

Comparison of fundamental physical properties of the model cells (protocells) and the living cells reveals the need in protophysiology

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Abstract: A hypothesis is proposed about potassium ponds being the cradles of life enriches the gamut of ideas about the possible conditions of pre-biological evolution on the primeval Earth, but does not bring us closer to solving the real problem of the origin of life. The gist of the matter lies in the mechanism of making a delimitation between two environments – the intracellular environment and the habitat of protocells. Since the sodium–potassium pump (Na^+/K^+ -ATPase) was discovered, no molecular model has been proposed for a predecessor of the modern sodium pump. This has brought into life the idea of the potassium pond, wherein protocells would not need a sodium pump. However, current notions of the operation of living cells come into conflict with even physical laws when trying to use them to explain the origin and functioning of protocells. Thus, habitual explanations of the physical properties of living cells have become inapplicable to explain the corresponding properties of Sidney Fox's microspheres. Likewise, existing approaches to solving the problem of the origin of life do not see the need for the comparative study of living cells and cell models, assemblies of biological and artificial small molecules and macromolecules under physical conditions conducive to the origin of life. The time has come to conduct comprehensive research into the fundamental physical properties of protocells and create a new discipline – protocell physiology or protophysiology – which should bring us much closer to solving the problem of the origin of life.

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*'Ring the bells that still can ring. Forget your perfect offering.
There is a crack, a crack in everything. That's how the light gets
in.'*
– Leonard Cohen

Potassium ponds

There is a statement we constantly come across in the scientific and popular-science literature: the ion composition of the internal environment of the body of humans and animals, in which all of its cells are immersed, is close to that of seawater. This observation appeared in the literature even 100 years ago, when it became possible to investigate the ion composition of biological liquids.

This similarity between the internal environment of the body and the sea is quite obvious: in both seawater and blood plasma there are one or two orders of magnitude more Na^+ ions than K^+ . It is this composition that can make one think that life originated in the primeval ocean (the memory of which has since been sustained by the internal environment of the body), and the first cells delineated themselves from seawater using a weakly permeable membrane, so that their internal environment became special, suitable for chemical and physical

processes needed to sustain life. Indeed, the ratio of the above cations in the cytoplasm is the exact reverse of their ratio in seawater: there is much more K^+ in it than Na^+ . In fact, physiological processes can only be possible in an environment where potassium prevails over sodium. Therefore, any theory of the origin of life must explain how such a deep delimitation (distinction) between the two environments could occur: the intracellular environment, wherein vitally important processes take course, and the external environment, which provides the cell with necessary materials and conditions.

For the protocell to separate from seawater, a mechanism must arise that creates and maintains the ion asymmetry between the primeval cell and its environs. We normally consider a mechanism of this kind as the isolating lipid membrane with a molecular ion pump, the Na^+/K^+ -ATPase, built into it. If life originated in seawater, the origin of the first cell inevitably comes down to the origin of the sodium pump and any structure supporting it – the lipid membrane – without which the work of any pump would make little sense. It seems that life is born in conditions that are really adverse to it and even ruinous.

The trouble with the idea of life originating in seawater has made one look for alternatives in the hope that the need for the

sodium proton pump can arise not right away but a bit later on, when the inside of the protocell had developed to the point where the creation of the pump would have become a necessity, including an energy system to drive it.

In 2007, we saw the simultaneous release of two articles (Mulkiđjanian & Galperin 2007; Natochin 2007), in which it was posited that life originated not in seawater as previously thought, but in smaller bodies of water with a K^+/Na^+ ratio necessary to sustain life, i.e. they contained more potassium than sodium in the same proportion as in the cytoplasm of modern cells. It is in these potassium ponds that all the stages of chemical evolution had to precede the origin of life. Mulkiđjanian and Galperin (2007) even proposed the ‘principle of chemical conservation’, under which in the cytoplasm of modern cells a number of characteristics of potassium ponds there has been conserved; these also include other metals and certain compounds that are vital to life as well as necessary potassium : sodium ratio. Given this approach, we can say that, in essence, potassium ponds were like a megacytoplasm – at least, when it comes to the above cations. If that is the case, the image of the primeval ocean that metaphorically still flows through our veins and can now be complemented by the myriads of potassium ponds conserved within the composition of all our body cells since primeval times.

The sodium ocean

Natochin (2007) has also supported the idea that the chemical composition of the primeval potassium pond has been conserved in modern cells (without, however, spinning this idea off into a principle of conservation), but he is primarily interested in the physiological significance of K^+ and Na^+ in this primeval water. Since the potassium megacytoplasm does not, until a certain point in time, come in contact with the sodium ocean, according to Natochin, it cannot maintain vitally important processes founded on the physiological antagonism between these cations. For instance, the megacell seen in the image of the pond would have been incapable of generating the resting and action potentials. Hence Natochin’s idea is about the key role of sodium ions in the origination of life, which can come into being only through the collision of the potassium and sodium worlds.

An advantage of the potassium pond over seawater lies, among other things, in the velocity of peptide synthesis being 3–10 times faster in an environment with potassium ions than in at the same concentration of sodium ions (Dubina *et al.* 2013), which can be explained by the different nature of the interaction of K^+ and Na^+ with the functional groups of peptide bonds (Jockusch *et al.* 2001). As for the origins of such ponds on the primeval Earth, Mulkiđjanian and Galperin (2007) and Mulkiđjanian *et al.* (2012) link their emergence to special conditions of condensation of geothermal water vapour in regions with considerable volcanic activity, where, for some reason, there was more potassium than sodium in the condensates. And Natochin (2007) believes that these ponds could emerge in a less violent geological setting – at the contact of fresh water with rocks rich in potassium compounds.

Issues and speculations

The idea of the potassium pond raises the following issues.

1. The water vapour condensates must in many ways resemble distilled water. If distillation was imperfect and there was more potassium than sodium in the condensate, having more K^+ than Na^+ by itself would clearly still not suffice. The quantity of the salts also matters.
2. Switek (2012), commenting on the above-mentioned ‘principle of chemistry conservation’, maintains that the time-frame for the existence of potassium ponds in turbulent regions with considerable volcanic and tectonic activity is definitely too short for the completion of pre-biological evolution.
3. Were potassium ponds the cradles of life or simply chemical reactors wherein peptides and other compounds needed for protocells were created and accumulated? Switek is convinced that we cannot assume that life originated in potassium ponds, since comparatively quickly a diffusional equilibrium of all the dissolved substances is established in them. For instance, potassium would be present in equal amounts both inside the protocell (should it have emerged here) and outside it, and the living cell must be a thermodynamically non-equilibrium system. Switek, if somewhat incompletely, expresses a simple thought in his own way: physical laws prohibit the emergence of a thermodynamically non-equilibrium sub-system (the protocell) within a thermodynamically equilibrium (or quasi-equilibrium) system (the pond).
4. According to Natochin (2007), it seems that life can emerge only in a potassium protocell/sodium environment system (i.e. in just a non-equilibrium system). But in this case, the mechanism for maintaining the non-equilibrium between the protocell and the environment would have had to emerge earlier, back in the potassium pond at that very time when there was no practical need for such a mechanism! It turns out that there should be synthesized in advance lipids in the potassium pond to form the lipid membrane at the next stage. Then in the same pond, sodium pumps and ion channels should also have been created for future use (some for Na^+ , and some for K^+). Lastly, all these elements should somehow come together to form a structure resembling a bio-membrane awaiting an encounter with seawater for either a day or millions or years. Without all these necessary preparations, the sea Na^+ , the cation of death, can easily penetrate the cell and extinguish any trace of life as soon as seawater floods a potassium pond.

The question inevitably arises as to why the sodium pump had to form in advance back of the potassium pump if there was no abundance of Na^+ in the protocell? If Mulkiđjanian and Galperin, in breach of the thermodynamic law of nature, are opting for the emergence of non-equilibrium (life) in the equilibrium pond, Natochin, on the other hand, is of the opinion that the protocell in the potassium pond had already prepared itself for its future life in the ocean. For, as far as he is concerned, the sparks of life emerge only when the protocell filled with potassium

finds itself in the sodium milieu. It is then that there emerges between the environment and the cell the sodium/potassium gradient, which like a starter, turns on the sodium pump, which was prepared for that purpose in advance.

Consequently, the idea of the potassium pond leads us to two implausible scenarios: while thermodynamics is resolutely against the origin of life in the potassium pond, the origin of the sodium pump in conditions where there is no natural need for it may require the agency of Providence.

5. The idea of the potassium pond, as fresh as it may seem and having failed to meet our expectations, again brings our attention back to that fundamental issue: The origin of life comes down to the origin of a fully functional membrane – a structure capable of effecting a non-equilibrium process of maintaining the special conditions of the intracellular environment. The potassium pond does not contribute anything novel to this issue. In fact, it creates new issues as to what do we do about the violation of the fundamental laws of physics and what about teleology, which holds that present events are determined by future needs?

According to some estimates (Pinti 2005), transitions in the physical properties of the evolving oceans lasted about 700 million years before origin of life, during which time cooling to reach temperatures suitable for thermophilic life forms (about 60–100°C) were established ~350 million years (Pinti 2005, Fig. 3.1) before the appearance of life (Schidlowski 2001). A possible explanation of this time lag between the acquisition of suitable aqueous temperature and the origin of life is that salts in the early oceans were dominated by sodium and so life could not have originated without the presence of optimum ratios of potassium as well. The evidence in favor of this view is the apparent coincidence the origin of life 3.8 Ga ago (Schidlowski 2001) with the beginning of the formation of granite rocks on the Earth's surface 3.8 Ga ago (Zhang et al. 2006). The coincidence is important because some types of granites contain high amounts of potassium compounds. The ratio of K/Na in potassium-rich granites usually reaches 1 and rarely exceeds this value (Whitney 1988). However, in India recently were found granites with K_2O/Na_2O ratios ranging from 1.1 to 69.8 (Rajaraman *et al.* 2013). In the modern ocean water and in the Archean era, the ratio was 0.02 (Pinti 2005). With this approach, life became possible only after Potassium Big Bang initiated by water erosion of basalt, potassium extraction and subsequent formation of granites on the planet. If this global interaction of water with continental crust occurred in reality, the necessity of potassium ponds as a theoretical prerequisite to the origin of life seems unnecessary but, also, can not be ruled out.

Fox's microspheres do not need potassium ponds

Let us now switch from risky assumptions to established facts. Over the last 90 years, we have had access to attractive cell models that can reproduce lots of interesting properties of living cells, going as far as demonstrating ion channels, resting potentials and action potentials. However, even a century would not suffice to discover a model for at least some pump

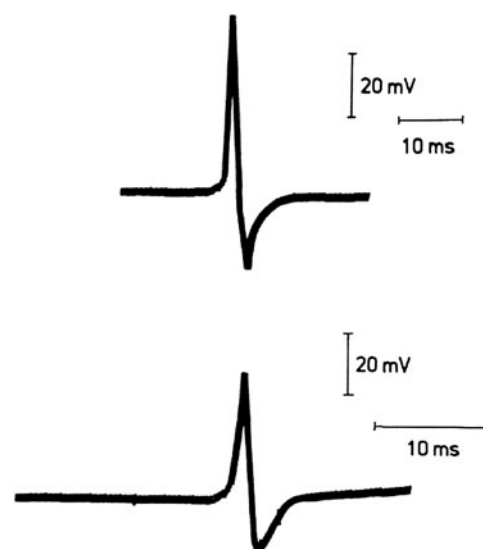


Fig. 1. An action potential that emerges in stimulating the crayfish's stretch receptor (above). An action potential recorded for the microsphere (Fox 1992). Reproduced with permission of Springer-Verlag.

that would have the properties of the sodium pump. Neither Miller's electrical charges, nor Fox's amino-acid condensation, nor building ready-made biomolecules into coacervates; none of this has managed to lead to the self-origination of the progenitor of the ion pump even in favourable lab conditions. We are witnessing some sort of intrigue here: entire protocells – gigantic protein complexes – easily self-originating in laboratories, while comparatively small protein pumps do not. We are left with absolutely no clues as to where to look for these key engines of life. Does not this mean that we are yet again confronted by thermodynamics as an insurmountable barrier in our way of prohibiting the ever-desired wonder – the emergence of non-equilibrium (i.e. life) in an equilibrium system, whatever it may be – a refined Sigma solution, a warm pond, a quiet lagoon or the oceanic abyss! Well, while science is still clueless about the origin of ion pumps, let us examine the known properties of Fox's microspheres.

Figure 1 illustrates two action potentials. One spreads along the crayfish's nerve fibre, and the other across the surface of the proteinoid microsphere. It follows from a comparison of these two notations that in both cases changes in the electric potential are qualitatively of the same type and quantitatively close. Here is an example to make things clearer. The gravitational forces on the Earth and Moon have qualitatively one physical nature – the differences are of only of a quantitative nature. Our case is the same: the physical nature of change in the potential in the axon and the microsphere is the same (in both cases the transmitters of the charge are ions), while the quantitative characteristics differ a lot less than the gravitational forces on the Earth and Moon.

Let us now ask ourselves the question as to what structural elements must the axon's membrane have for it to be able to generate an action potential. These elements are few in the excitable membrane and are well known: (1) the continuous lipid

bilayer; (2) the potassium channels; (3) the sodium channels; and (4) the Na^+/K^+ -pump. The functional role of the specific parts of this mechanism is as follows: the pump creates a concentration gradient of K^+ and Na^+ , due to which the membrane difference in potentials arise (the resting potential); the lipid phase acts as an isolator that suppresses parasite currents; the ion channels serve as specific electric conductors needed in this case for managing the size of the membrane potential. To restore the resting potential after excitation, the ion channels need to be closed and the pump turned on in order to reproduce the corresponding concentration gradients.

The workings of the mechanism for a neuron's action potential were explained by Hodgkin & Huxley 1952, who solved the problem using a mathematical model, for which the authors were awarded the Nobel Prize in Physiology and Medicine in 1963.

From the wide variety of mathematical techniques, the authors chose a mathematical method that is convenient for describing the flow of particles (in our case, ions), with these flows divided in space: some ions (Na^+) move in the membrane through special sodium channels, and others (K^+) through potassium channels. On the whole, as follows from the Hodgkin–Huxley model, for excitability to be possible, we need a membrane whose variable and selective permeability is described by an equivalent electric circuit wherein potential-dependent resistors match ion channels and capacitive reactance is ensured by a dielectric lipid membrane.

It is hard to overestimate the role of models, physical and mathematical, in science. Artificial membranes, for instance, have played a decisive role in the development of notions of the role of membranes in the life of a cell. Physical models of atoms and molecules, including DNA or mathematical models used in engineering and astrophysics all 'prove' that only a relevant model serves as then some key testimony in favour of a supposed mechanism for a phenomenon or an object's structure. The importance that models have played in science is illustrated by the Hodgkin–Huxley model, which for decades to come has determined not only our notions of excitable membranes, but the operation of membranes in general. However, the reverse is also fair – no mechanism can be considered proven if we cannot find a model that demonstrates, even in simplified form, its efficiency. Here we are interested in artificial cells, as models, which are stumbling blocks for those who believe that the spark of life can only come from a membrane–pump–channel 'lighter'.

Przybylski (1984) and Stratten (1984) raised the interesting question as to whether we can apply the Hodgkin–Huxley model to Fox's microspheres (Fox 1965). They showed that, as strange as it seems, there are neither logical nor physical bans on the use of this mathematical method to describe action potentials generated by these cell models, which may seem infinitely far from neurons. In other words, the Hodgkin–Huxley model is similarly well-compatible with both the nerve cell and the bunch of protein-like macromolecules. Unfortunately, Przybylski and Stratten did not go beyond this interesting statement.

The structure of the Hodgkin–Huxley model has turned out to be imperceptible (invariant) to the physical, chemical and

structural differences of compared objects, probably due because their common substrate base is proteins. At first glance, it seems like there is some kind of error or misunderstanding, but then you might recall the crucial advantage of mathematics as an instrument in scientific cognition – it is often inconceivably abstract in nature. Mathematical concepts, equations or quantities are not encumbered with specific material content. Mathematical transformations on which various models, theorems and corollaries follow are constructed have their own, special, logical laws and someday a derived mathematical result can have lots of physical interpretations. The laws of geometry, for instance, do not depend on whether a triangle is made from rusted wire or formed by laser beams.

What happened to the Hodgkin–Huxley model is that it has been created not as an abstract mathematical structure, but as an instrument for the analysis of the properties of a specific physical object, the excitable membrane of a neuron, and this within a specific historical context. The model's mathematical parameters were construed by its creators based on the properties of an axon, but under no circumstances this means that such a model can only be applied to an outgrowth of a neuron and does not allow other interpretations when it is applied to investigating other structures. In other words, the Hodgkin–Huxley model cannot monopolize the living excitable membrane as the only object to which it can be correctly applied – it, moreover, cannot be viewed as a mathematical proof of the presence of lipids, specific ion channels and active transport within the biomembrane. Any physical model is just one of the numerous embodiments of the spirit of mathematics, and the axon is no exception. All creators of mathematical models should remember that the model they have created will never hold true to the physical phenomenon whose analysis they were creating it for.

So what follows from the fact that the Hodgkin–Huxley model does not care what it is used for, e.g. to study the neuron or Fox's microsphere? We are dealing here with two possible inferences.

1. The membrane of a microsphere possesses the same properties as the membrane of a neuron, it, too, has a lipid membrane, which plays the role of an electric isolator, and specialized potential-dependent ion channels for Na^+ and K^+ ions. In reaching threshold depolarization, the microsphere's sodium channels open up, Na^+ ions stream into the microsphere, and an abrupt depolarization of the membrane occurs, following which potassium channels open up – so on and so forth. And next, to restore the resting potential we need a proteinoid ion pump, which will pump out of the microsphere redundant Na^+ ions and pump into the protocell the lost K^+ ions.

The second corollary is due to the fact that none of the above-mentioned structures in the protocells under examination exist.

2. The actual events taking place within the neuron's membrane have little to do with the theory (which we know well) which describes them; therefore a theory of this kind needs revision as to ensure that between the living cell and

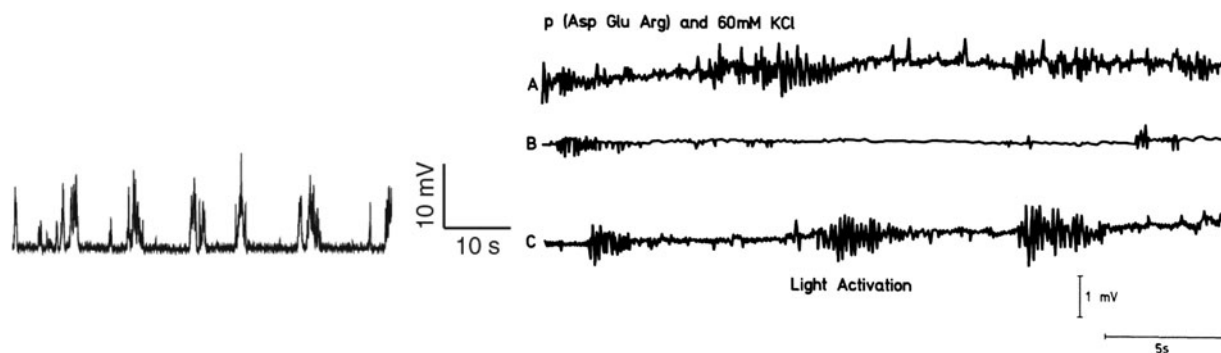


Fig. 2. Left: spontaneous activity of the ion channels of a cortical neuron under whole-cell patch-clamp conditions in a state of rest (Kodandaramaiah *et al.* 2012). Right: spontaneous activity of ion channels of Fox's proteinoid microspheres, recorded using an intraprotocellular potential recording technique. Microspheres prepared from proteinoid which is poly(asp,glu,arg) and suspended in 60 mM KCl. (A) 10 lux illumination; (B) in the dark; (C) 100 lux reillumination (Fox 1992). Discussion of the light effects is beyond the scope of this paper. Reproduced with permission of Nature Publishing Group and Springer-Verlag.

the cell model the impassable chasm disappears, whose existence we have ascertained. Microspheres and neurons are built of proteins whose physical properties (determined by non-covalent interactions) have remained unchanged for billions of years. Consequently, all protein formations must operate on the same principles. The theory of the action potential of a nerve cell accepted in the literature is just one of the possible interpretations of the Hodgkin–Huxley model, and we have ascertained its limitations. We may need a different unified mathematical model that could uniformly explain the electric properties both of living cells and cell models.

The radical character of these inferences explains why Przybylski and Stratten did not make them and why no attention has been paid to their observations. Over the last 30 years, they have been cited in just five articles by persons from their circle and not in connection with the paradoxical properties of the Hodgkin–Huxley model.

That said, microspheres, too, have something we can refer to as ion channels (Fig. 2, on the right). Let us compare these records. Here we may be dealing, given all the quantitative differences, with the same physical phenomenon; electrical charges from time to time break through a sort of barrier (the isolator), while the ion current now appears (the ion channels open up) and then disappears (the ion channels close). We can see that some structures of the living cell and microsphere are capable of changing their conductivity, at one time increasing resistance to the movement of charges and then decreasing it. As for the living cells, a recognized barrier to the movement of ions is the lipid pith of the membrane sandwich, and what makes it transparent are the protein inclusions capable of performing numerous functions. For as long as we are within the pale of the living cell, things look logical and harmonious, but once we switch our attention to microspheres, we get unusual and therefore interesting results that raise questions.

Indeed, in the case of microspheres, we cannot appeal to either the lipid membrane or the habitual ion channels, which are normally placed in the lipid phase. There are no lipids in

microspheres, while there are ion channels (calling them such), and they also need to be ion-specific, as is required by the Hodgkin–Huxley model. Figure 2 proves that there is no impassable chasm between the living cell and the model, which is to be expected, but there remains an obvious and deep rift in understanding the nature of this similarity.

Towards a protophysiology

The above discussion leads us to the following inferences.

1. The idea of potassium ponds is of particular interest, but it does not draw us closer to apprehending the origin of the living protocell.
2. Under existing notions, a key event in the origin of life is the emergence of a non-equilibrium physical process whose carrier, as is normally believed, is the lipid membrane with protein inclusions – channels and pumps. This minimal structure of life is in need of a continuous influx of energy from somewhere outside. Which means that, concurrently with a fully functional membrane, there also needs to emerge an energy supply system, which, on the whole, turns the origin of life into an act of creation – processes, this complex, of synthesis and assembly need to arise at one time, in one place, and in a certain order. Over the entire period of the existence of the issue of the origin of life as an experimental science (Oparin 1924), no laboratory has ever come up with a single-cell model that would have an actively operating membrane that consumes energy from an outside source and is capable of discerning physiologically significant cations which directs their movement through different channels, moving them to the opposite sides of the primeval membrane. We are still clueless as to what the ancestor of the modern sodium pump was. Even those working with lipid vesicles and the Na^+/K^+ -ATPase built into them, have not admitted that these little bubbles are the prototype of the primeval cell. The reason is clear; it is

hard to imagine that such a complex laboratory technology could be actualized in some primeval body of water.

3. The commonly accepted principles of the operation of a cell, which we come across everywhere in articles or textbooks, can only be applied to living cells and cannot, as it turns out, be used to explain the physical properties of cell models – proteinoid microspheres. The lack in the literature of a substantive, thematic comparison of the physical properties of living cells with the similar properties of their models gives rise to a strange thought about the existence of *two kinds of physics* of protein bodies: one deals with lipids, channels and pumps, and the other is a physics we do not yet know, which is in charge of the operation of Fox's protocells. For some reason, the physics are different, while phenomena they underline are similar: the resting and action potentials, channel conductivity and the ability to tell (to distinguish?) K^+ from Na^+ . The laws of physics are unitary, and, therefore, physical principles that determine the organization and operation of cells and protocells must be immutable since the beginning of time. There is one basis for this statement; what has been the foundation of life at all times has been proteins, whose properties are determined by their composition and structure, not the character of a geological epoch. However, it is apparent that the scientific community has not yet come to think likewise of the unity of physical laws governing the properties and interactions of proteins which engender the phenomenon of life. It may, therefore, make sense to introduce into scientific circulation the principle of the invariance of the physical properties of proteins. The physical properties of proteins do not depend on what structure they are a part of, living or protocell. The Hodgkin–Huxley model is unable to explain this invariance, since it makes physical sense in only one single case – in the case of a living cell as it relates to membrane theory.

To conclude, let us go back to the idea of potassium ponds. Ishima *et al.* (1981) provide unique data on the distribution of ions between proteinoid microspheres and the medium. As far as we know, this is the only work that features such data. Table 1 of the article states that the K^+ concentration inside the microsphere is 80 mM, and in the incubation medium it is 0.05 mM. It turns out the K^+ concentration in the microsphere is 1600 times that in the medium. The ability of Fox's microspheres to accumulate K^+ devalues the idea of the potassium pond as the cradle of life. We can have all kinds of ponds composition-wise, and the sodium ocean now does not seem too much of an adverse environment for protocells, which are capable of creating their own environment without lipid membranes and sodium pumps.

It is clearly enough that the microspheres act like ion exchange resin with reversible ion exchange properties. The adsorption principle of accumulation of ions is well known for living cells (Damadian 1973). Karreman (1973, 1977) and Chang (1977, 1978) built a quantitative theory of the resting and action potential based on the adsorption mechanism. Excitability of the microspheres is the best evidence in support of the adsorption approach to cell and protocell physiology. It is necessary critical revision the consequences of the Hodgkin–

Huxley model, discussed above. Adsorptive accumulation instead of pump, sensitive (excitable) protein/proteinoid adsorbent instead of ion channel. Potential-sensitive ion adsorption sites may look and behave like ion channels.

Existing cell models clearly tell us that all processes crucial to pre-biological evolution could be taking course inside protocells in relative isolation from the environment (even in the ocean), without the participation of structures and mechanisms that modern membrane cell physiology is insisting on. We can only wonder why they are not exploring most thoroughly the accumulating ability of microspheres and, possibly, other cell models. The important role of comparative investigations into cells and cell models is not limited to the issue of the origin of life. They could also change the way we think of processes taking place in living cells, which we believe we understand rather well. The paradoxes of the Hodgkin–Huxley model are a vivid testimony to how limited our knowledge is.

Accidental observations made as part of ascetic research into microspheres and other cell models ought to be consolidated and expanded tangibly under the umbrella of a special scientific discipline – protophysiology. There are four fundamental physical properties of protocells that are subject to thorough research using the latest methods: (i) semipermeability; (ii) the ability to accumulate some substances and remove others from one's internal environment; (iii) the ability to generate electropotentials; and (iv) osmotic stability. Studying the role of sorption processes should be included in research on the origin of life when comparing known and future cell models with Archaea, normal bacteria and extremophiles of different type (thermophilic, halophilic, polyextremophilic cells, etc.). Such study would encourage a focus on understanding the fundamental physical process organizes physical basis for chemical, biochemical and structural evolution. I am convinced that knowledge obtained as a result will bring us closer to solving the problem of the origin of life. Unique internal environment of a protocell with its special features is, in fact, the Protocell World inside of which life was really born and began its evolution. It is essentially a Protocell World which weaves known RNA World, DNA World and Protein World into unity. Protophysiology may have practical importance, as artificial cells are used for synthesis of various chemicals and pharmaceutical agents, as well as for targeted delivery of drugs to treat cancer and other diseases.

Biophase as the main subject of protophysiology

The Russian school of cell physiology founded by Nasonov and Troshin in 1930s (see Troshin 1966; Matveev 2005, 2010 for details) considered the living cell as a non-membrane phase compartment with different physical properties in comparison to the surrounding medium, and this physical difference plays a key role in cell function. First micrographs of plasma membranes (obtained with an electron microscope) dealt a heavy blow to such approach and brought victory to the plasma membrane, as a key structure for cell organization and functioning. In this historical context, studies in recent

years dedicated to non-membrane phase compartments in the living cells sound sensational.

According to a new take on an old phase, non-membrane phase compartments play an important role in the functioning of the cell nucleus (Aumiller *et al.* 2013), nuclear envelope (Adams & Wente 2013) and then of cytoplasm (Hyman & Brangwynne 2011). Brangwynne (2013) sees the compartments even as temporary organelles. According to available data, the phase compartments can play a key role in cell signalling (Li *et al.* 2012). Hyman *et al.* (2014) believes that formation of the phases, their ability to self-separation has a general biological significance and is involved in a variety of life processes, including the origin of life.

Since Fox's microspheres do not contain lipids, our recognition of them as non-membrane phase compartments would be the most simple, even obvious approach. Based on the historical perspective and current data, it can be assumed that any protocell at the dawn of life on Earth should be a phase system because this kind of physical system has the potential to create special internal conditions necessary for the origin of life and for the first steps of molecular evolution. Conditions for formation of protein biophase (protocell) and its fundamental physical properties are priorities for the protophysiology.

The only essential condition for the transition of a protein solution to a biophase is a singular point in time and place such that a large-scale interaction of protein with water leads to dramatic modifications of physical state of water and biophase becoming the incubator of life. The idea of bound water appeared in the 19th century and is the subject of interest in different sciences. The most famous example of systems containing bound water is hydrogels. Protein–water interaction is the subject of many papers however a little bit of them is useful for cell and protocell physiology because phase properties of bound water as physical system is preferably important for the physiology. The most important feature of bound water, which has been known since the 19th century, is its poor solubility for all solutes. Due to this, sodium concentration will be lower in biophase than in sea water. This exclusion effect is very important for origination of special intraprotocellular physical conditions needed for life processes and further molecular evolution. It is surprising that 150 years later (after first ideas on bound water), it was able to visualize appearance of water layer near hydrophilic surfaces that excludes colloidal particles due to poor solubility of water in the layer (Chen *et al.* 2012; Sulbarán *et al.* 2014). The same phenomenon was observed near living cell surface as well (Zheng *et al.* 2006). Visualization of existence of the exclusion zone around Fox's microspheres (and other biophases) would be an important achievement for the science on the origin of life. In the view of non-membrane phase approach, the usage of liposomes and other membrane (non-biophase) cell models to solve the issue of the origin of life is a deadlock way of the investigation.

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