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Point of View

The origin and evolution of life and the role of inorganic environmental factors in its rise and development remain key problems of natural science. The assumption that the first forms of life—protocells—appeared not in the "sodium" sea, as has been always believed, but in "potassic" water basins is substantiated in the article below. Later, protocells with the potassic cytoplasm adapted to the sodium water environment. The role of sodium in animal evolution is the core subject of the article.

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The Physiological Evolution of Animals: Sodium Is the Clue to Resolving Contradictions

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A tight insoluble knot of life: the flesh,
Pierced by respiration and pulsation.
The planet was freezing.
Life was flaring up.
Our forefather, who dragged out to land
His fishlike frame from chilly waters,
Brought with him the whole ancient Ocean
With all its breathing ebbs and flows,
Full of the waters' primary warmth and salt—
The living blood that streams in our vessels.

These lines from M. Voloshin's poem [1, p. 204] had been published before experimental data on the marine origin of life were obtained. The poem was written in the first decades of the 20th century, and its author was amazingly accurate in posing natural-science questions. Analysis of every line makes it possible not only to answer them in strict terms and facts of hypothetical steps of evolution but also to discuss the things that were encoded by Voloshin in the first lines—"The planet was freezing. "Life was flaring up"—and without which life could not rise.

How was the intuitive portrait of the past generated?

When reading the *Old Testament*, the reader is invariably impressed by the coincidence between the days of creation and data about the macroevolution of vertebrates. What I mean is the poetic image of the sequence of events rather than strict figures, dates, and structures. When *Genesis* appeared, there were no paleontological or primary data that could help us penetrate into the distant past. This reference to the *Old Testament* is not paying tribute to the current trends or reviving anti-Darwinian court proceedings but rather an attempt to penetrate into the depths of consciousness and the nature of intuitive images, based on something invisible and unconscious. However, it is impossible to grasp it in the consciousness of those who are dead and gone. The first chapter of *Genesis* presents the sequence of creation: God created fishes, "creeping things," and "winged birds"; and living creatures filled the waters in the seas; and cattle, creeping things, and beasts filled the earth. This implies not a single-step formation of different kinds of living creatures but development in time and in the sequence that is familiar to us from strict documentary sources.

FROM POETIC IMAGES TO QUANTITATIVE INTERPRETATION

Voloshin's poetic image of the evolution of life is very close to data from the special literature, monographs, and textbooks devoted to the evolution of life on the earth. Let us consider a few works.

A.G. Ginetsinskii, one of the leading specialists in this field, who, after Academician L.A. Orbeli's death in 1958, succeeded to his post as the director of the Sechenov Institute of Evolutionary Physiology, USSR Academy of Sciences, wrote [2, p. 9]

> At present, nobody doubts that life appeared in the water of the Paleozoic ocean, which contained certain proportions of mono- and divalent cations that may be with good reason called biological. For primitive prototypes of the animal kingdom, as well as for modern coelenterates, seawater was both the external and internal environment. It was under these conditions that cells adapted to the proportion of ions typical of the waters of ancient seas.

This concept is substantiated by L. Prosser, the author of the multivolume *Comparative Animal Physiology*: in his opinion, life began in the ocean. According to geochemical data, the ionic composition of seawater has not changed significantly since the early Cambrian,

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although its overall saltiness has probably increased slightly [3, p. 177]. The same idea is developed by S.E. Shnol' [4, p. 95]:

> Life takes its origin from the sea. The chemical composition of seawater is determined by the composition of the earth's crust and the physical and chemical properties of its components and, consequently, by the chemical composition of the planet. Almost all the authors emphasize that it is highly probable that life began in the sea. I also view this as practically doubtless.

When reading these lines and noting the unanimous interpretation of the early stages of evolution, the reader is ready to acknowledge the indisputable logic of these considerations. To revise these concepts, we need serious arguments.

Moreover, data of paleontology and comparative physiology make it possible to translate Voloshin's poetic images into the language of numerical values. "Our forefather, who dragged out to land his fishlike frame from chilly waters," brought with him the whole ancient ocean with the salt of the waters—"the living blood that streams in our vessels …." The time described in the poem may be roughly dated back to hundreds of millions of years ago, when *Chordata* and *Agnatha* already lived in the ancient Cambrian ocean. Over the past hundreds of millions of years after our forefather had left this environment for land, the physicochemical frame—the electrolytic composition of the internal environment's fluids, which surrounded cells in those distant times—has largely remained the same.

The events that had taken place long before the time described by Voloshin—the formation of protocells—need comprehension. According to modern data, life had begun many millions of years before the Cambrian. The newest data, which vary within billions (!) of years, make it possible to date roughly the appearance of eukaryotic cells. Some calculations show that this happened about 2.7 billion years ago [5], while others date their appearance back to 950–1259 million years ago [6]. These calculations are based on studies of the amino-acid sequence of 129 proteins of 36 eukaryotes and are designed for determining the time of the divergence between the main kingdoms of living organisms: fungi, plants, protests, and multicellular animals. It is believed that the latter separated from choanoflagellates 761–957 million years ago, while the divergence between protostomes and deuterostomes is estimated at 642–761 million years ago [6].

The problems of the evolution of life are studied by paleontologists, geochemists, zoologists, biochemists, and molecular biologists [5–9]. The problems of the evolution of functions in animals are actively developed by specialists in evolutionary physiology [10–12]. To understand the conditions under which living organisms appeared, it is necessary to have data on the origin, soil chemical composition, and evolution of the earth and ocean [13, 14]. However, deep penetration into the mechanisms of the origin and evolution of life is impossible without many-sided physiological studies on the development of its phenomena.

At the turn of the Cambrian, the osmotic pressure in the cells and fluids of the internal environment of aquatic animals was the same as in seawater; these animals had no osmotic regulation, and the osmotic pressure of their extracellular fluid was equal to that of the water environment. In the modern ocean, *Myxini* live, whose morphophysiological organization is close to that of distant ancestors of *Agnatha. Myxini* are the only large group of vertebrates that belongs to stenohaline[1] aquatic organisms [3]. They can live only in seawater; if the saltiness changes, they die.

The transfer of fish from the ocean to rivers and lakes was connected with the appearance of hyperosmotic regulation—a physiological system in the organisms of aquatic animals responsible for removing extra water and seeking and accumulating ions that were abundant in the ancient ocean but scarce in freshwaters. In the Ordovician of the Paleozoic era, ostracoderms ("shell-skinned" fish) appeared in these waters, which managed to overcome the salt barrier and to develop physiological adaptation mechanisms in order to leave seawater and adapt to freshwaters in rivers. Shell-skinned fish brought the salts of ocean waters with them in their blood. Although the concentration of salts in their blood was lower than in modern seas, the proportion of ions remained close to the environment from which they had migrated. The body of these ancient fish was covered not with a light scale but with a shell. The explanation seemed obvious: they were adapting to a new habitat and needed protection from predators. However, H.W. Smith, who studied the characteristic features of the adaptation of these originally marine fish to freshwater, proposed a nontrivial hypothesis [15]. He assumed that their main enemy was not hypothetical predators but fresh water. Along the osmotic gradient, it penetrated into the body and could dilute the blood and cause osmotic shock in cells. Waterproof covers were necessary to protect the fish from fresh water, and their kidneys ensured the removal of water from the blood. This principle of water–salt metabolism has survived from the forefather of vertebrates into modern aquatic animals and is present in modern forms of organisms. Many ways and mechanisms of adapting to different saltiness of the external environment have become clear [11, 16].

Voloshin, a poet; Smith, a chemist; Ginetsinskii, a physiologist; and other natural scientists, whose works we could continue to quote, believed that life appeared in the sea and, hence, the proportion of ions and the ions themselves in the blood plasma and extracellular fluid are close to those that existed in the ancient ocean. However, modern data undoubtedly show that life began much earlier than in the Paleozoic and the date of

[1] Greek *stenos* narrow + *hals* salt.

the rise of multicellular organisms has shifted millions and even billions of years back. According to modern concepts, life on the earth began slightly less than 4 billion years ago, while the earth itself had formed 4.5 billion years ago. Those who study the rise of life focus mainly on the mechanisms of forming organic molecules and macromolecules and transferring hereditary information [9]. Meanwhile, there is another problem concerning the evolution of life: the inorganic environment, in which life has been developing since its first steps in a close interaction with organic substances.

One of the central problems of the earliest stage of evolution, which is at present little spoken of, is the ratio of physicochemical factors of the environment to organic molecules—the elements that underlay the formation of life molecules and then the assemblage of the first cell. I mean the exclusively important initial stage of evolution—the formation of the initial cell, the first little bundle of life with a minimum of components that nevertheless ensured the possibility to live and reproduce itself, even if in the most primitive form. Let us mention the most important condition: an individual should be independent, separated from the external environment; i.e., it needs an envelope, a membrane, which would protect it from the environment. Of course, this envelope was not necessarily a complex plasmatic membrane found in modern living forms; it could be a pellicle, which isolated a viable individual from the inorganic or alien organic environment.

To form a membrane, a system of synthesis is necessary. When could it emerge? Only when the RNA world appeared [17], i.e., the system of synthesizing the first peptidic molecules—chemical organic elements of life, without which it cannot develop [9]. Even if we proceed from the hypothesis that organic molecules were imported to the earth from outer space (suppose that we have proof that microorganisms can live for a long time in outer space and then adapt to the earth's environment), we ought to recognize that physicochemical conditions on the earth must be optimal for the development of life, the ionic composition, pH, and osmolality being of primary importance.

Therefore, there is no life without organic elements, but neglecting inorganic components of the environment, in which macromolecules are synthesized, is fraught with an incorrect assessment of the conditions under which life took its first steps (in real time, of course, these were far from being the first stages). Hence, it is very important to penetrate into the essence of events that took place 3 to 3.5 billion years before the Cambrian. It is necessary to propose a modern concept of arguments to substantiate the hypothesis that life began in the sea. However, under thorough physiological analysis, the thesis that life originated from the marine environment not only seems questionable but also open to contest on the basis of many facts and logical constructions of the physiological evolution of living beings.

THE ENVIRONMENT OF PROTOCELLS

The salt composition of the environment where the first forms of life appeared could comprise salt solutions, in which the concentration of ions was friendly for the functioning of elements of gene matter (RNA or DNA), chemical reactions of protein synthesis, and other vital biochemical processes. Of course, we have no direct data that would allow us to judge about physicochemical conditions of the environment where life began; however, we may make certain conclusions about its electrolytic composition. Nature's conservative attitude to fundamental principles of constructing living systems most probably showed in the qualitative similarity of the concentrations of ions, which ensure vital functions in the series of next generations, from the first forms of life to modern individuals.

To understand the physicochemical conditions of the environment where life formed, it is necessary to analyze the geological past of the planet's surface. As for inorganic ions, tissues of living beings contain macroelements: cations of potassium, sodium, calcium, and magnesium. Paleogeochemical data are necessary to estimate the ionic composition of the environment in the epoch when living beings appeared. Certain information about the environment in which animal cells appeared may be derived by comparing petrological chemical indices of the sodium and potassium content in argillaceous deposits of different periods of the earth's history [18]. Clays and their derivatives (argillites and aleuropelites) are characterized by a high sorption capacity, which makes it possible to judge about the proportion of sodium and potassium cations in probable environments of the development, if not the origin, of initial forms of living organisms. Studies have shown that in superficial rocks, potassium ions usually prevailed compared to sodium ions. The highest content of potassium in rocks is characteristic of the early Proterozoic (the first third of the Riphean) [18], when the initial forms of animal cells—eukaryotes—probably developed. During the next 3 billion years, there were periods when the ratio of sodium ions to potassium ones in the superficial rocks changed: the content of sodium became equal to that of potassium or even higher. These are the Sumian–Sariolian (more than 2.5 billion years ago) and Kalevian (approximately 2 billion years ago) periods.

To answer the key question about the environment where cells appeared, it is necessary to discuss experimental data on the inorganic cations that ensure the operation of a protein synthesis system. The systematized data show that to synthesize a protein in vivo and in vitro, 5–20 mM of Mg^{2+} ions and about 100 mM of K^+ ions are necessary, while Na^+ ions are not only incapable of substituting K^+ ions but also "are their antagonists and inhibit the operation of the protein synthesis system" [19, p. 152]. It is believed that in the ribosome, magnesium and potassium ions favor complex formation and the fixation of components of the protein syn-

Table 1. The concentration of certain cations (mM/l) and osmolality (Osm, mosm/kg H_2O) in blood plasma or hemolymph in animals and humans

Subject of inquiry	Osm	Na	K	Ca	Mg
Mussel *Mytilus edulis*, 34‰*	1100	479 ± 4	10.9 ± 0.2	18.8 ± 0.7	56.7 ± 2
Freshwater pearl mussel *Margaritifera margaritifera*	32 ± 1.2	14.7 ± 0.4	0.37 ± 0.02	1.86 ± 0.08	0.41 ± 0.02
American cockroach *Periplaneta americana*	421 ± 3	116 ± 8	11.3 ± 1.3	3.6 ± 0.6	4.9 ± 0.7
Starry sturgeon *Acipencer stellatus*	304 ± 3	122 ± 2.5	1.3 ± 0.1	2.2 ± 0.2	1.7 ± 0.2
Sockeye salmon *Oncorhynchus nerka*	289 ± 4	141 ± 1.4	2.0 ± 0.2	2.8 ± 0.4	1.1 ± 0.1
Common frog *Rana temporaria*	222 ± 2	108 ± 1.5	3.4 ± 0.2	2.12 ± 0.08	1.2 ± 0.04
Russian tortoise *Testudo horsfieldi*	295 ± 6	135 ± 2.5	5.5 ± 0.4	2.7 ± 0.03	1.3 ± 0.12
Rock pigeon *Columba livia*	295 ± 3	147 ± 1.7	2.4 ± 0.3	2.6 ± 0.04	0.68 ± 0.03
Wistar rat	294 ± 3	140 ± 0.2	3.9 ± 0.4	2.3 ± 0.06	1.03 ± 0.05
Great gerbil *Rhombomys opimus*	–	155 ± 1	7.3 ± 0.26	1.4 ± 0.07	0.9 ± 0.05
Human	287 ± 2	143 ± 1	4.5 ± 0.1	2.27 ± 0.07	0.85 ± 0.03

* Saltiness of the habitat of the mussels is % of seawater of the Barents Sea.

thesis system and act as catalysts in peptide bond synthesis [19]. The cell of modern vertebrates retains the same optimum of cations: about 13 mM of Mg^{2+} ions and 100 mM of K^+ ions [20].

It is worth answering one of fundamental questions: which differences between sodium and potassium determined the choice made by nature? The two cations have the same charge, equal to 1, but the radii of hydrated and dehydrated Na^+ ions are 5.6 and 0.98 Å, respectively, while those of K^+ ions are 3.8 and 1.33 Å, respectively. At the same time, there are no substantiated ideas concerning the physicochemical parameter that determined the preference of K^+ as the intracellular cation. The above data only make it possible to conclude that the dominance of potassium ions was an obligatory condition in the intracellular electrolytic environment.

It is logical to assume that the initial forms of cells, from which life on the earth began, could have qualitatively similar physicochemical parameters. These initial forms may be called *protocells* [21] to distinguish them from modern or fossil organisms. We have proposed this term for the physiological characteristic of cells at the initial stage of their development, when they had no plasmatic membrane in the modern sense of the word. They could have only an envelope that prevented the newly formed organic molecules of this organism from transferring into the environment and held them inside the cell. It is reasonable to suppose that the concentration of monovalent ions and the osmotic pressure under the protocell's envelope (membrane) were equivalent to those in the environment. Any deviations in these parameters from the habitat would require special membrane macromolecules, similar to ion pumps, ion channels, and water channels. However, such molecular devices require the presence of protein syntheses systems in the cell. We can hardly assume that such systems existed at the early stage of life, and they had not existed prior to its beginning.

What was this envelope, or membrane, like? We may very cautiously assume that it was roughly analogous to the structures found in modern cells and organisms. There are organisms with simpler structures compared to the plasmatic membrane. The pellicle that covers microbial communities and certain types of membranelike formations, such as diaphragms over endothelial pores of glomerular capillaries or slit membranes between the podocyte "pedicels" in the kidney, may exemplify this. Note that we merely suppose that certain isolating envelopes probably existed as precursors of the plasmatic membrane. Its modern perfect organization shows that it has a long history.

Measurements of the concentration of ions in cells and the extracellular fluid in modern animals testify to the fact that the main intracellular cation is potassium; in the hemolymph, blood plasma, and extracellular fluid of the majority of animals, sodium dominates. Let us compare the concentrations of the cations in extracellular fluids (Table 1) and tissues (Table 2) of various animals, from mollusks to mammals. If potassium ions dominate in cells, the share of sodium in tissues is lower and that of potassium is higher than in the hemolymph or blood plasma, which shows the prevailing accumulation of potassium in cells. The same tendency is observed in the cells of protozoans. For example, in the amoeba *Amoeba proteus*, the content of potassium is 0.376; of sodium, 0.026; of calcium, 0.063; and of magnesium, 0.217 μM per 1 mg of dry matter [3]. Thus, in the amoeba cell, the content of potassium ions is 14.5 times higher than that of sodium ions. In muscle cells of mammals, the concentration of potassium ions is about 150–160, while that of sodium ions is 12–16 mM/l; i.e., the ratio of K to Na is more

Table 2. The concentration of certain cations in water environments and the lithosphere of the earth, in plants, and in tissues of animals and humans (according to [22])

Subject of inquiry	Na	K	Ca	Mg
Lithosphere (% to mass)	2.83	2.59	3.63	2.09
Human (% to dry matter)	0.47	1.09	4.67	0.16
Rat, kidney, cortex	73.9 ± 5.2	80.9 ± 1.9	–	9.2 ± 0.25
Atlantic cod *Gadus morhua*, muscle	26.3 ± 2.8	129 ± 4	–	12.1 ± 0.46
American cockroach *Periplaneta americana*, muscle	21.7 ± 4.8	85.6 ± 2.6	–	23.3 ± 0.7
Common sawfly *Rhadinoceraea micas*, muscle	72.3 ± 6.8	190 ± 12	–	34.2 ± 4.8
Plants (μM/g of dry matter)	0.4	250	125	80
Ocean water	457	9.7	10	56
Black Sea (Karadag)	227	5.4	7.2	25.5
White Sea, Chupinskii Inlet	310	6.9	8.5	37
Lake Baikal	0.18	0.025	0.39	0.12

Note: The concentration of cations is in mM/l; in tissues, in mM/g of damp matter; in other cases, as specified.

than 11. Consequently, cells with very different levels of development and specializations show significant similarity in their physicochemical organization. It is highly probable that in animal cells, from unicellular forms to higher multicellular organisms, the potassium cation continues to dominate. This is vitally important for preserving the physicochemical properties of the intracellular environment from the moment of its formation to the present day, i.e., over billions of years. Rare exceptions (for example, sodium nucleus-free red blood cells of certain animal lines) only confirm the above regularity, because sodium replaces potassium in the cell when there is no need to synthesize protein at the latest stages of the life of a given type of cells.

Therefore, the main point in our assumption is that, contrary to prior beliefs, the first cellular forms emerged not in the “sodium ocean” but in potassic water basins, rich with magnesium ions, which contain organic substances, including nucleic and amino acids. Macromolecules could be synthesized and organic products could be preserved under the protocell envelope in such water basins. Apparently, their subsequent development and propagation took place in a potassium environment with a minimal content of sodium ions; the cell’s content and its environment were isoosmotic to each other. This environment was probably shallow reservoirs on the earth’s surface. The aforementioned petrographic indices of the sodium and potassium content relate to clayey deposits [18]. Curiously enough, poetic images (or evoked associations) again coincide with the hypothetical environment where the first living forms—protocells—appeared. Compare: “And Jehovah God formed man of the dust of the ground” (*Bible*); “God formed the first man out of clay, / That’s what we hear every day” (A. Zarif’yan [23, p. 59]); and “What did ancient folks believe? / Lilith had been prior to Eve …. / Not from a rib, nor out of clay, / From the silver of the breaking day” (V. Shefner [24, p. 211]).

THE ROLE OF SODIUM IN THE CELL’S FUNCTION

Upon the emergence of protocells, a *plasmatic membrane* began to form, which was another stage of physiological evolution. The potassium protocell had been developing in potassic basins. Geological evolution was accompanied by volcanic eruptions; as the earth’s crust and the architecture of the earth’s surface changed, the content and ratio of ions in basins changed too. As is known, volcanic ash is characterized by high concentrations of sodium ions. In basins where sodium dominated, only those cells that had acquired a plasmatic membrane and were able to preserve the potassic cytoplasm in the sodium salt environment could survive; in other words, the membrane should have a sodium pump. We may assume that sodium ions stimulated the formation of the plasmatic membrane, which replaced the cellular envelope, and natural selection helped these cells survive.

The appearance of the membrane, which separated fluids with different ionic compositions, was connected with the formation of sodium pumps and sodium channels in this membrane; this could become the starting point of cellular differentiation and then organogenesis. In our opinion, the key role in these cases belonged to sodium-dependent physiological processes. They included the appearance of membrane electrogenesis and the formation of sodium-dependent cotransporters in the plasmatic membrane, which ensured the transportation of glucose, amino acids, and certain inorganic ions to the cell. These molecular mechanisms opened new possibilities in the cell’s evolution and participation in the formation of multicellular organisms.

Let us consider the physiological role of sodium-dependent macromolecules, which gave an evolutionary advantage to multicellular animals compared to other groups of living beings. Na^+, K^+ ATPase of the

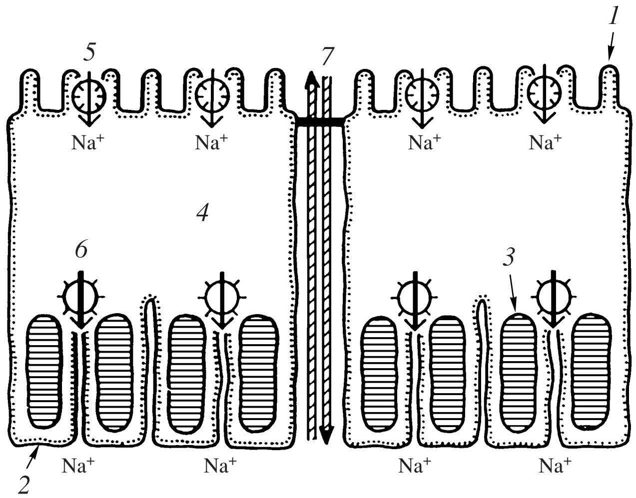

Fig. 1. The scheme of sodium transportation in the asymmetrical cell of the osmoregulating epithelium.

(*1*) apical plasmatic membrane, (*2*) basal plasmatic membrane, (*3*) mitochondrion, (*4*) cytoplasm, (*5*) sodium channel, (*6*) sodium pump, and (*7*) the intercellular junction zone; arrows indicate the direction of the flow of ions depending on the gradient of their concentration.

plasmatic membrane ensured the restoration of the cellular potassic cytoplasm in the sodium external environment. The Na^+/K^+ gradient favored the emergence of an electric potential on the plasmatic membrane of this cell, which later made it possible to realize a number of functions; in the first place, it became a prerequisite for the formation of the *nervous cell*; i.e., it ensured fast information exchange and control of cells in the organism as a whole. Synthesis of physiologically active substances in such a cell ultimately resulted in the development of neurosecretion; humoral control of functions in a multicellular organism; and the endocrine system, which formed in close connection with the nervous system.

By removing sodium ions from the cell, Na^+, K^+ ATPhase created prerequisites for a sodium-dependent mechanism of supplying organic and inorganic substances to the cell. In the course of further cellular differentiation, this mechanism participated in the formation of special epithelia, which played a leading role in organogenesis during the formation of sorbing epithelia of integuments, the alimentary canal, excretory organs, and salt glands.

Sodium-dependent processes could also stimulate *cellular differentiation.* The presence of sodium pumps and sodium channels in the plasmatic membrane was perhaps the starting point for cellular differentiation [21]. In initial cells, sodium-dependent macromolecules are randomly distributed in the plasmatic membrane. However, there are cases when ionic channels are concentrated in one part of the membrane, while ionic pumps are in the other part (Fig. 1). An accidental redistribution of the channels and pumps may lead to the formation of a polarized, or asymmetric, cell, whose membrane will concentrate mainly ionic channels in one part and sodium pumps in the other (Na^+, K^+ ATPhase). This process could precondition the formation of epithelial cells. As for modern organisms, asymmetry is inherent in the skin cells of amphibians and in the cells of some parts of the alimentary tract and excretory and other organs. In multicellular organisms, there are also symmetrical cells (for example, red blood cells and myocytes), whose heterogeneous plasmatic membrane is characterized by a uniform distribution of channels and pumps. Consequently, macromolecules of the plasmatic membrane, involved in the transportation of sodium ions, could become a source of a new morphofunctional cellular organization—cellular differentiation—and lead to the formation of epithelial cells. Coelenterates already have different cellular layers. Between the cellular layers that form the ectoderm and the endoderm, there is the mesogloea, whose ionic composition in medusae is close to that of the environment—ocean water. The extracellular fluid (hemolymph) in marine organisms, for example, in mollusks and ascidians, is also close to the concentration of sodium and potassium ions in the marine environment.

SODIUM AND OSMOTIC REGULATION

Sodium is not only the main cation of the blood plasma and extracellular environment in the majority of animals; its ions with the accompanying anions determine the osmolality of fluids in the internal environment. Our calculations show that the share of sodium ions in the total concentration of osmotically active substances in average seawater is 49.5%; in the osmolality of the blood plasma in mammals (humans, dogs, and rats), 49%; and in other classes of vertebrates (birds, reptiles, amphibians, and fish), 44.6–48.8%, although blood osmolality in these organisms can differ sharply, from 1152 to 222 mosm per 1 kg of H_2O. Moreover, in invertebrates—marine and freshwater bivalve mollusks, which have the lowest osmolality among all the organisms under study (in pearl mussels, it is 32 mosm per 1 kg of H_2O; see Table 1)—the share of sodium in osmolality is about 47%. Similar sodium shares in the osmolality of extracellular fluids in such different organisms and with such different absolute values of sodium ion concentrations (from hundreds to tens of mmol per 1 l), as well as the presence of the potassic cytoplasm in all the cases, show that the physicochemical structure of animal cells is universal.

Most probably, vertebrates penetrated into freshwaters from the ocean environment through estuaries. Later, the eukaryotes that were able to survive in the sodium environment and ensure the ionic asymmetry of cells transformed into multicellular organisms and created their own monocellular environment—a system of "sodium" fluids of the internal environment, which were isoosmotic to the content of the intracellular fluid.

This stimulated a rapid development of the animal kingdom. Note that this solution of the physicochemical problem of bodily aquatic phases is fundamentally different from the solution of the same problem in the kingdom of multicellular plants.

The seemingly simple but still unsolved question is why the concentration of potassium ions in cells and that of sodium ions in the extracellular fluid in the majority of animals vary from 0.1 to 0.5 M. In the opinion of a number of scientists, the ancient ocean was less salty than the modern one, and the blood of vertebrates has preserved the same values of the concentrations of sodium and other ions, i.e., the same saltiness, as in the ancient ocean [2, 15]. When vertebrates had to adapt to a higher osmolality of the external environment, they increased not the concentration of sodium and chlorine ions in their blood but that of organic osmolites, such as urea. In particular, such a mechanism of adaptation formed in elasmobranchii (sharks and skates), which have a very high concentration of urea in their blood; as a result, the osmotic pressure of fluids in the internal environment has become higher than in ocean water, and the necessary quantity of fresh water penetrates into the internal environment along the osmotic gradient through bodily parts, such as gills. Marine poikilosmotic animals have found another way out: they accumulate organic osmolites in cells (for example, amino acids) to remain isoosmotic to seawater and thus withstand the osmolality of the extracellular environment, which is similar to that of the ocean. As a rule, scientists did not explain these facts and referred to the total concentration of substances in seawater or another environment, where the main groups of living beings had appeared and undergone adaptation [3].

In the organisms of humans and other mammals, osmolality regularly increases only in the inner kidney medulla, when, under the impact of vasopressin, osmolality in certain animals increases up to 9000 mosm per 1 kg of H_2O (more than 200 atm!); in humans, maximal osmolality in the kidney tissues is about 1450 mosm per 1 kg of H_2O. In this kidney zone, hyperosmolality is largely due to an increase in the concentration of urea and not in that of sodium and chlorine ions. It is of importance for cells what substance causes hyperosmolality. A considerable increase in osmolality, caused by NaCl, damages DNA molecules, while the same concentration of urea does not [25]. We may make the following physiologically important conclusion: there is a zone of sodium concentrations in the pericellular environment in which physiological functions can be performed without additional adaptations, but a further increase in sodium concentration leads to dysfunction.

The blood in the vessels of marine bony fish, marine turtles, and birds is practically three times less osmolar than ocean water. Naturally, even perfect skin coverings and excretory organs of these animals do not prevent water losses; they constantly need fresh water to maintain the osmotic pressure of the blood and bodily fluids at a lower level than in the environment. There are no freshwater sources in the ocean, and it requires too much energy to migrate to rivers and lakes for fresh water; consequently, animals should form their own special *desalinators*—organs and cells for making fresh water from salty seawater. In the marine environment, these animals drink salty water; sodium salts are absorbed by the blood through the intestine, and then cells in gills (salt glands) remove concentrated fluids from the blood and thus desalt it and maintain a constant level of osmolality, which is lower than in seawater.

In the course of evolution, homoiosmotic animals formed desalinators, whose function is part and parcel of the activity of the *sodium transportation system.* Desalinators of animals of different groups actively remove sodium salts in the direction opposite to the electrochemical gradient and thus desalt the blood. This function is performed by chloride cells in the gills of marine bony fish, the rectal glands of sharks, and the nasal and lacrimal glands of reptiles and birds. The operational principle of this system—Na^+ salt secretion—is universal for all the above organisms. Only in mammals, the main organ that stabilizes the osmotic pressure is the kidney. It retains water and removes excess osmotically active substances.

SODIUM AND HOMEOSTASIS

Certain physicochemical parameters of the environment in which life began were critical for the development of living beings and underlay the formation of basic functions in the initial precellular forms of life, the first cells, and then multicellular organisms. There is no doubt that the aquatic environment was the stage on which chemical processes of life played. Upon the formulation of the notion of physicochemical parameters of the internal environment relative to the external one, or the notion of homeostasis [26], hypotheses appeared concerning interrelations between organisms and their habitats during the formation of internal environment fluids.

Humans and animals form blood, extracellular fluid, and lymph systems [2, 20, 24] and develop mechanisms of stabilizing their volume and composition. Since the time of C. Bernard, the central idea of physiology has been the recognition that the stability of the internal environment ensures free life. Excretory organs are of special importance in keeping and maintaining a constant composition of the internal environment. In the majority of cases, their function is based on a two-phase principle: first, protein-free fluid ultrafiltration occurs from the blood plasma or hemolymph through the kidney tubule; and then a part of sodium ions, many physiologically important organic substances (glucose, amino acids, etc.), and certain anions return in necessary amounts to the fluids of the internal environment using sodium-dependent mechanisms, and the remaining part is excreted.

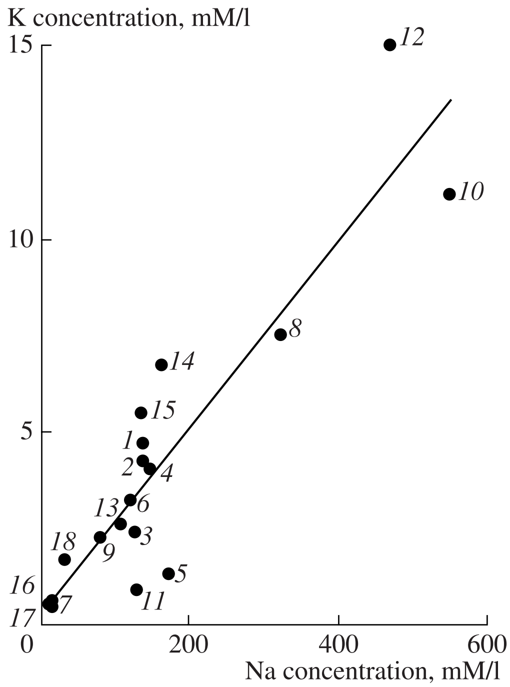

Fig. 2. The ratio of sodium ions to potassium ions in the blood plasma and hemolymph of animals and humans.

(*1*) pink-eyed white rat, (*2*) human, (*3*) *Acipenser güldenstädti*, (*4*) *Columba livia*, (*5*) *Gadus morhua*, (*6*) *Lampetra fluviatilis*, (*7*) *Margaritifera margaritifera*, (*8*) *Mytilus edulis*, (*9*) *Mytilus trossulus*, (*10*) *Myxine glutinosa*, (*11*) *Oncorhynchus nerka,* (*12*) *Pecten islandicus*, (*13*) *Rana temporaria*, (*14*) *Rhombomys opimus*, (*15*) *Testudo horsfieldi*, (*16*) *Unio pictorum*, (*17*) *Unio tumidus,* and (*18*) *Viviparus viviparus*; the correlation coefficient between the sodium and potassium ion concentrations is 0.906, $p < 0.01$.

The choice of sodium ions as a countercation to the intracellular potassium cation was of special importance in the evolution of humans and animals. In the initial forms of eukaryotes that gave rise to the kingdom of plants, sodium was not used to perform these basic functions (see Table 2). In the extracellular fluid of practically all invertebrates and vertebrates, sodium is the dominating cation. In addition, in a very broad range of its concentrations—from 15 to 500 mM/l—the ratio of potassium ions to sodium ions remains stable (Fig. 2). In vertebrates, especially in humans and other mammals, not only are the physicochemical parameters of the blood plasma and extracellular fluid stabilized but also the environment where the nervous system functions. This is ensured by a special fluid outside the blood–brain barrier. There are other similar physiological mechanisms, such as the blood–aqueous barrier; the endolymph and perilymph are formed in the inner ear.

As for the dominating cation in animal cells and the extracellular fluid, there are only a few exceptions, which only confirm the main idea of this article. They include the hemolymph in certain species of insects and the endolymph of the inner ear in mammals, in which the concentration of potassium ions is higher than that of sodium ions. However, the content of potassium ions even in the muscles of these insects is higher than in the "potassic" hemolymph. For example, in the muscle of the common sawfly, the K/Na ratio (tissue/hemolymph) is 2.7. To ensure such a high gradient of potassium ion concentration in insects with the potassic hemolymph, nonstandard adaptive mechanisms were employed: high values of hemolymph osmolality are due to the fact that organic substances accumulate in the hemolymph. Owing to this, prerequisites are created for maintaining a higher level of potassium in cells.

The protocell could exist in stabilized physicochemical environmental conditions (osmolality, potassium ion concentration, and pH), the parameters of which were practically similar to those of the intracellular content. With regard to physiology, the characteristic feature of the evolution of multicellular animals is the development and continual improvement of the system that regulates the parameters of the fluids of the internal environment. Our studies have made it possible to discover the most strictly stabilized physicochemical parameters of vertebrates' blood plasma, which have reached the highest level in humans. They include osmolality and the concentration of sodium ions and ionized calcium in blood plasma. The explanation is obvious: the osmolality of blood and the concentration of sodium ions determine the volume of each cell and bioelectric processes on the plasmatic membrane. Potassium ions regulate many processes inside the cell.

SODIUM IN THE EVOLUTION OF PHYSIOLOGICAL SYSTEMS OF ANIMALS

The definition of the notion of *life* includes the energy-consuming process that implies the maintenance and self-reproduction of the characteristic structure of an individual. In modern terms, this means the appearance of the genetic matrix as the base of synthesizing protein molecules and other components of living structures. It is highly probable that the basic principles have survived from the primary forms into the most complex modern organisms; the same is true with respect to the ratio of organic substances to inorganic ones in these beings. I mean the basic principles rather than the innumerable adaptive forms that have developed on this basis.

We may single out two components in physiological evolution: *obligatory*, or stable, and *adaptive*. In other words, changes in the environmental conditions required an adequate response from the organism, which should be based, however, on stable and invariably preserved features. Physicochemical conditions that are necessary for the functioning of the living cell apparently belong to the preserved and protected; only those features change that ensure a better adaptation to new conditions of existence.

Physicochemical factors of living cells belong to basic principles of general physiology. However, the literature does not answer the question why the sodium ion was chosen as the main extracellular cation in animals. Moreover, scientists do not even pose this question.

Meanwhile, searching for an answer to this question, like Ariadne's thread, may give a clue to the nature of stimuli for cellular differentiation and the formation of the nervous system and fluids of the internal environment. In addition, ionic differences of the pericellular environment make it possible to consider certain key aspects in the differentiation between plants and animals. As is known, in the cells of eukaryotes, both animals and plants, potassium ions dominate; outside the cells, sodium ions prevail only in the majority of animals, while in plants, they belong to microelements (see Table 2).

The most popular hypothesis concerning the formation of the eukaryotic cell is the symbiotic approach [27]. At one of the evolutionary stages, along with the world of prokaryotes, new kingdoms of living beings began to develop, which determined the rise of plants, fungi, and animals. With regard to physicochemical conditions inside the cell, including the dominant cation, they sought for mechanisms that would allow them to adapt to the external environment. We may assume that animals formed in a pericellular environment where sodium ions dominated, while the cell itself contained the potassium ion; the strategy of plants comprised searching for such structures of envelopes that would withstand osmotic forces. However, scientists have been ignoring these differences, and we have to explain their essence.

In multicellular animal organisms—coelenterates and worms, mollusks and insects, as well as different classes of vertebrates—there are several fluid phases. One of them is intracellular fluid and another is extracellular fluid. They are separated from each other by the cell's plasmatic membrane. The concentrations of individual ions and nonelectrolytes in the two fluids are different, but the total concentration of osmotically active substances is practically the same, and the values of the osmotic pressure are similar. The adaptation of the isoosmotic cell to the sodium environment of the ocean provided exclusive opportunities for the development of animals. Later, these living beings migrated from the sea to fresh waters and land. In all the cases, the osmolalities of the intracellular and extracellular fluids changed simultaneously, but the principle of their functioning remained the same.

Studies on the evolution of living systems and the search for solutions to the problems of the evolution of the physiological system will also answer the question about tendencies in the evolution of individual functions, from their manifestations in primitive forms to their development in higher ones. Experimental analysis of another group of data will make it possible to answer the question of what initial conditions favored the appearance of this or that function and determined the vector of its development. As for general physiology and the physiology of water–salt metabolism, we need data on the role of physicochemical factors in the mechanisms of physiological functions in the living beings of different levels of development. This was the base on which fundamental properties of living systems formed and which predetermined the direction of evolution. Enzymatic reactions, which determine the very possibility of life; interactions between life molecules; the development of cells and multicellular organisms; and the action of signaling molecules formed on the same basis. As the evolution of life advanced, physiological functions of increasingly complex organisms developed under similar physicochemical conditions and any qualitative changes in the most significant physicochemical factors, which determine physiological functions of cations such as sodium and potassium, became increasingly difficult.

The majority of works on evolutionary physiology are devoted to analyzing the mechanisms of developing physiological functions. The term *evolutionary physiology* was proposed by A.N. Severtsov in 1914, when the first works on the evolution of functions had already appeared. The first generalizations in evolutionary physiology appeared in the early 1930s; this trend began to develop especially rapidly in the mid-20th century. A wide range of problems, associated mainly with the evolution of the functions of the nervous and digestive systems, water–salt metabolism, and kidneys, was studied.

Orbeli formulated a system of ideas of evolutionary physiology as an independent trend in physiology [10]. Within the framework of his concept, two tasks of evolutionary physiology were determined. One of them was studying the evolution of functions, i.e., the development and improvement of functions of different systems of organisms. The other implied understanding why the evolution of this or that function proceeds in one direction and not in another one. The approaches proposed in this article are beyond the scope of both tasks.

The origin of the cell, the role of physicochemical factors, and the role of sodium ions can hardly be considered as the evolution of physiological functions, such as respiration, blood circulation, digestion, excretion, and so on. The discussion and solution of the task posed in this article can hardly be attributed to the evolution of functions. The development of evolutionary physiology in Orbeli's constructions makes us pose another task, which may be called *physiological evolution.* It may be formulated as the formation of physiological processes and functions under the rise of organic elements of life and independent self-reproducing organisms in inorganic nature, which were capable of realizing the minimum of physiological functions that underlie life. These are physiological functions of the initial cell; the ways by which cellular functions appeared and developed; and the formation of respiration, digestion, excretion, and other organs and systems. Solving basic physiological problems—those of physiological evolution—will stimulate anew studies and generalizations within the framework of evolutionary physiology.

TWO STRATEGIES OF DEVELOPMENT

We may assume that initial organisms—ancestors of eukaryotes, common to plants, fungi, and animals—lived in a potassic environment. At that time, precursors of each of the above groups had to choose a strategy of adaptation to the environment, in which sodium ions had begun to dominate.

Previously, nobody paid attention to the fact that the content of potassium in plants per unit of the mass of dry matter exceeds the total amount of sodium 600 times [22]. In animals and humans, this ratio is qualitatively different. For example, in humans, the share of potassium is two times higher than that of sodium (see Table 2). In the plasmalemma of plants, potassium, calcium, and anionic channels have been discovered; some of them are potential-dependent and are activated by stress, light, and other factors. Just as in membranes of animal cells, the water permeability of plasmatic and vacuolar membranes in the cells of plants is regulated with the participation of aquaporins. In tracheophytes, there are intracellular pre- and postphloemic and pre- and postxylemic transportation systems [28]. In animal cells, the situation is different: the most important role in their plasmatic membrane belongs to molecular mechanisms for sodium transmembrane transport, such as sodium channels and pumps, as well as developed vascular networks of blood and lymph circulatory systems.

It seems highly probable today that there were two strategies of adaptation to the sodium environment. One of them implied the creation of the plasmatic membrane and considerable energy consumption associated with the constant removal of sodium ions and the preservation of the potassium environment. In this case, the osmotic pressure on both sides of the membrane should be the same and sodium ions should dominate in the external environment. Such are the external environments of mono- or multicellular marine organisms and the extracellular environments of freshwater or land animals or homoiosmotic marine organisms. The osmotic pressures inside and outside the cell remained equal. The other evolutionary strategy could imply preserving the potassic cell and providing it with an envelope that would be able to withstand the high osmotic pressure inside the cell. Under these conditions, the cell may be surrounded be water or some other liquid, but the organism in question has no internal environment and does not lose energy for homeostasis. Plants have been evolving in this way.

The system of equal osmolalities in the intra- and extracellular fluids and the ionic gradient under equal osmolalities implied considerable energy consumption, but later this led to the formation of a centralized regulation system, the nervous system, and a special system of stabilizing the composition and volume of fluids of the internal environment. In humans and other mammals, this function is largely performed by the kidneys, which receive 25% of the minute blood volume at a time. (I mean arterial blood here, but there are certain vertebrates in which both arterial and venous blood is supplied to the kidneys; in others, the kidneys receive mainly venous blood.) Kidneys purify blood and return necessary substances to it. This implies a constant loss of more than 10% of cellular respiration energy, this energy only stabilizing the conditions under which all bodily cells can work. As a rule, the Malpighian glomeruli of a person who weighs 70 kg filter about 25 000 mM of sodium every day, 99% of which is again absorbed by blood. This colossal work is aimed only at keeping stable physicochemical parameters of the blood and extracellular fluid.

* * *

The newest works on the evolution of life ignore one of the key physiological questions concerning inorganic factors of the environment where life began. Apparently, the formation of protocells with the potassic cytoplasm and their future adaptation to the external water environment, in which sodium ions dominated, were significant events in the evolution of life. This stage was connected with the formation of the plasmatic membrane for developing ionic asymmetry relative to the external environment. The problem of developing multicellular organisms was solved differently in plants and animals. In animals, the presence of sodium ions in the pericellular environment was the starting condition of electrogenesis, favored the formation of the asymmetrical cell, and stimulated tissue differentiation and the formation of the epithelium. Electrogenesis in cellular membranes preconditioned the formation of the excitable cell and the development of the nervous system, favored the coordination of functions, and ensured responses to momentary changes in the external environment. The formation of the polarized cell, whose plasmatic membrane concentrated sodium channels in its one part and sodium pumps in the other, ensured the development of absorption, digestion, excretion, and respiration. The formation of the system of the fluids of the internal environment with the dominance of sodium ions in them was a prerequisite for the development of homeostasis—the system of stabilizing the fluids of the internal environment at a constant physicochemical level. Thus, the choice of sodium as the counterion of potassium stimulated biological progress in the kingdom of animals.

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