration of K<sup>+</sup> (UK/PK  $1.5 \pm 0.7$ ) and lower concentration of Mg<sup>2+</sup> (UMg/PMg  $0.77 \pm 0.21$ ) compared to blood plasma. In the hagfish urine, the taurine concentration is higher than in plasma but that of threonine, proline and glucose is lower; there is a Na<sup>+</sup>-glucose cotransporter providing glucose reabsorption.

Paleontological chronicle shows the presence of glomerular filtration in the first cyclostomes that emerged almost 0.5 billion years ago. Obviously, its emergence was preceded by developing freshwaters as new habitats, and this indicates another purpose of the two-stage mechanism of urine formation (as a combination of filtration and reabsorption) not associated with the need for osmoregulation. Descendants of marine fish managed to penetrate into freshwaters and adapt to living therein; while one of the fish groups migrated back to the ocean, the others remained in freshwater, and some became migratory, dipnoan [33].

Urine formation begins from glomerular filtration, which is alike in organisms of different developmental levels, but its volume

increases during the evolution of vertebrates. Its significance for hyperosmotic regulation consists in the formation of solute-free water in the tubules. The necessity of this renal function arose in adaptation of animals to freshwater habitats and in terrestrial animals to drinking freshwater. Hypoosmotic urine formation depends on active  $\text{Na}^+$  transport from the tubular lumen and a drop in osmotic permeability of the epithelium. Renal conservation of water and the formation of osmotically concentrated urine involve aquaporins [34] and the renal countercurrent multiplication system [27]. In renal tubules of marine teleosts, urine is isosmotic to blood plasma along the entire length of the nephron, while in lampreys and in the terminal tubular segments of freshwater fish it is hypoosmotic due to creating a low osmotic permeability of the tubular wall by means of intense reabsorption of  $\text{Na}^+$  and  $\text{Cl}^-$  from the tubular fluid across the water-impermeable wall.

So, the kidney of vertebrates and excretory organs of marine invertebrates show an analogous functional pattern: the fluid approximating in its content the internal environment enters the lumen of the protonephridia, metanephridia or coelomoduct, and then all the essential ingredients are reabsorbed while the unneeded are excreted with urine. A combination of the two processes, ultrafiltration of an isosmotic fluid (equivalent to a protein-free filtrate of fluids of the internal environment) to the tubular lumen and reabsorption from it of a compositionally and concentrationally “ideal” fluid, can be considered as a theoretically optimal pattern of the excretory organ’s design. A possible alternative to this mechanism is a completely secretory kidney which would excrete from blood (hemolymph) to urine toxic or excess substances that were consumed with food. A huge diversity of organic and inorganic compounds vitally important to a living organism defies description, while that of unneeded or harmful is even less imaginable. An elimination of the latter via the secretory mechanisms would require a special organ with a capacity of instant recognizing and evaluating the incoming substances, as well as regulating their excretion, which seems to be infeasible. The above consideration of the constructive variants of the metazoan excretory organs assumes that

nature had initially chosen a combination of ultrafiltration with subsequent reabsorption of a compositionally ideal fluid as a basic model, while secreting (and excreting) strictly specific substances through a special excretory organ had emerged later and was used as an additional mechanism (Table 2). A main trend in the progressive evolution of the kidney consisted in an intensification of renal blood flow, ultrafiltration and proximal reabsorption [35] which provided a stabilization of the physicochemical parameters of fluids of the internal environment in ever-complicating organisms [6].

The above assumption finds support in the similarity between structural organizations of the excretory organs which, like the kidney, function in the majority of metazoan organisms. Following the initial step of glomerular filtration or capturing the coelomic fluid, the ultrafiltrate reaches the nephron’s proximal segment (or its analog in other excretory organs) where reabsorption of the extracellular fluid being “ideal” for the given organism takes place (Table 1). In the kidney, in the same tubular cells, there is a system of secreting some organic and inorganic substances which should be removed at a permanent basis in amounts exceeding those the ultrafiltrate can receive during ultrafiltration [26]. It has turned out that in some cases the kidney can secrete isotonic fluids either [36].

In terms of the classical views on renal functions, as formulated in the 19th–20th centuries, the mammalian nephron implements a “blind” and relatively constant in its volume process of glomerular filtration with an “obligatory” continuous reabsorption of substances in the proximal segment which comes to 2/3 of the ultrafiltrate volume. Then follows a subtly regulated “facultative” reabsorption in the distal nephron. Effectively, these few lines contain the whole output of the studies implemented by the end of the 1950s [33, 37].

As soon as the beginning of 1970s, it became clear that in the evolution of the vertebrate kidney some basic functional characteristics that have previously been deemed invariable are subject to considerable revision. In endothermic or homeothermic animals (mammals, birds), as compared to ectothermic or poikilothermic

**Table 3.** Reabsorption regulatory factors in the renal proximal tubule

Object	Factor	Effect type	Reference	
Human	mannitol	↓	41	
	sucrose	↓	41	
	acetazolamide	↓	26	
	GLP1	↓	45	
Rat, <i>Rattus norvegicus</i>	angiotensin II, 10 <sup>-12</sup> M	↑	51	
	angiotensin II, 10 <sup>-7</sup> M	↓	51	
	noradrenaline	↑	51	
	endothelin	↑	51	
	dopamine	↑	50	
	atriopeptide	↓	51	
	oxytocin	↓	48	
	exenatide	↓	43	
	Frog, <i>Rana temporaria</i>	exenatide	—	46

Arrow direction indicates increased (↑) or decreased (↓) reabsorption in the proximal tubule, or no effect (—).

vertebrates (fish, amphibians, reptiles), the volume of glomerular filtration sharply increases; moreover, the volume of the fluid and solutes reabsorbed in the proximal nephron also drastically increases [38]. This fact has been ignored in the first half of the 20th century as a major trend in progressive evolution of the kidney was thought to consist in the formation of the renal urine concentrating mechanism [33, 39]. This function really emerges in the avian kidney and reaches a high degree of its development in mammals being associated with the emergence of a novel structural organization, namely a subdivision of the kidney into the cortical and the medullary substances, as well as an emergence of the countercurrent multiplication system [40].

#### PROXIMAL REABSORPTION AND ITS ROLE IN THE KIDNEY EVOLUTION

Let us consider the facts that fundamentally change our idea on the evolution of renal functions, such as the intensification of glomerular filtration and a parallel increase in proximal reabsorption [38], as well as an alteration in the structure of renal blood supply. In marine fish, amphibians, reptiles and birds, blood is supplied to the kidneys from the two sources, arteries and the renoportal vein [31]. Completely arterial blood supply to the kidney

exists in freshwater fish and mammals. Increased cardiac output was assumed to boost glomerular filtration and therefore to automatically increase the volume of the fluid fraction reabsorbed in the proximal tubule.

These alterations in the kidneys during the evolution of vertebrates can receive fundamentally otherwise interpretation. The kidneys are aimed at providing and maintaining homeostasis, primarily at stabilizing osmolality and volume of fluids of the internal environment, their ion concentrations, arterial pressure, blood pH. The idea of “obligate” fluid reabsorption in the proximal segment was replaced by understanding that functions of the proximal nephron, specifically changing the volume of the fluid to be absorbed therein, can be “facultatively” regulated. Since the epithelial properties of this nephron’s segment allow isosmotic reabsorption only, changing the volume of the fluid to be absorbed can only be a consequence of changing the amount of reabsorbed solutes. In Smith’s studies [41], it was demonstrated that the presence of nonabsorbed substances in the ultrafiltrate, e.g., osmotic diuretics (mannitol, sucrose), leads to decreased proximal reabsorption and increased diuresis (Table 3). Depression of the Na<sup>+</sup>/H<sup>+</sup> exchanger in the proximal segment decreases fluid reabsorption therein [42]. A glucagon-like peptide

1 (GLP1) is involved in the regulation of  $\text{Na}^+/\text{H}^+$  exchange; its mimetic, exenatide, was shown to decrease fluid reabsorption in this tubule [42–44]. Therefore, reabsorption of solutes in the proximal tubule is not obligatory but adjustable, remaining meanwhile isosmotic (Table 3).

In the rat kidney, exenatide accelerates excretion of water following an oral water load or ions following a NaCl load. This allows revision of a role proximal reabsorption plays in the regulation of homeostasis. It has turned out that after drinking plain water the GLP1 concentration in human blood rises up to the same level that it reaches after glucose uptake. GLP1 secretion during hyperglycemia stimulates insulin secretion. GLP1 is one of the incretins, and its preparations play an important role in treating type 2 diabetes mellitus.

A comparison between these data and the basic views on the evolution of renal functions suggests another role of proximal reabsorption in the evolution of vertebrates. As compared to the kidney in cyclostomes, fish, amphibians, and reptiles, in homeotherms, particularly mammals, both the rate of glomerular filtration and the volume of fluid reabsorbed in the proximal segment increase several times [38]. The functional role of the proximal segment in the kidney evolution is important not only for absorbing a compositionally “ideal” fluid but also for redistributing the fluid volume between segments through the regulation of this function. In the proximal segment, fluid remains isosmotic to blood plasma due to a high permeability of the epithelium, especially at the intercellular contact sites. In lower vertebrates, the volume of the reabsorbed fluid in this segment is low, while in mammals it increases several times [46].

A regulatory decrease in proximal reabsorption in rats can be induced by oxytocin, angiotensin, peptide hormones, and exenatide (Table 3). The homeostatic effect consists in the fact that a larger volume of fluid enters the distal segment at the same level of glomerular filtration, while it is the distal segment and the collecting ducts where the effect of hormones that regulate water and some ion absorption is focused [27]. The evolutionary advantage of this way of regulation is that an increase in the volume of fluid reabsorbed in the proximal segment results in the fact that a larger volume of fluid in the distal segment falls

under the effect of selective regulators, hormones. Thus, in mammals, in the proximal segment of the nephron the volume of absorbable ions and water can be adjusted within a wide range and this strongly affects their renal excretion.

#### REGULATED REDISTRIBUTING OF FLUID FLOWS IN THE NEPHRON

In the evolution of the renal structure and function in vertebrates, some features can be distinguished that have a key significance in adaptation to various conditions of the water–salt regime. Distinctions in kidney microanatomy underlay a highest evolutionary achievement of the renal functions, namely the ability to concentrate urine osmotically which became able due to the emergence of the loops of Henle and a division of the kidney into the cortex and medulla [40].

Microanatomy of the vertebrate kidney indicates that the nephron loops, including those of Henle, are present in the kidney of lampreys [47] and cartilaginous fish. They were found in different segments of the nephron being involved in the implementation of some renal functions other than urine concentrating. However, in the kidney evolution what we designated here under the term “functional loops” of the nephron is of exceptional significance. We mean the possibility to alter (increase or decrease) reabsorption of water and/or solutes in one segment of the nephron and hence the volume of the tubular fluid entering the following nephron segments against the background of stable glomerular filtration. This mechanism ensures interaction between individual renal structures and plays an important role in the implementation of the kidney’s homeostatic functions. Such loops include the juxtaglomerular apparatus the formation of which recruits the glomerulus and the macula densa [26]. The functional significance of this loop, where the distal tubule (macula densa) touches the glomerulus, is that depending on the composition of the tubular fluid near the macula densa the glomerulus responds by changing the volume of fluid filtrated in the given nephron. Another loop pattern is the functional proximodistal loop in which the volume of fluid reabsorbed in the proximal tubule and the amount of solutes that reach the distal segment can be adjusted, thus affecting diuresis



[48]. The third type of the functional loops forms in the outer medulla between the thick ascending limb of the loop of Henle and the collecting duct. Due to changes in the rate of prostaglandin secretion in this kidney area, reabsorption of ions in the thick ascending limb of the loop of Henle changes, leading to increased loading the collecting ducts with fluid. This can occur against the background of a constant level of glomerular filtration and intact diuresis but would be accompanied by increased reabsorption of solute-free water [49]. Such functional features of the mammalian kidneys are of great importance for homeostasis which eventually determines the context for successful functioning of different organs, including the brain [10].

Redistributing of substances between the ascending limb of the loop of Henle and the collecting ducts influences the final urine composition. It was revealed under conditions of various human pathologies [49]. L.A. Orbeli [12] considered changing the function in conditions of pathology as one of the basic methods of evolutionary physiology.

#### PROXIMAL REABSORPTION FOR PRESERVING AN IDEAL BLOOD CONTENT AS A PRINCIPLE OF KIDNEY PERFORMANCE

As shown above, the emergence of glomerular filtration and its functional analogs did not owe to the initial adaptation to freshwater habitats and the need for water excretion but was equally characteristic of initially marine invertebrates and vertebrates. The question arises as to why in both cases the mechanism of urine formation includes two components, ultrafiltration (or capturing the coelomic and other internal environment fluids) and subsequent reabsorption of essential substances, but is not based on direct secretion of unneeded substances. The kidney arose in evolution as an organ for stabilizing the composition and volume of fluids of the internal environment, i.e. for homeostasis. This equally concerns those living beings that inhabit sea- and freshwater, live on land and air. The answer may be that there should necessarily exist a physiological mechanism that would maintain an “ideal” composition of fluids of the internal environment, their osmotic pressure, all

their vitally important substances. These substances come with food, form during metabolism, partly penetrate through the coverings, and are excreted in breathing, through the intestine and kidneys. Due to an immense diversity and variability of chemical compounds that surround animals and can anyhow enter an organism, let alone their metabolites, it is impossible to deactivate and excrete all of them, so that filtration and reabsorption of only what is vitally important proved to be more efficient. From this, it follows that the “ideal” composition and concentration of substances needed at any moment of life can only be ensured through combining ultrafiltration (or its analog) and returning an “ideal” fluid to the internal environment, i.e. exactly through what occurs while reabsorbing fluids during urine formation in the proximal segment of the nephron. The function of the distal segment consists in subtle adjusting predominantly inorganic components of the internal environment.

The very idea of a combination of the first (ultrafiltration) and the second (reabsorption) steps of urine formation has been facing for decades a rejection by the research community. Above, we discussed in detail the purpose of the glomerular kidney and its advantages despite high energy costs of its performance. Yet another question then arises as to why evolution accepted this pattern of the renal functional organization as a major tendency so unreservedly. It is clear that the need for uninterrupted reabsorption of a huge variety and different amounts of substances requires as huge energy costs. Moreover, throughout evolution these costs are rising continuously, as judged by an increase in arterial blood flow and the elimination of the mammalian renoportals system. It should be inferred that evolutionary advantages given by a stability of physicochemical parameters of the internal environment, despite increasing energy costs for performing renal functions, led during evolution to a choice in favor of homeostasis. This, in turn, enabled the creation of an optimal context for successful brain performance and a broad range of adaptations in ever-changing living conditions. At the same time, we cannot help noting that even at high energy costs the mammalian kidney is continuously building up its spare capacities. There is an additional recovery system, which is actualized in the form of a compensatory hypertrophy when a

kidney or part of it is lost. In the case of a removal of one kidney, the remaining copes completely with its function of providing homeostasis. These facts indicate that in the process of evolution optimal conditions are created for high-performance kidney functioning in different situations.

The idea of a combination of filtration and reabsorption as a basic principle of urine formation was first advanced by K. Ludwig in the 1840s. However, yet in the beginning of the 20th century this idea was still debatable. J. Barcroft, when discussing this problem, describes the situation in the first decade of the 20th century: "Thirty years ago, meetings of scientific societies were less crowded than nowadays ... the privilege and a sort of entertainment for the youth at that time was listening to how the elders entered into lengthy debates ... I remember how I once spoke out this very assumption to Langley in one of the conversations that we used to have from time to time on the issue of gland secretion,—bearing in mind the assumption that the kidneys can both secrete and absorb; this idea seemed too "bulky" to his generation, and he immediately said: "It's too complicated," to which I could only answer that, after all, it's no more complicated than the kidney itself" [5].

A great diversity of the excretory organs in invertebrates, including arthropods, complicates direct application of the filtration hypothesis to insect Malpighian tubules, but cumulative argumentation and the pattern of arterial blood supply in insects support the assumption that the initial process in Malpighian tubules also includes fluid filtration [17]. An important advantage of reabsorption (reverse absorption) over sorption (absorption) is that in many cases it is underlain by ion exchange. For example,  $K^+$  secretion occurs in exchange for  $Na^+$ , while many transport phenomena in the nephron (reabsorption of glucose, amino acids, ions etc.) are provided by a symport or antiport of ions, which are more advantageous energetically than a primarily active transport. The advantage of reabsorbing vitally important substances, which can enter the blood flow in large excess, is used with the involvement of the mechanism of tubular transport maximum ( $T_m$ ), when reabsorption is limited by a strictly definite amount of substances while the rest is excreted with urine.

## ISSUES OF PROXIMAL REABSORPTION

Isosmotic reabsorption in the proximal segment of the nephron conceals many riddles. In this segment, almost all significant components of fluids of the internal environment are transported to the blood. Water is absorbed right after the reabsorbed solutes, thus retaining iso-osmolality at both sides of the tubular wall. Various physicochemical processes which cut energy costs (e.g., ion exchange and  $Na^+$ -dependent co-transport) play an important role in the molecular mechanisms of absorption [8]. The need for returning all the essential substances to the blood and exactly at a ratio that matches the optimal content of fluids of the internal environment (reabsorption of a compositionally "ideal" fluid) imparts an exceptional complexity to this process. Multiple studies offer an analysis of the molecular mechanisms of reabsorption of some essential organic and inorganic substances (ions, water, monosaccharides, amino acids etc.). The principles have been found that limit transport of these substances in the renal tubules ( $T_m$ ). There are numerical values of this parameter for glucose and some amino acids [41]. However, another issue arises as to how nature managed to solve this task at the dawn of the evolution of metazoans, when the first proto- and metanephridia had already emerged and absorption of an "ideal" fluid with all its innumerable essential components of the internal environment had already been occurring.

In all types of nephridia, the proximal segment retains its structural and functional constancy, the ability to return to the internal environment an "ideal" fluid and to secrete some organic molecules to the tubular lumen [26, 41]. The distal segment was formed to promote adaptation associated with osmoregulation and excretion of some excess ions [33]; it is present in most invertebrates, agnathans and higher vertebrates (Table 1). Still unresolved is the issue of the molecular mechanisms that underlie absorption of an ideal multi-component fluid in the proximal segment which comprises almost all ions, microelements, amino acids at a requisite concentration. The rate of this process is regulated with the involvement of hormones and neurotransmitters (Table 3) [50, 51].

## EVOLUTION OF EXCRETORY ORGANS AND GENERAL PROBLEMS OF EVOLUTION

The main problem now is not to understand how to improve, sophisticate a cell or an individual organism, as these questions were answered by the Darwin's discovery of natural selection [2]. The formidable task is to realize how to "assemble" the first cell, the first individual, the first kidney etc. Specifically, how to actualize the principles that underlie a holistic understanding of the phenomenon, the creation of a fundamentally new living entity in the inorganic world, even at unlimited possibilities.

The scientific rationale behind the stages and mechanisms of evolution gives no answer to the question on the origin of the first cell. Up-to-date literature allows understanding of the origin of hereditary systems [52], the formation and further development of bioenergetics and so on but disregards the key question as to how and in which form the first cell, the first organ, the first living thing have emerged. The toughest issue is how the choice of a solution to the problem was predetermined that had eventually found its realization hundreds of millions of years thereafter.

These issues give rise to many ensuing ones. If proceeding from the fact that life was developing on Earth, even regardless of whether the elements of the cell-to-be emerged on our planet or were imported from the space, then (1) there should necessarily exist a medium suitable for living, (2) there should necessarily be an optimal bunch of initial components for the formation of a protocell. The first issue seems to have already been solved: physicochemical elements of the intracellular medium are supposed to be the same at both sides of the cell membrane because in the initial period there were no macromolecules that could provide the cell with its own internal medium distinct from the external [15]. From this point, the stage of biological evolution may have begun according to the Darwin's principles. "Man selects only for the sake of his benefit, nature—only for the benefit of the protected being" [2]. And also "Each creature reveals a tendency to become more and more improved in relation to its surrounding conditions. This improvement will inevitably lead to a gradual

increase in the organization of most living things all over the world" [2].

If excluding the idea of the Creator and his Plan [53], then we would need not only to resolve the mechanism of the origin of Man, Brain and Mind but also to explain how 4 billion years ago, while selecting atoms and molecules essential for life in all its manifestations, there were found and retained those whose potential managed to unfold billion years thereafter. We believe that the above facts and arguments with regard to the evolution of renal functions may answer at least some questions as to the reasons behind a choice of exactly the given type of the kidney's organization in animals and humans.

## CONCLUSIONS

1. The above consideration of varied organs referring to the excretory system of metazoans indicates that they represent homeostatic organs whose function is to retain the ideal composition of fluids of the internal environment and to eliminate everything that does not meet these purposes. The latter function may concern either excess concentrations relative to limiting normal values or physiologically insignificant and harmful substances. The morphofunctional organization of the kidneys and their analogs is based on a combination of ultrafiltration of the protein-free fluid to the tubular lumen followed by reabsorption of all the vital substances in the form of a compositionally and concentrationally "ideal" fluid of the internal environment.

2. The excretory organs of the secretory type (glands) or the aglomerular kidneys characterize those animals whose kidneys cannot ensure their osmoregulation in full. In this case, the salt glands form to excrete osmotically concentrated secrets (NaCl, KCl) and to supplement the kidneys' function aimed at stabilizing other parameters of the internal environment, as typical of birds, reptiles and elasmobranchs. The fish aglomerular kidney participates in homeostasis along with the intestine and gills. Marine fish drink seawater, which is hyperosmotic relative to the blood; then the salts of divalent cations are secreted off the renal tubular cells, while the surplus of monovalent ions is excreted via branchial cells.

3. In the mammalian kidneys, a novel mechanism is realized responsible for redistributing fluid flows within the nephron. At the same level of glomerular filtration but quantitative re-distribution of reabsorbable substances between the proximal and distal segments, it alters the amount of substances entering the distal segment and accessible there for the selective adjustment of reabsorption under the effect of hormones and neurotransmitters.

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### COMPLIANCE WITH ETHICAL STANDARDS

This study did not involve any human or animal subjects as research objects.

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