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ON THE MICRO-SPACE THEORY OF OSMOSIS

OSMOFILTRATION, “ACTIVE” WATER-FLOW IN MEMBRANE-GAPS

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The author revised the conclusion drawn from his former experiments and recognized a complex phenomenon that he named *osmofiltration.* This phenomenon occurs by way of the inhibition of the diffusion within a micro-gap bordering the experimental membrane in the model. It may have a significance as a basic biological phenomenon in similar biological membranous structures, and even, it may have a medical importance. The author discusses his working hypothesis about the micro-space theory of osmosis.

Keywords: Hindered diffusion – membrane-gap – osmofiltration

INTRODUCTION

As it is explained by the “unstirred layer effect” [4], the transmembrane water transport is influenced in an asymmetric way by stirring the solution on one side of a membrane series and not on its other side. As a result of the stirring the diffusion from the layer bordering the membrane is facilitated, because it becomes narrower and thins out [5]. An osmotic water transport occurs towards the unstirred compartment. This effect, when it is not eliminated, can even disturb osmotic investigations.

Quite independently, the author formulated a working hypothesis to look for the true mechanism of the osmosis. Professor E. Ernst was placed in a dilemma: whether the osmosis was caused by the collision of the solute molecules to the membrane (van’t Hoff’s model), or the driving force of the osmosis is generated by the difference of the mobility of the solvent molecules (i.e. the difference of the solvent vapour pressures) [6] at the two sides of the membrane.

Author reported the results of an experiment (Fig. 1A) to III. Meeting of the Hungarian Biophysical Society [8]. An osmotic system contained twin membranes separated by a central compartment filled up with solution. Lateral compartments

Dedicated to professors J. Ernst’s and T. Zoltán Csáky’s memory.

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were filled up with pure water. Each membranes were covered by impermeable sheets of different surface areas, i.e. both membranes were bordered by narrow gaps of different surface areas. In these experiments a water transport occurred which seemed to be irregular and was correctly explained later. It was caused by the different inhibition of the diffusion along the membranes in a structure of twin membranes with gaps, as it was explained by the author [9, 10]. These publications summarize all components of the complex phenomenon discussed and named *osmofiltration* in the present paper. The most important experiments will be reviewed for a better understanding, then the osmofilter structure and its function will be shown in biological subjects as it was found in the experiments performed in the Pharmacological Institute (Lexington, KY, USA, director: Prof. T. Z. Csáky).

MATERIAL AND METHODS

An investigation was launched into the problem – according to the working hypothesis – that what happens if two osmotic membranes are opposed and arranged in a system made of membranes of identical properties but of different free surfaces.

The membranes – made of parchment paper – were covered at both sides with multiply perforated Plexiglas in experiments **A** and at their outer sides only in experiments **B**. The difference between the total areas of the perforations was equivalent to the difference of the free surface areas. Each membrane was of 225 cm^2 total area. 215 cm^2 and 125 cm^2, respectively, of the membranes were covered by Plexiglas. The space between the two membranes was filled by common acacia gum dissolved in

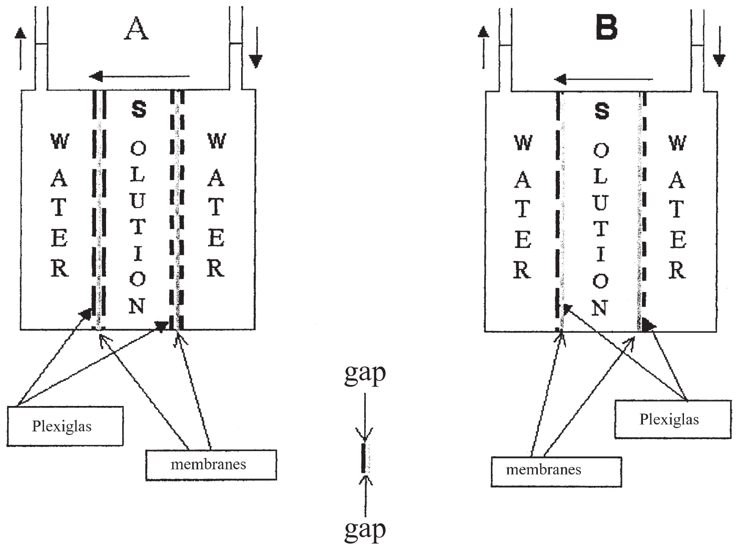

Fig. 1. Sketch of the experimental device

water in a concentration of 20% m/v. One gram of dry acacia gum contained 33 mg amount of inorganic substance as determined by incineration and flame photometry. So the acacia gum solute consisted of large molecules of organic and small ions of inorganic substances. The lateral, open compartments contained distilled water. The water transport across the membranes was determined by measuring the amount of water that left the lateral compartment through the bore-hole in the top cover of the compartment. Pressure difference might be read by measuring the height difference of the water levels in the glass tubes attached to both bore-holes in the top cover. The transported volume, the flow rate and pressure difference produced by the osmosis might be recorded in this way. Always a number of experiments was done, e.g. the transported volume or the flow rate was determined in 15 experiments, the pressure difference was measured in another 10 experiments (series **B**).

In comparative experiments dextrane solution or some crystalloid solution replaced the acacia gum solution in the central compartment. In an experiment a double layer of cellophane was used instead of parchment paper.

RESULTS

An intensive and long-lasting water flow was measured in the direction shown by the arrows and a pressure difference was generated between the lateral compartments both in experiments **A** and **B**. In experiment **B** a water transport was found throughout one or two weeks. The flow rate was 2.7 ml/day in the beginning and it decreased day by day. When the transported water was piled up in the vertical glass tubes, an average pressure of 154 mmHg (~21 kPa) was built up in a day over a range of 122 mmHg (~16 kPa) to 200 mmHg (~26 kPa).

As it is well known, parchment paper is quite easily permeable for dissolved inorganic substances, but even for some not very large organic substances like polysac-

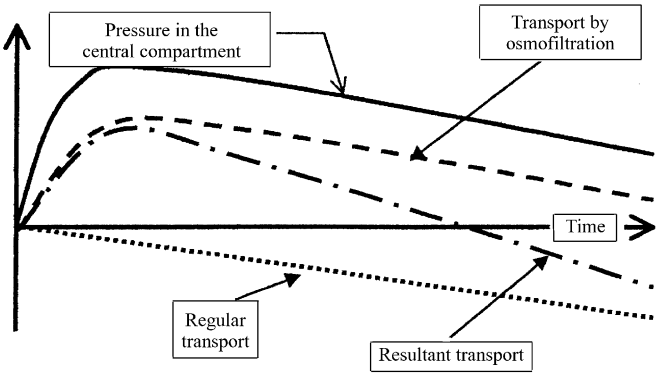

Fig. 2. Functional diagram of the two-membrane system

charides. As a consequence of the permeation of solutes their concentration in the lateral compartment became more close to the membrane of the larger free surface (5.4%) than to the membrane of smaller free surface (1.9%). In spite of this water transport occurred from the higher concentration towards the lower one, i.e. in a *reverse* direction. Later on the concentration difference increased, the water transport stopped, then it was reversed as it was expected according to the actual concentration difference and osmotic pressure (Fig. 2). The irregular direction of the transport was the consequence of the difference of the *covered* surface areas of the two sides of the membrane system.

The analysis of the solute content from the side chambers was done immediately after cessation of the water transport. The *relative* ash content, i.e. ash content % of the dry material, of the solute – wich consist of the small inorganic particles – from the chamber contacting the membrane of the larger covered surface (4.5%) was 1.4 times higher than that one from the compartment bordered by the membrane of smaller covered surface (3.6%). That was the reason for the conclusion that the cause of the dominant water transport through the system in the first period of the experiment was the process which goes on in the membrane-gap structure. As it can be seen from the difference of the solute contents a part of the larger organic molecules is retained in the narrow gap between the membrane and the Plexiglass (Fig. 3).

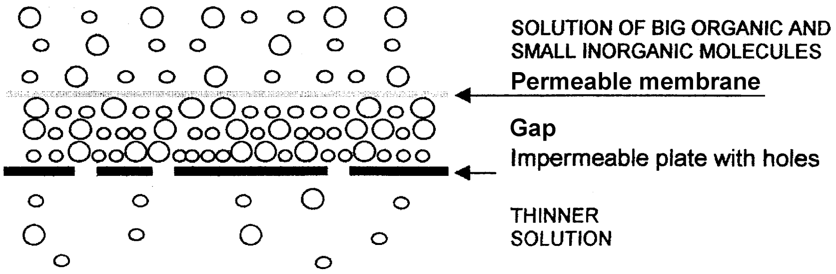

Fig. 3. Schematic representation of the membrane-gap function

Successful experiments were done, when the central compartment was filled with dextrane, or $K_4Fe(CN)_6$, or Na_2CO_3, or NaCl dissolved in water. The efficiency decreased in the order as given above, and it was always lower than with acacia gum. A minimum effect was found when the membrane was made of double layer cellophane: with dextrane solution of 20% in the central compartment four mm height difference developed between water-columns in the vertical tubes, that corresponded to a pressure difference of 40 Pa.

DISCUSSION

Considering the microstructure of the membrane-gap it seems to be relevant that the parchment paper is not strictly semipermeable. Some of its pores are permable for big organic molecules, although they are hindered from penetration, i.e. the *Staverman* reflection coefficient (selectivity coefficient) $\sigma < 1$. Small inorganic particles penetrate easily ($\sigma \ll 1$). Another important property of the system that the gap between the solid plate and the adjoining parchment paper has a size which makes possible the free movement of small inorganic particles, but it hinders the movement of big organic molecules; in other terms the gap itself has a selectivity. The mass of highly concentrated colloid or semicolloid particles “sucks” (absorbs) the water intensively from the membrane, at the same time this “mass” works as a filter. The high pressure removes the water from the gap, together with the small particles. It is important that the gap can resist the internal pressure, otherwise the qualitative and quantitative conditions of this process called *osmofiltration* would be altered. It is obvious that the component processes are serially coupled.

An interesting consideration from the viewpoint of energetics that evaporation and osmosis are spontaneous phenomena. The water evaporates even without an “artificial” heating. The heat required to evaporate water molecules is taken from the surroundings which cools down and so does the water.

Osmosis is caused by the triple interaction of the membrane, the water and the solute (or its hydrate water). The solute “provokes” the osmosis close to the rectifying membrane. The working hypothesis for the mechanism of the osmosis is that the water molecule “evaporates into the microspace” adjoining the solute molecule even without an energy import. The “evaporating” water molecule obtains energy from the water-membrane side and it crosses the microspace as a provisional “vapour molecule”. (There are hollow spaces among the water molecules. A neutral point or microchannel may be formed in the electrostatic or molecular field of the surrounding water molecules. These cavities come about within the so-called free volume, or within the quasicristalline structure of ice-like clusters of water molecules [1, 2]. Hydrophillic solute particles can destroy this structure causing a simultaneous volume contraction which results in an absorption of water molecules from the membrane side.) When a “vapour” molecule hits the hydrate water of a hydrophillic solute particle, its energy is transferred to the solution. The final result is that a coupled energy- and water transport occurs in the direction water → solution.

As a consequence of these it is expected, that the solution of higher concentration would warm up during the osmosis.

(In an egg, the osmotic concentration of the yolk is higher than that of the white of egg. Therefore, there may be an incessant osmotic inflow towards the yolk through the film between the yolk and the white of egg. That may be a possibility to explain the temperature difference between the yolk and the white of egg, too. The yolk is warmer as observed F. Vető [17].

At the same time, the inner pressure increases due to the osmotic water influx and the consequence is an outward water flux. Therefore, there is a steady water circulation there.[1] Pócsik, I. [16] carried out a detailed investigation into the coupled water- and heat transport [Q*] due to the hydrostatic pressure difference across the pores of a protein membrane.

Probably, there are simultaneously separate channels for water influx and outflux, and these two fluxes have different energy requirements. The energy difference is supplied by metabolic processes in a living structure. These considerations may hold for a membrane-gap structure. An "active" water transport occurs at the membrane of the gap, without which the osmofiltration could stop.

Experiments were done in other laboratories [3, 4, 5, 11, 12, 13] on systems consisted of two (or more) membranes. All these membranes had different permeabilities for water and for solutes as well. The water (together with the solutes) was transported in the direction from the lower permeability towards the higher one. The system of membranes author used represented another category. They could be used to investigate the mentioned transports in the first phase of the experiment because of the identity of the membrane materials.

The hindrance of the diffusion by the layer of smaller permeability at the border of the membrane-gap results in a transport apparently having an extraordinary direction. The membrane-gap is a system that transports in a given direction on its own.

The water transport may be described by the methods of irreversible thermodynamics if we consider the membrane-gap structure as an open system. According to this, each flux should be taken into consideration with its sign (direction). In case of a single-membrane experiment the total flux:

$$J_v = L_p(\Delta p - \sigma\Delta\pi) = L_p\Delta p - L_p\sigma\Delta\pi$$

J_v the volume flux
Δ_p the hydrostatic pressure difference
$\Delta\pi$ the osmotic pressure difference between the liquids at both sides of the membrane
L_p the filtration coefficient of the membrane
σ the Staverman reflection coefficient (the selective coefficient of the membrane).

As it could be seen that even a transport through a single membrane is composed of two fluxes of opposite direction due to the concentration difference and to the hydrostatic pressure difference. A flux of four components occurs in a double-membrane system across the free, uncovered parts of the membrane. In addition, there are fluxes along both membranes through the gaps due to the osmofiltration caused by the different areas of the covered parts of the membranes (J_{vof}) which is a flux in the direction solution → membrane → side chamber.

The total flow of water through the system (J'_v) is determined by six components:

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[1] This sort of circulation may play a role in the transport of solutes – e.g. medicines – toward cell membranes.

$$J'_v = \Sigma\, J_v + \Sigma\, J_{vof} \quad \text{therefore:} \quad J'_v - \Sigma\, J_v = \Sigma\, J_{vof}$$

The total flow (J'_v) can be measured. ΣJ_v can be calculated from the experimental parameters according to the flow equation. So we get the total of the fluxes of osmofiltration ΣJ_{vof} wich depends on the ratio of gap areas. In this way the osmofiltration flux J_{vof} may be determined even for a single membrane, too.

The basic conclusion is that it is the actual concentration- and pressure difference of the narrow layers close to the membrane – and not the average of the concentration- and pressure difference of the whole liquid phases separated by the membrane – which determines the direction of the total flux. Consequently, the presence of a small amount of solute – which is in a very little volume of the gap – is concentrated enough and is sufficient to result in an osmofiltration and to maintain it. That seems to be "economic" for living systems.

Both the "unstirred layer effect" [4] and the osmofiltration are related to the (opposite) influence on diffusion. It seems that osmofiltrationn is more efficient biologically, because the concentration in the membrane-gap may be increased to saturation. The membrane-gap structure has got similar power within cells and living organisms. The size of such structures may range from microscopic to submicroscopic, having of various shape and function. They may be investigated from various aspects. Electronmicrographs show double membranes where the main water transport occurs in an animal (human) organism. There is a higher material exchange. A double membrane may behave as a membrane-gap structure, if the second membrane (in the direction of the flow) is impermeable, or less permeable for water and solutes, but discontinuities are the filtration holes.

The possible role of osmofiltration will be discussed below considering what we have said above.

Blood vessels and extravasal liquid flow

The wall of the capillaries contains at least a double membrane because both the inner and outer side of cell the layer constituting the capillary wall is covered by cell membranes. This double membrane is discontinuous (e.g. lymphocytes may penetrate it in case of an inflammation). The extracellular flow originates from this field. Osmofiltration may be a helper to transport the extravasal liquid towards the cells and lymphatics.

A slow and incessant flow of water and solutes occurs in some tissues of living organisms (bone, cartilage, connective tissue, joints) in spite of their poor supply with blood vessels, to the required extent. The blood vessels of these hard tissues are similar – in principle – to the structure and function of the membrane-plexiglass gap in the experimental setup.

This view may provide a better understanding of the pathological processes in rheumatological diseases and it may play a role in finding new therapeutic methods. In addition, the author believes that the changes of the membrane-gap structures can result in some other diseases.

Water uptake from the intestines of the frog Rana catesbeiana

The author learned about asymmetric osmosis in Professor T. Z. Csáky's laboratory, Lexington, USA, in 1973. The asymmetric osmotic transport through the walls of the frog ileum was discussed in a former excellent publication by Bentzel, Csáky, and Loeschke [14]. The piece of tissue dissected from the intestine was attached to the ends of the transfusion system in a way that the pressure of the liquid expanded the tissues in the wall of the intestine and the structural details became more recognizable. They examined both the outward (from the *mucosa* towards *serosa*) and inward transports. The outward transport was always greater than in opposite direction under the effect of the same concentration difference. As above authors mentioned, the difference due to the direction of the transport may be the consequence the different ways of the flows of liquids. The different ways of transport correspond to different osmotic permeability's (different filtration coefficients, L_p).

A quarter of a century later the author scrutinized the micrographs from above paper recently and came to the conclusion that a structure and function of the osmofiltration exists within the structure between the epithelial cells and basement membrane. A sort of "drain" of the basement-membrane liquid is clearly seen in EM photos of mentioned paper [14]. A gap field increased by infoldings and ranging to the basal cell membrane approaches the "drain" with filtration holes. (Unfortunately, because of technical hindrance there is no possibility to demonstrate these valuable pictures in present paper.)

Considering the absorption of water, first of all the biochemical activity of epithelial cells should be mentioned, when the cells absorb aqueous solution. The increased concentration results in a small increase of volume of the cells under the brush border membrane. Therefore the tight junction close up and an osmofiltration-like process begins which drives the solution towards the basal cell membrane through cells and intercellular spaces. (No diffusion barrier is required for adjacent cells having the same rate of production of molecules, because the equality of the production rates replaces the barrier.)

The membrane-gap structure of the main osmofiltration originates from the basal cell membrane. The basal membrane of the epithelial cells very likely provides the classical osmotic function of an osmofilter, and the field between the basal cell membrane and the basement membrane together with the infoldings corresponds to the gap of the osmofilter. The pressure can increase within the gap. The discontinuities of the basement membrane layers are situated opposite to the infolding, the filtration can occur through these discontinuities. The infoldings contain osmotically efficient molecules produced by epithelial cells, therefore the gap fills up and the osmofilter functions incessantly. After all, the pathway of the water exists from the intestinal lumen beyond the epithelial basal cell membrane, where the osmofilter absorbs and transfer it to the "drain" along basement membrane. In addition to the resistive properties of this pathway, it is for sure that the liquid flow caused by osmofiltration plays a role in the asymmetry observed in osmotic transports. This effect is the driving force of the absorption of aqueous solutions.

It is worth to mention that osmofiltration plays a role not only in cases of net volume flow against osmotic or hydrostatic pressure, but it can cause both increase or decrease of the normal osmotic transport.

Internal flow of liquid in eyes

Biological transport of liquids may have their origin both in biophysical and biochemical processes, these two scientific areas are very closely related in this respect. Osmofiltration occurs in the presence of a solute which is provided by biochemical processes.

A system of water uptake and transfer similar to that described in the previous chapter exists in the trabecular system of the eye, through which the 80% of the vitreous humour production is transferred from the *iridocorneal angle* to the veins. The structure through which the absorption and transfer occurs is the *trabeculum* within which longitudinal collagen and elastic fibres provide the pathway of the flow (Fig. 4). The diagrammatic figure based on electronmicrographs [7] shows a part of the *trabeculum.* The internal part of the *trabeculum* is a relatively closed space, which is connected through filtration opening and joint "drains" to the further pathways of the liquid flow.

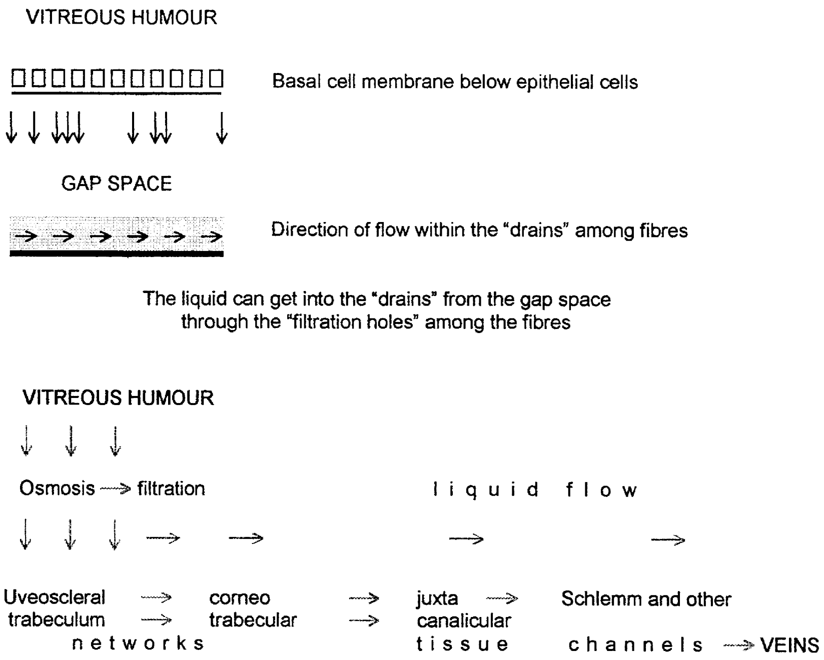

Fig. 4. Structural and functional model diagram of the *trabeculum*

The energy for the osmofiltration is provided by biochemical processes producing osmotically efficient molecules. Osmosis occurs at the basal cell membrane and creates a pressure difference required to the movement of liquids.

In producing of vitreous humor, the gaps among the biochemically active epithelial cells may play a structural role in the *rear iridocorneal angle.* The size of the gaps are determined by tight junctions and gap junctions connecting the cells. No presence of an impermeable layer adjoining the membrane-gap is required here. The free diffusion of the solute penetrating the membrane of a cell counteracts the function of the neighbouring cell, which is similar but of opposite direction, and increases the concentration in the gap. This is a way how vitreous humour may be produced.

The author examined water absorption (in Lexington, 1973) and he used dissected and isolated pieces of frog intestine which was attached to the ends of the tubes of the transfusion system. An organic dye was dissolved in the liquid that was in contact with the intestine during toxicological experiments. In consequence, the intestine became hard, a hard layer was formed at the side of the *intima* and the size of the intestine increased significantly, the water absorption decreased. This observation can be compared to the development of the pathological changes of the multilayer *lamina cribrosa* around the optic nerve due to glaucoma, and it may explain the development of a *papilla excavation* in the retina at the back of the eye.

Possible osmofiltration in the kidney glomerulus, too?

The structural diagram of the glomerulus (Fig. 5) [15] shows a striking similarity to that of the experimental device, as it is proved by a comparison of the blood vessel wall in the glomerulus with the structure of the membrane – plexiglass cover plate system in the experimental device (Fig. 1).

There is a continuous filtration from the arteriole of the *glomerulus* in case of normal function. However, the hydrostatic pressure in the filtrate space increases in a pathological case, when the ureter is clogged (by a calculus, or a tumour) and the urine cannot flow off. The epithelial cells move closer to the basal membrane and the

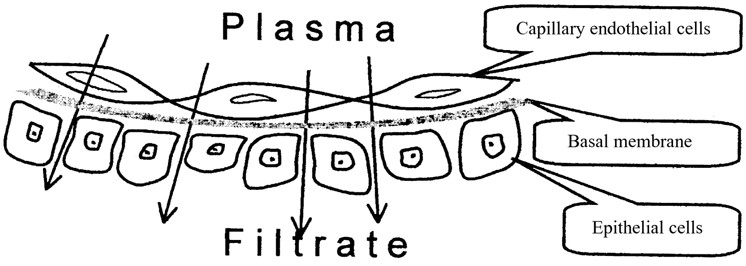

Fig. 5. Structural diagram of the glomerulus

gap space between them will have just the size which is required for the osmofilter function. In such a case of *hydronephrosis* the pressure in the filtrate may exceed even the blood pressure.

Plant osmofiltration?

One may speculate how it is possible that a plant can strike root into extremely hard soil or rocks, and the sap in trees can climb higher than it is calculated from known parameters. It is not unthinkable that membrane-gap structures may actuate osmofiltration in plants, too.

Biomedical significance

A further investigation into the problem of the membrane-gap structure and its osmofilter function may have a biomedical significance from the point of view of medication or prevention of consequences of the malfunction of the mentioned structures. This is a problem of the present day, because the survival of hibernation, frostbites or desiccation, success of resuscitation or transplantation may depend on the soundness and right functioning of these structures. That is why a hard frozen frog may be restored to life when thawed, or the dissected heart of a clinically dead person may be stored for a while then implanted into another person – it will restart and do its pumping work after an involuntary pause.

One can ask if the development of membrane-gap structures and the outset of their osmofilter function could be a stage of the transition from the inanimate nature to living beings? It is possible that osmofiltration is a basic vital process and the membrane-gap is a basic structure of living organisms.

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