Review

Contributions of Alexey Vereninov to the Study of Ion Permeability and Homeostasis

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Key Words

Electrophysiology • Ion permeability • Cell volume • Membrane potential • Mathematical modeling

Abstract

The review is devoted to Alexey Vereninov's research in the field of cell permeability and ion homeostasis. Particular attention is paid to the modeling approach developed in the study of frog striated muscles and subsequently applied to other cell types. Biologically relevant ways by which the muscles were studied and the model that was constructed determines the overall nonreductionist approach of the research. In the first study of excitability in invertebrate tissues and organs Vereninov used techniques and methods derived from his teacher Dmitry Nasonov. From 1963 to 1971, he tested membrane and phase concepts of bioelectrogenesis using isolated fibers and whole sartorius muscles. Both mechanistic and theoretical models have been used in the study of "salt potentials". The properties of muscles considered in these models are relevant to both the phase and membrane models. Vereninov's work, therefore, led to reconciliation between the two theories. In studies performed in 1971 and 1976 Vereninov presented a nonreductionist approach to the study of ion and osmotic balance in animal cells. Using the concept of cell permeability, he developed a method to analyze potassium permeability by combining electrophysiological and ionic data. In 1980-1983 Vereninov applied Ussing's criterion to study the role of passive permeability in the ion balance of muscles in a bi-ionic system. These studies were carried out under specific ionic conditions that allow the distinction between passive and active components of ion transport. Under these conditions, sodium was actively transported, and potassium exhibited both active and passive transport. Moreover, in a bi-ionic system with rubidium and potassium, some unidentified transport component was also present. Therefore, instead of Ussing's criterion, the application of which is restricted to specific experimental conditions, the analysis of ion fluxes was carried out using mathematical modeling.

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Introduction

The review covers the experimental and theoretical aspects of Alexey Vereninov's work on permeability and ionic homeostasis in animal cells. The methodological aspect of his work is essential in his early studies. Vereninov's publications between 1963 and 1983 show a wide range of scientific interests of the author. A deep knowledge of physics and mathematics allowed him to conduct physiological experiments and mathematical modeling. To resolve methodological issues Vereninov further developed a non-reductionist modeling approach inherited from his predecessors and teachers. Using muscles and later blood cells allowed him to avoid some methodological problems connected with cell isolation. Later, when studying processes at lower levels of the organization, he chose the most relevant methods that did not compromise the biological specificity of the work. Thus, in the study of cancer and apoptosis, he used a morphological method that may be considered non-reductionist in the methodological sense.

One of the first physiologists who realized the role of the cell as the basis of the most important biological processes was Dmitry Nasonov. Considering the ability of the cell to maintain constant volume, Nasonov avoided mechanistic explanations, relating it to one of specific cellular functions. At that point, the ideas of Nasonov were supported by the concept of cell permeability developed by A. Troshin [1]. Later, the topic of ionic homeostasis in animal cells became central in Vereninov's research. However, that direction of cellular research was formed only after some time: he started with an investigation on the problem of excitability using invertebrate tissues [2]. Meanwhile, in studying neurons, he mastered electrophysiological and histological methods, including vital cell staining. Following the general trend of cellular studies, he used the sartorius muscle as a laboratory model for the study of cell permeability. Due to specifics of intracellular organization, muscles occupy an intermediate position between cells and tissues. This view was supported by experiments of S. Krolenko, who studied the functional activity of frog muscle fibers, which can be considered as a unit of muscle organization. He demonstrated that the fibers are identical to the whole muscle in regard to their electrophysiological and osmotic properties. It should be noted that nerves and muscles are considered as cells only in particular physiological contexts [3].

Vereninov and his colleagues made microelectrode impalements of muscle fibers to measure transmembrane potential (TP), while the ion content and ion fluxes were measured on the entire muscle. In this approach, electrophysiological and electrochemical parameters of the cell were associated with the phenomenon of bioelectrogenesis either by mathematical modeling or the analysis of empirical data. These studies allowed to relate the passive mechanism of membrane transport with its role in generation of TP [4, 5]. Later Vereninov achieved a closer match between theoretical and experimental types of analysis using Ussing's criterion of passive membrane transport [6].

By studying short-circuited to zero frog skin, Ussing and Zerahn found that the total current was equal to sodium flux; but when a potential difference was reappeared, the sodium flux decreased [7]. Mathematical description of this effect given by Ussing revealed a relationship between the unidirectional ionic flux ratio, the ratio of ion concentrations inside and outside skin, and the value of TP. By analyzing this relationship, it is possible to decide whether or not the membrane transport has an active component [8]. Thus, the analysis revealed the presence not only of active sodium pumping but also mainly passive iodide transport in frog skin [7, 8]. The first application of Ussing's criterion to analysis of cellular membrane transport was done on neurons [9].

Ussing further contributed to the elucidation of the mechanism of sodium transport in isolated frog skin by showing that both influx and efflux of ^{24}Na is hyperbolically dependent on the external sodium concentration [10]. He called this mechanism "exchange diffusion", which means coupled bidirectional movement of like ions. In 1959, Keynes and Swan, using the radioisotope method in the muscle suggested the existence in muscles of a specific sodium carrier. The carrier mechanism of the exchange diffusion was confirmed in experiments with the substitution of external NaCl by LiCl or choline chloride. The presence of a carrier

can be inferred from the inhibition of ^{24}Na efflux after the removal of external Na$^+$. Sodium efflux in media containing 10 mM K$^+$ has a hyperbolic dependence on sodium concentration when a fraction of sodium is replaced by lithium. The efflux rate decreased even more when potassium was excluded from the lithium Ringer's solution. The Michaelis constant for ^{24}Na efflux was found to be 38 mM in a medium with 10 mM K$^+$ [11]. Adrian and Slayman showed depolarization after ouabain treatment by measuring TP in Na-loaded muscles. The authors interpreted a decrease in TP by inhibition of electrogenic component of the Na$^+$ efflux that was present in muscles with elevated Na$^+$ concentration [12]. However, a reduction of TP by ouabain can be more accurately explained by a decrease in the electrochemical K$^+$ gradient created by the sodium pump. Despite misconceptions about the particular mechanism of ion transport, these studies served as a methodological basis for analyzing ion fluxes and their role in bioelectrogenesis.

Electrophysiological studies of A. Vereninov on invertebrate muscles

The first electrophysiological study of A. Vereninov was comparative and devoted to the application of a generalized strength-duration equation suggested by Nasonov and Rozental' [13]. The theoretical problem underlying this study was the choice between capacitance and diffusion mechanisms of excitation [2]. The use of invertebrate muscles for solving this problem was dictated by their longer stimulation time, which allows the determination of parameter n in equation $i=a/t^n+b$. In this equation, the dimensionless parameter n varies from 0.5 (as in Nernst's diffusion equation) to 1 (as in the classical strength-duration equation). The values of n determined from the linear part of the strength-duration curves on a double logarithmic plot were 0.8 -0.9 in invertebrates and 1 in fibers of frog striated muscle or nerves. The latter value, however, was taken from other studies [2]. These data suggest the presence of the diffusion mechanism that is indicated by a lower n value [14]. However, longer stimulation time of invertebrate muscles indicates that t and n are not independent parameters, and therefore a lower n value cannot be used as an argument to support the diffusion hypothesis.

Studies of electrical and osmotic phenomena using the conception of cell permeability

Initially, the concept of cell permeability was used for the explanation of the phenomenon of plasmolysis and de-plasmolysis in plant cells (see [1] for review). Later, this concept was applied to explain osmotic phenomena in erythrocytes and striated muscles. Experimentally, permeability was studied by measuring muscle volume in the presence of substances with different lipid solubility. The lipoid theory of Overton defined permeability as the ability of substances to penetrate lipid membranes. Overton predicted high permeability for urea based on its role as a waste product [15] or its effect on the contractility of muscles [16]. These mechanistic explanations marked the beginning of the modern membrane theory, which postulates the presence of pores and specific transport mechanisms. Contemporaries used data obtained by Overton for mechanical explanation of bioelectric phenomena. Thus, Höber used these data to explain the polarizing effect of monovalent cations on the muscle. However, he could not confirm the effects of calcium on muscle excitability [17].

Overton's statement about the low dissolving power of water for intracellular solutes met with criticism from both proponents and critics of the membrane theory. A. Hill determined osmotic pressure by measuring the vapor pressure of muscle and erythrocytes to test the presence of osmotically inactive water [18]. His statement that most water in muscles was in free state supported membrane theory while the finding that only half of water was osmotically active, as determined by traditional volume measurement, contradicted

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it. Hill explained this discrepancy by changing membrane permeability during prolonged treatment, which leads to the loss of potassium from muscles [18]. Nasonov and Aisenberg addressed the same problem, pointing to the role of non-electrolyte permeability. They found that adding 5 mM of egg albumin, 50 mM of dextran, 0.3 M of sucrose or 2 M of urea caused a 20% decrease in cell water content against the expected 50%. When expressing the concentration of added substances in volume percent, all these dependencies were in the same range. They concluded that volume changes in muscles are controlled by the colloid mechanism (see [1] for the review).

Bioelectric phenomena were a central point in both membrane and phase theories. In a series of experiments, Höber measured bioelectric potentials on the surface of muscles in presence of different cations and anions. He assumed that the potential difference (PD) between KCl-treated and untreated muscle parts is mainly determined by alkali cations while anions and divalent cations are less important [17]. The polarization effect of cations on the muscle membrane was specific and ranged for iodide salts in the order: $K^+ \gg Rb^+ > NH_4^+ > Cs^+ > Na^+ > Li^+$. Due to the weakness of the lipoid theory, he used Bernstein's theory, which also focuses on the role of inorganic ions [19]. Since the effects of Rb^+ and Cs^+ do not fit within the theory, which assumes that permeability is determined by the radius of hydrated ion, Höber explained this disagreement by a hypothesis unrelated to the concept of membrane permeability. Among the factors responsible for this effect, he suggested the role of variations in the chemical activity of the protoplasm [17].

Nasonov and Alexandrov made further assumptions concerning the mechanism of ion activity changes. However, in contrast to the theory of Bernstein, they supposed that ions are released from their complexes with proteins during cellular activity. Thus, according to the phase theory, K^+ diffuses from the altered zone to the intact part of the damaged muscle. This view contrasted with Bernstein's theory, where the injury-induced current arises between the internal and external faces of the membrane. Therefore, both theories only partially explain the complex bioelectric phenomena. To address these issues, A. Troshin proposed a mechanistic explanation of PD generation in the intact muscle. His explanation was based on two different phenomena – diffusion and sorption. Diffusion of ions occurred in the aqueous phase of protoplasm, while sorption occurred in the colloidal phase. Also, based on these phenomena, he constructed a model that successfully predicted the effect of inorganic ions on PD in intact muscle [1].

Historical view of a non-reductionist approach in Vereninov's studies

Vereninov's non-reductionist approach was developed in the classical physiological traditions. Wedensky's and Nasonov's electrophysiological school had the most significant influence on this approach. Wedensky studied the generalized systemic response of nerves to stimulation during narcotic action [20]. He demonstrated that the optimum response was observed under intermediate stimulation of muscular preparation, while an increase and decrease in stimulation intensity led to a drop in the tetanus response. Treating these observations as separate phenomena, he combined them into one mechanistic concept of parabiosis. Parabiosis is a change in nerve conduction under intense stimulation, narcotics, and other non-specific factors [21]. Developing a similar concept of paranecrosis, Nasonov and Alexandrov studied the effect of sub-threshold stimuli on cells. Having found parallels between the processes of excitation and injury, they united it under one concept of paranecrosis (see [1] for review). Simultaneously, Russian physiologist E. Bauer gave a non-reductionist description of life phenomena, referring to mechanistic analogies rather than physical theories. Explaining the functioning of the living cell, he presupposed that it uses two types of work [22]. The internal work is done against equilibrium and transforms the free energy of substances outside the organism into the "structural energy" inside the organism. A second type of work is external, providing the stability of the whole system and thus participating in adapting the system to a changing environment. The mechanical analog of the first type of work is the work performed by a piston in an internal combustion engine. To explain the second type of work, he proposed the principle of "stable non-equilibrium". He

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theoretically analyzed this principle by considering proteins' function in enzymatic catalysis.

The non-reductionist approach can be demonstrated in experimental studies, where Vereninov examined the restoration of TP after depolarization caused by loading the muscle with KCl. In this study, he considered the variability of individual fibers. Variation of TP in muscles during recovery suggests the possible mechanisms that involve changes in potassium and chloride permeabilities [4]. In contrast, data usually obtained from molecular biology studies are not analyzed this way. Instead, biologists often use approaches based on model organisms that establish relations between the general characteristics of biological objects and the characteristics of a single organism. These approaches regard the organism as a model system for objects of the same or broader class even though they are quite specific in representation of their target [23]. Such models can also represent organs, tissues, and cells. However, the methodological and epistemic status of these models requires the use of other corresponding types of reduction. In experiments started in 1981, not included in this review, Vereninov mainly used cultured cells to study transmembrane ion fluxes. After 1990, his studies were carried out on rat thymocytes and human lymphocytes since the physiological parameters of these cells do not change significantly *in vitro*. Since 2004, the physiological parameters of the transformed myeloid cell line U937, which also served as a prototype of cancer cells, have been actively studied.

Using muscle as a model object presents methodological difficulties that can be solved mechanistically. The frog striated muscle can be separated into individual fibers representing the cellular level. A larger surface-to-volume ratio in the fibers compared to the whole muscle gives an advantage in the analysis of ion fluxes. For example, by measuring $^{42}K^+$ fluxes during continuous fiber stimulation, Hodgkin and Horowicz calculated the amount of potassium transported during a single action potential [24]. Thus, although the entire sartorius muscle represents a more relevant object, isolated muscle fibers met the research needs in Vereninov's early electrophysiological studies, especially since it was assumed that PD is generated inside the protoplasm.

Use of physical and mechanical models in study of "salt potentials"

Studies of "salt potentials" were originally done by Höber on injured muscles, but later he used intact muscles to test the membrane theory [17]. Boyle and Conway also used this method and found no meaningful difference between injured and intact muscle, provided that similar absolute changes in PD were observed with KCl addition to intact and injured muscles [25]. Also, using an intact muscle, Thoshin obtained the same order of cation selectivity as did Höber. However, in interpreting this order, Troshin assumed that it is due to specific interactions of ions with colloid particles and diffusion in the cytoplasm. This hypothesis was tested by measuring electric potentials in a complex coacervate system [1]. Troshin registered PD on the surface of solid coacervate consisting of gelatin and gum arabic. After the equilibration of coacervate with NaCl solution, one part of it was exposed to a test salt solution. The values of negative PD between the exposed and untreated part of coacervate ranged in the following order: $K^+ > Rb^+ > NH_4^+ > Cs^+ > Na^+ > Li^+$ [1]. The obtained sequence was analogous to the dependence obtained in studies on muscles [17] but significantly different from those obtained with the same electrode system without coacervate [1]. In absence of coacervate the following sequence was obtained: $Cs^+ > Rb^+ > K^+ > NH_4^+ > Na^+ > Li^+$ which corresponds to the diffusion of ions in the electrode system and is related to the radius of hydrated ions. These studies demonstrated the generation of PD in colloidal non-membranous system, imitating the properties of cytoplasm.

An acellular system that imitates phase properties of the muscle protoplasm was used by Vereninov to test the phase theory. The correlation between their physical properties was studied by locally applying a KCl solution to the surface of muscle fiber and a rod-shaped cation-selective material and measuring PD. To imitate longitudinal conductance that may compromise the value of salt potential generated by the membrane mechanism, the sample was made either solid or containing a long cavity filled with NaCl solution. The recorded PD between treated and untreated parts of the surface was not significantly affected by

the presence of conductive NaCl solution within the cavity, suggesting the role of phase properties in its generation [26]. Thus, a physical model imitating the phase properties of a muscle demonstrated the role of specific ion sorption in the mechanism of PD generation. However, the data do not lead to an unambiguous choice between the phase and membrane theory of electrogenesis.

The non-reductionist modeling approach of Alexey Vereninov was clearly expressed in the construction of a model based on the cable properties of the muscle fiber. The choice of this model was dictated by the availability of an appropriate mathematical treatment and the ability to test its properties experimentally. According to R Taylor's cable theory, the decay of PD along the fiber follows the exponential law. This dependency can be measured in electrical circuits [27]. Therefore, the first part of the model is theoretical, used for explanation of the phenomenon while the second is predictive, used for "testing" of the underlying mechanisms. The testing was done using an electrical model constructed of resistive elements and voltage source. When applied in biological research, this mechanistic practice can be considered reductionist because it focuses on a particular mechanism. However, taken together with theoretical explanations it provides the most relevant way to analyze complex living systems. A similar approach can be found in an earlier study by Hodgkin and Keynes, who investigated potassium currents in nerve fibers [9].

Theoretical and mechanistic analytical aspects in Vereninov's study are closely connected. The modeling was performed using the following parameters: external longitudinal resistance r_e =1300 kOhm/cm, internal longitudinal resistance r_i =120 kOhm/cm, membrane resistance r_m =51 kOhm*cm, and space constant $\lambda= (r_m/(r_e+r_i))^{0.5}$ = 0.19 cm. Based on these parameters, resistor values were selected and used to construct a mechanistic model of the muscle fiber. Voltage was applied parallel to the r_m resistor. The result of calculation matches the measured gradient of PD on the muscle fiber and distribution of the electric potentials on the resistor ladder network [27]. The excellent approximation of electric gradient on a muscle fiber by the cable equation confirmed the hypothesis of membrane mechanism of bioelectrogenesis. However, the changes in electrical gradient after adding or removal of KCl from the fiber surface suggest longitudinal diffusion of KCl within the fiber.

Thus, the generation of salt potentials depends on the transverse movement of ions, while their dissipation depends on longitudinal diffusion. Moreover, in interpreting these results, Vereninov did not exclude the participation of active transport and the movement of ions according to Donnan equilibrium. Testing of these possibilities became the main topic of his research since 1971. Meanwhile, in two studies discussed above Vereninov tested the effect of chloride on the "salt potentials". He locally applied a solution with increasing concentrations of K^+ and varying concentrations of Cl^- to the surface of the muscle fiber. For that purpose, Cl^- in solution was replaced with SO_4^{2-}. He found a higher rate of generation and dissipation of the salt potential in Cl^--free media [26]. In exploring this effect, he adhered to the point of view of the phase concept, according to which potassium acts by intracellular binding. This view contradicted Boyle and Conway's data that showed gradual changes in salt potentials caused by the addition of KCl to the medium [25]. By contrast, Vereninov's view was supported by the data of Hodgkin and Horowicz showing that membrane potential of muscle fibers did not hyperpolarize upon potassium removal when chloride concentration in the medium was kept constant [28].

Mathematical modeling of TP in frog skeletal muscle

Vereninov developed an interest in mathematical modeling rather early in his career. His first theoretically oriented work, written together with T. A. Vinogradova was published in 1971. In that work, the authors considered a model of skeletal muscle membrane potential based on the assumption of passive distribution of ions between the muscle and external media [4]. As had been first shown by Boyle and Conway in 1941 [25], K^+ is the major player in the generation of a negative TP, while chloride has a depolarizing effect. The depolarizing role of chloride was confirmed in a microelectrode study by Hodgkin and Horowicz [28] who loaded fibers with K^+ and Cl^- and incubated them in media with controlled Cl^- concentrations.

A decrease in the external K^+ concentration did not lead to considerable changes in TP. That observation could not be explained by the membrane theory, and therefore, the authors postulated a non-Goldman type of K^+ permeability, which later became known as "anomalous." The latter effect was further evaluated by Adrian [29]. By modeling the voltage-current relationships, he showed that depolarization (evidenced by high internal Cl^-, according to the Goldman formula) is attenuated by outward K^+ rectification.

The novel feature in Vereninov's approach was the investigation of TP recovery by replacement of hypertonic Ringer with the addition of KCl with isotonic Ringer. It resulted in S-shape kinetics of repolarization, and the authors hypothesized that delayed K^+ rectification was the reason. To prove this mechanism, extensive electrophysiological measurements on individual fibers would be required. However, as Vereninov and Vinogradova realized, the task could be significantly simplified if the experiments were supplemented by mathematical modeling. The algorithm used in their work was similar to that of Adrian [29] and additionally incorporated the data of Hodgkin and Horowicz [24, 28]. It allowed for the integration of ion fluxes in time, thus producing ion concentration dynamics [4]. This first mathematical model used by Vereninov was restricted to passive permeability, also called channel-like diffusion. This simplification permitted the computation of TP changes based on the Goldman equation [30, 31], and the model adequately described the long-term behavior of the volume and TP. In a later work, Vereninov extended this model to include the active ion transport term [32], which together with the Goldman leakage term became standard and was adopted by subsequent researchers [33, 34].

The mathematical model of ion transport based on the Goldman equation includes variable intracellular ion concentrations. Since ions contribute to osmolarity, the complete model should take into account the changes in cell volume. To better describe cell volume under realistic conditions, Vereninov included an additional parameter in the model - the total concentration of impermeable osmolytes in the medium. In this model, he developed the approach proposed by Boyle and Conway [25], who used a hypertonic KCl solution and assumed that K^+ and Cl^- move into the muscle until the osmotic balance with the medium is reached. By measuring dry and wet weight, both Adrian and Boyle and Conway [25, 29] found that muscle osmotically equilibrated in KCl and became swollen unless the external medium was hyperosmotic. But, as Vereninov pointed out, the stability of cell volume in hypertonic KCl solution contradicts the membrane theory while being consistent with the classical permeability concept of Nasonov and colleagues. This concept assumes that muscle is freely permeable to both ion species, so the electroneutrality of intracellular compartment is obeyed. Vereninov's approach anticipated the modern understanding of muscle volume behavior, which incorporates both the classical permeability theory and specific membrane transport mechanisms [35, 36].

Modeling of TP changes in the frog skeletal muscle after addition of KCl

The data in Table 1 illustrate the successful application of Vereninov's method in evaluating potassium and chloride permeabilities [4]. The Table lists the measured TP along with ion concentrations in the frog sartorius muscle. The ion concentrations were taken from other studies [29, 37]. In this study, muscles were first placed in Ringer solution supplemented with 100 mM KCl and then returned to normal Ringer buffer. The ratio of permeability coefficients for K^+ and Cl^- is found from the equation:
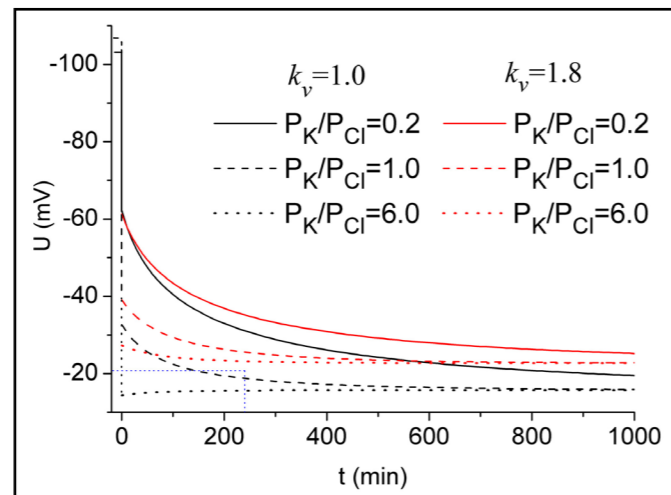
$$\frac{P_K}{P_{Cl}} = -\frac{[Cl]_i - [Cl]_o \exp(UF/RT)}{[K]_i \exp(UF/RT) - [K]_o} \tag{1}$$

where the subscripts i and o denote the concentrations in the cytoplasm and the medium, respectively, U is the membrane potential, F is the Faraday constant, R is the gas constant, and T is the temperature. To find the absolute permeability coefficients from the P_K/P_{Cl} ratio, Vereninov and Vinogradova made use of the Hodgkin and Horowicz result that P_K varies in a wider range than P_{Cl} [28]. One limitation of Vereninov's model was the neglect of volume changes upon KCl addition or removal.

In later modifications of the computational method, Vereninov corrected this shortcoming. The latest version of the program is available on https://vereninov.com/cellionfluxes/. The changes in media osmolarity are introduced there through parameter k_v.

The parameter k_v can be defined as the coefficient of proportionality between ion concentrations before and after cell volume change. In calculations that assume that the cell behaves as an ideal osmometer, internal osmolarity can be replaced by external ones. Vereninov suggested using this parameter in the study of lithium transport in U937 cells [38]. In subsequent studies, it was used to characterize osmotically induced changes [39, 40]. To demonstrate the role of k_v in the changes of TP caused by the addition of 100 mM KCl to the medium, its value was set either to 1 or 1.8 and the calculations were carried out using a program for the computation of pump-leak flux balance in animal cells [41]. The

Fig. 1. Changes of TP in a membrane Donnan system caused by the addition of 100 mM KCl to an external medium. Calculations were performed using the Web version of the program for the computation of pump-leak flux balance in animal cells [41]. The following initial parameters were used (in mM): $[K]_i$=139, $[Na]_i$ =23.6, $[Cl]_i$ =3.8, $[K]_o$ =102, $[Na]_o$ =120, $[Cl]_o$ =221, P_K was set to 0.002 min^{-1} (solid line), 0.01 min^{-1} (dashed line), or 0.06 min^{-1} (dotted line) while P_{Cl} was kept constant at 0.01 min^{-1}. Parameter k_v (the ratio of new to old osmotic strength after changing the medium) was set either to 1 (black lines) or to 1.8 (red lines). Parameters h_p and t_{max} were set as appropriate. The values of all other parameters were set to zero. The initial values of TP (U) were calculated using the above settings except $[K]_o$ =2.5, $[Cl]_o$ =121. The vertical lines at zero time (t=0) represent the transition from the initial value to the first calculated value after changing the medium. The blue dotted lines highlighted the experimental value of TP obtained at 4 h.

rate of TP changes at k_v = 1.8 is slower than at k_v = 1.0 for all three permeability ratios (Fig. 1). These differences are not due to variations in absolute P_K values since similar changes in kinetics were obtained using different P_{Cl} values. Also, for each ratio of permeability coefficients, lower TP values were obtained at k_v = 1 (Fig. 1, black lines).

Vereninov and Yurinskaya demonstrated a similar hyperpolarizing effect of hyperosmotic treatment in an *in silico* study using experimentally determined characteristics of U937 cells [40]. Essentially, this effect appeared only at low P_{Cl} values, when the outward fluxes of K$^+$ were higher than the outward fluxes of Cl$^-$. In muscle, this effect is observed over a wider P_K/P_{Cl} range, which is associated with low internal chloride concentrations. Thus, the hyperpolarizing effect of KCl at low P_K/P_{Cl} values appeared only at the end of the incubation period (Fig. 1) when Cl$^-$ concentration rises to considerable level. Fig. 1 demonstrates the usefulness of k_v for analyzing the changes in TP, especially at high P_K/P_{Cl} values. The curve corresponding to high permeability ratio has an initial drop and immediate reversal (Fig. 1, black dotted line). This biphasic behavior is due to initial volume changes. If the calculation is performed for isosmotic conditions, i.e. by replacing 100 mM external Na$^+$ with 100 mM K$^+$ this feature is not observed unless unpermeant osmotic B_o is added to the external media. The best agreement between predicted data and microelectrode measurements is obtained at k_v and the permeability coefficient ratio is set to 1 (Fig. 1, black dashed line). From the good match between this curve and the experimentally obtained value of -21 mV (Table 1), it follows that the effect of volume changes on TP is insignificant. However, considering the higher P_K/P_{Cl} value obtained from TP values (Table 1), it is important to consider the initial volume changes.

Table 1. Internal K$^+$ and Cl$^-$ concentrations, TP and the ratio P_K/P_{Cl} at the beginning and at the end of the incubation of muscles in Ringer solution supplemented with 100 mM KCl and after a return to normal Ringer solution. Values of $[K]_i$ and $[Cl]_i$ are taken from the studies [29] and [37]. The values of TP at the beginning and the end of the incubation period are taken from the original study [4]. The permeability ratios P_K/P_{Cl} was calculated using Equation 1 at the beginning (3-6 min) and at 240 min of incubation. The calculated values are fully corresponding to values obtained in the original study. Data on repolarization in Ringer solution (second row) were obtained before the occurrence of TP transients. The Ringer solution consisted of ions in the following concentrations (mM): K$^+$ 2.5, Na$^+$ 120, Cl$^-$ 121, Ca^{2+} 1.8, H$_2$PO$_4^-$ 0.85, and HPO$_4^{2-}$ 2.15

Media interchange	$[K]_i$, mM	$[Cl]_i$, mM	U$^{3-6}$, mV	U^{240}, mV	$P_K/P_{Cl}$$^{3-6}$	P_K/P_{Cl}^{240}
Ringer 3h → Ringer+KCl 4h	139	3.8	-26	-21	1.3	2.0
Ringer+KCl 4h → Ringer 4 h	218	102	-22	-35 – -38	0.6	1.4 – 1.7

Modeling of TP changes in KCl-loaded frog skeletal muscle after return to normal Ringer

Adrian in his modeling study estimated the dependence of voltage-current relationships on the internal Cl$^-$ concentration. The calculation of ion fluxes was performed in media with low external Cl$^-$ concentration when outward Cl$^-$ fluxes were dominant in the total membrane current. This was the condition at which the highest heterogeneity of repolarization was observed in real experiments [29]. It is known that Adrian carried out these experiments in solutions with constant osmotic pressure, which may also have influenced the scatter of the results. To test the effect of medium osmolarity on the kinetics of repolarization, Vereninov performed experiments in both isosmotic and anisosmotic conditions. When KCl-loaded muscles were placed in Ringer solutions with an additional 100 mM NaCl or 50 mM Na$_2$SO$_4$, the variability of electrical response was higher than in muscles returned to normal Ringer solution [4]. The disturbance caused by the osmotic factor suggests the presence of additional mechanisms making the repolarization response more complex. These mechanisms may be active ion transport or the effect of osmolarity on the P_K/P_{Cl} ratio. The latter effect was considered by Vereninov in a modeling study assuming a normal Ringer solution as the external medium. In that study, he used experimental data of ion concentrations which stabilized after prolonged incubation in Ringer + 100 mM KCl (Table 1). As in the study described in the previous section, the P_K/P_{Cl} values were altered by changing P_K. The results of modeling at the P_K/P_{Cl} = 1 closely approximated the experimental data obtained in a normal Ringer solution, while a decrease of P_K/P_{Cl} led to a delay of repolarization [4].

Therefore, it would be interesting to test the effect of k_v on repolarization kinetics. The results of calculations performed using the web version of the program at k_v = 0.6 (Fig. 2) revealed an S-shaped repolarization kinetics obtained in the original study [4]. However, the current version of the program does not allow one to set k_v to 1 under hyposmotic conditions. The reason for this difficulty was clarified in the study of I. Vereninov and co-authors [38]. They suggested that the reason for the program error is the excess of the internal osmolyte concentration over the external one. Another source of error is an exceedingly high value of TP. An alternative test for the effect of initial osmolarity change can be done by supplementing the medium with 100 mM NaCl, which is equivalent to setting k_v to 1. Thus, as expected, no significant changes in the shape of the curves were observed in that study. Consequently, the kinetic of repolarization is independent of initial volume changes. Again, as in the study of Vereninov [4], a good agreement between calculated and experimental data was obtained at P_K/P_{Cl} = 1 (Fig. 2, dashed line). The value obtained in modeling study approximates values calculated using Equation 1. Thus at the beginning of incubation and just before TP transients occurred, P_K/P_{Cl} value is close to unity (Table 1). The original study also revealed sharp transient increases in P_K that are responsible for S-shaped kinetics of repolarization. However, the mechanism of this increase remained unknown.

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Analysis of bidirectional cation fluxes in frog striated muscles

The Goldman equation established the relationships between TP and the instantaneous values of unidirectional ion fluxes [30]. This equation was used in the modeling study by Vereninov and Vinogradova [4] for the calculation of TP generated by passive fluxes of K^+ and Cl^-. The effect of sodium in that study was minimized by the use of excessive amount of KCl. Another way to selectively study a particular transport pathway is to decrease the concentrations of interfering ion species. This approach, lowering the internal ion concentrations, was originally used by Overton [15] who treated muscles with hyposmotic saline or isosmotic sugar solutions. Later Harris and Steinbach further developed this approach by applying pure water or isotonic sugar solution to muscles and measuring the ion efflux rate. They found that fluxes of cations are electrically balanced by fluxes of anions [42]. The anion flux, however, may account for the anomalous dependence of TP on the external potassium concentration. Martirosov and Lev found considerable differences between measured and calculated values of TP when muscles were first exposed to a hyposmotic sucrose solution for 3 h and then incubated in various potassium-containing solutions for 15 min [43]. This method was applied by Vereninov in the study of the anomalous dependence of TP on potassium concentration [5, 44, 45].
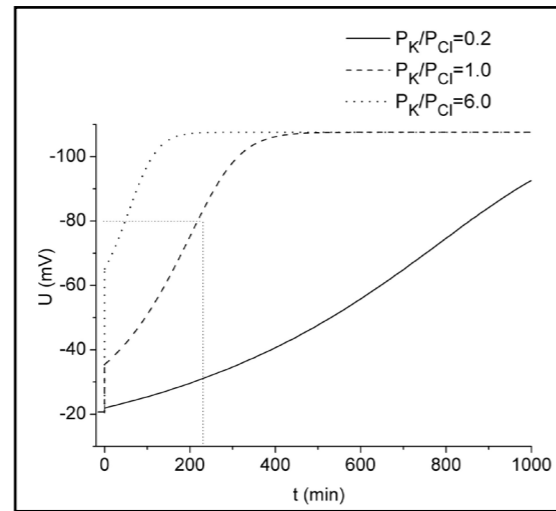

Fig. 2. TP changes after the return of a Donnan system equilibrated with Ringer solution supplemented with 100 mM KCl to normal Ringer solution. Calculations were performed as in Figure 1 with the following initial parameters (in mM): $[K]_i$ =218, $[Na]_i$ =23.8, $[Cl]_i$ =102, $[K]_o$ =2.5, $[Na]_o$ =120, $[Cl]_o$ =121, k_v = 0.6. P_K was set to 0.002 min^{-1} (solid line), 0.01 min^{-1} (dashed line), or 0.06 min^{-1} (dotted line). The vertical lines at zero time represent the transition from the values of TP in depolarized muscles to the first calculated value after changing the medium. The values of TP in depolarized muscles were taken from the calculations in Figure 1 at a time point of 180 min. The small dotted lines highlighted the experimental value of TP obtained at 4 h.

Study of net potassium fluxes in K- and Na-depleted muscles

Martirosov and Lev used the Nernst diffusion equation for the theoretical assessment of TP [43]:

$$U = \frac{RT}{F} \ln \frac{a_{Ko}}{a_{Ki}} \qquad (2)$$

where a_{Ko} and a_{Ki} are potassium activities in the medium and in the cytoplasm. Using Equation 2, Lev calculated TP based on the measured ion concentrations and compared it to the experimental values of TP. They found that the formula overestimated TP in sucrose medium by 7 - 8 mV [43]. Martirosov and Lev explained the discrepancy by the absence of chloride in the external solution. Equation 2 is written in terms of activities instead of concentrations, but authors made no distinction between them.

Vereninov performed his studies under the same conditions as Martirosov and Lev but extended the second incubation by 6 h. By measuring the dry and wet weight at the beginning and the end of the incubation, he showed that water content decreased by about 30% in a medium with 2.5 mM K^+ but remained unchanged in the presence of 75 mM K^+ [44]. The intracellular potassium concentrations increased from 60 mM to 75 mM during incubation in a sucrose medium with 1.25 mM K_2SO_4 and from 60 mM to 65 mM in a medium with 38

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Table 2. Changes of internal concentrations of K^+ ($[K]_i$), TP, and the ratios of influx to efflux rate constants for ^{42}K ($k_{o→i}/k_{i→o}$) during 6 h incubation in media with different K^+ concentrations after equilibration in ion-free media (112 mM sucrose, 3 h). Media with 1.25 mM and 38 mM of K_2SO_4 additionally contained 8 mM $CaSO_4$ and 1.5 mM K-phosphate buffer. The indexes indicate that the data were obtained at 15-minute and 360-minute incubation periods. The experimental values of $[K]_i$ and TP were obtained in original works [5, 45], with the exception of $[K]_i$ values in Ringer solution taken from [29]. The values of $k_{o→i}/k_{i→o}$ were calculated using data in table and Equation 3

Incubation media, mM	$[K]_i$, mM	measured U, mV	calculated U, mV	calculated $k_{o→i}/k_{i→o}$
Ringer	139	-91	-102	38.6
sucrose 112 K_2SO_4 1.25	$60^{15}→75^{360}$	$-66^{15}→-81^{360}$	$-80^{15}→-86^{360}$	$14.2^{15}→25.8^{360}$
K_2SO_4 38	$60^{15}→65^{360}$	$-9^{15}→-23^{360}$	$+5.2^{15}→+3.5^{360}$	$1.4^{15}→2.5^{360}$

mM K_2SO_4 (Table 2). For the calculation of TP, Vereninov used Equation 2 while based on concentrations. As shown in Table 2, the theory overestimated TP when medium contained 1.25 mM K_2SO_4, but underestimated it when concentration of K_2SO_4 increased to 38 mM. To explain these differences, the ratio of the influx to efflux rate constants was calculated using Equation 3 derived from Ussing's equitation (Equation 5, see below):

$$\frac{k_{o→i}}{k_{i→o}} = e^{-\frac{UF}{RT}} \qquad (3)$$

where U is the measured TP. The calculated data shown in Table 2 fully agree with the original calculations of Vereninov and were very close to his experimentally data [5]. In that study, the analysis was done using a flux constant ratio and the difference between the calculated and measured values of TP at the beginning and end of incubation [5, 45]. The high $k_{o→i}/k_{i→o}$ values in muscles incubated with 2.5 mM K^+ suggested that K^+ moves towards electrochemical equilibrium [5]. In the medium with 1.25 mM K_2SO_4, the difference between the theoretical and experimental values of TP during a 6 h incubation decreased. Furthermore, in the medium with 75 mM K^+ the ratio $k_{o→i}/k_{i→o}$ remained low, and the difference between the theoretical and experimental TP values increased at 6 h time point (Table 2). Therefore, the ratio $k_{o→i}/k_{i→o}$ indicates the position of the system relative to the equilibrium state and thus characterizes the net ion movement. However, low flux constant ratio at 75 mM K^+ may result from high bidirectional ion flux and does not reflect the absolute rate of ion flux. This phenomenon can be explained by the high rate of K^+ self-exchange, which is electrically silent and can only be detected by the isotope method. This effect was considered in the later works of Vereninov. I. Vereninov and co-authors considered this problem using a program version designed for the study of coupled Li^+ transport in U937 cells. They found that in normal conditions, coupled Na/Na and Cl/Cl fluxes constitute 70 and 90% of the corresponding overall turnover rate [38]. These studies demonstrate that analysis of net ion fluxes using the values of $k_{o→i}/k_{i→o}$ requires caution until the full set of parameters is available. In this case, modeling is a reliable tool for analyzing the system since all the components are taken into account.

Testing the hypothesis of independent ion fluxes for bidirectional transport of Na and K

If a bi-ionic system consists of two cations and is little or not influenced by anions, TP can be estimated using the following equation [28]:

$$U = \frac{RT}{F} \ln \frac{[K]_o + α[Na]_o}{[K]_i + α[Na]_i} \qquad (4)$$

where the subscripts o and i indicate the concentrations in the cytoplasm and media, respectively, and α is permeability coefficient in muscles taken as 0.01. By using this equation and varying composition of external media, Martirosov and Lev estimated the effect of Na^+ permeability on the value of TP. The calculated TP value (-64.0 mV) in hypotonic Na_2SO_4-containing media was consistent with that measured (-63.5±2.2 mV) and was lower than the -71.5 mV value calculated using Equation 2. The agreement between the numbers calculated from Equation 4 and experimental data indicated a predominantly passive distribution of ions in the system [43]. Vereninov and Toropova conducted a detailed study of Na^+ and K^+ transport under similar conditions using the methodology applied in previous study. TP was measured in fibers located on the same muscles as the ones used to calculate theoretical influx to efflux ratios [6]. This allowed discrimination between active and passive mechanisms of ion transport using Ussing's criterion of independence [8].

H. Ussing initially applied the independence criterion to distinguish between passive and active mechanisms of chloride transport [8] and sodium transport [7]. By applying this criterion to K^+ passive distribution, the following equation was developed [9]:

$$\frac{M_{o \to i}}{M_{i \to o}} = \frac{[K]_o}{[K]_i} e^{-\frac{UF}{RT}} \qquad (5)$$

where $M_{o \to i}$ and $M_{i \to o}$ are the inward and outward K^+ fluxes, having units of μM K^+ per g of dry weight per hour. This criterion assumes that the flux ratio is determined by the chemical gradient $[K]_o/[K]_i$ and the value of TP. The following equations were used to replace ion fluxes with the flux rate constants that are more convenient for measurement [6]:

$$k_{o \to i} = \frac{M_{o \to i}}{[K]_o} \qquad (6)$$

$$k_{i \to o} = \frac{M_{i \to o}}{[K]_i} \qquad (7)$$

By substituting Equations 6 and 7 into Equation 5, Equation 3 can be obtained and used for the calculation of efflux and influx constant ratios ($k_{o \to i}/k_{i \to o}$).

Bidirectional fluxes for ^{86}Rb were studied in muscles depleted of sodium and placed in sodium-free medium while ^{22}Na fluxes studied in muscles placed in potassium-free media. The operation of the sodium pump stops under these experimental conditions. Although the bi-ionic system favors passive ion distribution, the difference between calculated and experimental $k_{o \to i}/k_{i \to o}$ values for both ions suggests active transport. Moreover, inhibition of the ^{22}Na efflux by ouabain suggests an active pump (Table 3). However, even in the presence of ouabain, ^{22}Na flux constants did not provide unambiguous evidence for the independence of sodium transport [6]. This reasoning cannot be applied to ^{86}Rb transport either, but available data allow deducing a mechanism that is not directly testable by Ussing's relation. It should be noted that the interference of Rb^+ and K^+ in bi-ionic system and the close similarity of these ions [12] do not account for this mechanism.

The experimentally determined $k_{o \to i}/k_{i \to o}$ values in the $K^+ + Rb^+$ system were approximately 2-time lower than would be expected from passive distribution of K^+ (Table 3). This fact may be interpreted as a coupled Rb/Rb electroneutral exchange. Although the exact mechanism of K/K exchange in muscles is unknown, it may be similar to Na/Na exchange, which occurs in muscles with high sodium content [11]. Also, K/K exchange operates in ATP-depleted erythrocytes, where it is catalyzed by the sodium pump [46]. The possibility of K/K exchange was discussed in this review in connection with data in Table 2 showing that the calculated $k_{o \to i}/k_{i \to o}$ values for ^{42}K fluxes in a medium with 75 mM K^+ are lower compared to the 2.5 mM K^+ medium. Data in Table 3 also confirm this fact for ^{86}Rb. The decrease in $k_{o \to i}/k_{i \to o}$ values is at least partially due to an overall increase in transport rates. Identifying the exact mechanisms of ion transport by using Ussing's criteria is impossible. However, mathematical modeling helps solve this problem, which was a matter of further research by Vereninov.

Table 3. The application of the Ussing criterion to distinguish between passive and active fluxes of Rb^+ and Na^+. Ratios of influx to efflux rate constants ($k_{o \to i}/k_{i \to o}$) were determined in muscles incubated in Ringer solution or equilibrated in K^+-free sucrose media and transferred to the incubation media containing either 1.25 mM or 38 mM of Rb_2SO_4 or 1.25 and 38 mM of Na_2SO_4. Each of sulfate-containing media additionally contained 8 mM $CaSO_4$ and 1.5 mM K^+ phosphate buffer. Influx rate constants were determined at 83 min when muscles were loaded with ^{86}Rb or ^{22}Na. Efflux rate constants were determined after their transfer to isotope-free media. All data were obtained from the original study [6]

equilibration media, mM (4 h)	incubation media, mM (5 h)	radioisotope	determined U, mV	calculated $k_{o \to i}/k_{i \to o}$	determined $k_{o \to i}/k_{i \to o}$
Ringer	Ringer	^{86}Rb	-91	39	67
		^{22}Na	-91	39	0.25
sucrose 112	sucrose 112, Rb_2SO_4 1.25	^{86}Rb	-58	10	4.9
	Rb_2SO_4 38		-27	3	1.1
sucrose 112	sucrose 112, Na_2SO_4 1.25	^{22}Na	-76	21	0.45
	Na_2SO_4 38		-76	21	0.13
	Na_2SO_4 38+Ouabain		ND	ND	0.29

Discussion

The non-reductionist modeling approach plays an important role in biological studies. Some of the most successful applications of this approach are found in the studies of cellular ion homeostasis; unlike other models that assume specific transport mechanisms, Vereninov's approach only relies on experimentally measurable parameters, which allows it to solve problems more generally and makes model more flexible. His initial interest in passive ion transport in the frog striated muscle was dictated by theoretical consideration and its accessibility for experimentation. A muscle is an autonomous tissue that remains viable for a long time without external energy sources. For that reason, a muscle can maintain ion homeostasis; however, it may involve other mechanisms in this process. A modeling study conducted in 1971 fully validated the permeability values for two ions that were assumed to be passively transported. A later modeling study included active and other mechanisms of ion transport. Results calculated in the newest version of the program completely match the original data and suggest that the membrane transport system operates as a complex integrated mechanism that is described by a correspondent model. This type of model stands in contrast to simple mechanistic models used to represent particular mechanisms [47]. Mechanistic models may be called predictive because they explain (or predict) only an aspect of the phenomenon for which a particular mechanism is responsible. However, it is often challenging to separate mechanical and theoretical aspects in modeling. For example, Hodgkin and Huxley used the mechanism called "charged particles" in a model of nerve impulse propagation. In this model, charged particles describe both sodium permeability and the gating mechanism for sodium [48]. A simple mechanical model has also been used to test the "long channel" hypothesis, initially in the study of K^+ efflux in giant axons [9].

In developing an integrated model, Vereninov combined theoretical and practical aspects of analysis. Initially, his research was concerned with the phenomenon of ion permeability, emphasizing the passive component of ion transport. His model was object-specific and restricted to a particular type of cells. At the same time, it may be considered non-reductionist similar to "model organisms" [23]. Later, he turned to problem-specific modeling, which has a reductionist purpose. The integrated modeling approach has a mechanistic basis and provides virtually unlimited possibilities for studying ion transport mechanisms. Therefore, the goal of this integrated approach is to offer as many of the system's parameters as possible. The main reason to qualify his early modeling studies as non-reductionist is their

wealth of theoretical considerations. Although his model of ion transport was generally based on the same theoretical principles as those of others, the specific implementation of the modeling problem was quite original. One of the problems that Vereninov's and other models have tackled is the influence of impermeant ions on the ionic and osmotic balance of the cell. Recently Model et al. (2023) examined the role of impermeant ions in electrolyte balance using four mathematical models, including Vereninov's (https://vereninov.com/cellionfluxes/). Although all models used different methods for calculating changes in TP and ion concentrations, the obtained results were quantitatively and qualitatively similar [31]. In another work, Vereninov's code has been used to identify the factor responsible for cell vacuolation under certain experimental conditions [49]. The code is currently being used for teaching human and animal physiology courses at Kent State University.

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Statement of Ethics
The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors have no conflicts of interest to declare.

References

1   Troshin AS: Problem of cell permeability, ed Revised and Supplemented. Oxford, Pergamon Press, 1966.
2   Vereninov AA: [Force-duration relationship in short-range liminal electrical stimulation]. Biofizika 1959;4:40-47.
3   Blaustein MP, Kao, JP, Matteson, DR: Cellular Physiology and Neurophysiology. Philadelphia, PA, Elsevier, 2012.
4   Vereninov AA, Vinogradova TA: [Discontinuous changes in transmembrane potential during the washing of potassium chloride loaded muscle fibers according to data on the distribution of fibers by intracellular potential]. Tsitologiia 1971;13:869-878.
5   Vereninov AA, Vinogradova TA, Toropova FV: [The "anomalous" relationship between the concentration of potassium in the medium and the membrane potential of muscle fibers with a decreased intracellular potassium concentration. II. Rate of forward and reverse ^{42}K transport through muscle fiber membranes in saccharose-sulfate solutions with potassium concentrations of 2.5 and 75 mM]. Tsitologiia 1976;18:66-73.
6   Vereninov AA, Toropova FV: [Membrane potential, permeability coefficients and the ratio of the influx/efflux rates for alkaline cations across the muscle fiber membrane of the frog in a bi-ionic system]. Tsitologiia 1983;25:297-305.

7   Ussing HH, Zerahn K: Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. Acta Physiol Scand 1951;23:110-127.
8   Ussing HH: The distinction by means of tracers between active transport and diffusion: the transfer of iodide across the isolated frog skin. Acta Physiol Scand 1949;19:43-56.
9   Hodgkin AL, Keynes RD: The potassium permeability of a giant nerve fibre. J Physiol (Lond) 1955;128:61-88.
10   Ussing HH: The active ion transport through the isolated frog skin in the light of tracer studies. Acta Physiol Scand 1949;17:1-37.
11   Keynes RD, Swan RC: The effect of external sodium concentration on the sodium fluxes in frog skeletal muscle. J Physiol (Lond) 1959;147:591-625.
12   Adrian RH, Slayman CL: Membrane potential and conductance during transport of sodium, potassium and rubidium in frog muscle. J Physiol (Lond) 1966;184:970-1014.
13   Nasonov DN, Rozental DL: [Time factor in evaluation of tissue irritability]. Fiziol Zh SSSR Im I M Sechenova 1953;39:405-422.
14   Nernst W: Zur Theorie des elektrischen Reizes. Pflügers Arch 1908;122:275-314.
15   Overton E: Beiträge zur allgemeinen Muskel- und Nervenphysiologie. I. Ueber die osmotischen Eigenschaften der Muskeln. Pflügers Arch 1902;92:115-280.
16   Overton E: Beiträge zur allgemeinen Muskel- und Nervenphysiologie. II. Mittellung. Ueber die Unentbehrlichkeit von Natrium- (oder Lithium-) ionen für des Contractionsact des Muskels. Pflügers Arch 1902;92:346-386.
17   Höber R: Über den Einfluss der Salze auf den Ruhestrom des Froschmuskels. Pflügers Arch 1905;106:599-635.
18   Hill AV: The state of water in muscle and blood and the osmotic behaviour of muscle. Proc R Soc Lond B Biol Sci 1930;106:477-505.
19   Bernstein J: Untersuchungen zur Thermodynamik der bioelektrischen Ströme. Pflügers Arch 1902;92:521-562.
20   Wedensky NE: Die fundamentalen Eigenschaften des Nerven unter Einwirkung einiger Gifte. Pflügers Arch 1900;82:134-191.
21   Wedensky NE: Die Erregung, Hemmung und Narkose. Pflügers Arch 1903;100:1-144.
22   Bauer ES: Theoretical biology. Reprint of the 1935 edition with preface, biographical critical essay, and abridged English translation. Budapest, Akadémiai Kiadó, 1982.
23   Levy A, Currie A: Model Organisms are Not (Theoretical) Models. Brit J Philos Sci 2015;66:327-348.
24   Hodgkin AL, Horowicz P: Movements of Na and K in single muscle fibres. J Physiol (Lond) 1959;145:405-432.
25   Boyle PJ, Conway EJ: Potassium accumulation in muscle and associated changes. J Physiol (Lond) 1941;100:1-63.
26   Vereninov AA: [Kinetics and electrochemical nature of negative changes in muscle fibers under the influence of potassium chloride]. Nerv Sist 1963;4:40-45.
27   Vereninov AA: [Peculiarities of the study of salt and alternating potential differences on isolated fibers under conditions of the local effect of an agent]. Tsitologiia 1963;5:694-700.
28   Hodgkin AL, Horowicz P: The influence of potassium and chloride ions on the membrane potential of single muscle fibres. J Physiol (Lond) 1959;148:127-160.
29   Adrian RH: Potassium chloride movement and the membrane potential of frog muscle. J Physiol (Lond) 1960;151:154-185.
30   Goldman DE: Potential, impedance, and rectification in membranes. J Gen Physiol 1943;27:37-60.
31   Model MA, Mahajan A, Düsterwald KM, Dmitriev AV: Nucleic acids regulate intracellular ions and membrane potential. Paracelsus Proc Exp Med 2023;2:13-29.
32   Vereninov AA, Sr., Vereninov AA, Jr.: [Ionic, electrical and water balance in the animal cell. The system with active cation transport, Goldman's channels and the Na + K +2Cl-type symport]. Tsitologiia 1991;33:4-17.
33   Ataullakhanov FI, Korunova NO, Spiridonov IS, Pivovarov IO, Kalyagina NV, Martinov MV: How erythrocyte volume is regulated, or what mathematical models can and cannot do for biology. Biochemistry (Moscow) Suppl Ser A Membrane Cell Biol 2009;3:101-115.
34   Dmitriev AV, Dmitriev AA, Linsenmeier RA: The logic of ionic homeostasis: Cations are for voltage, but not for volume. PLoS Comput Biol 2019;15:e1006894.

35   Lindinger MI: A century of exercise physiology: key concepts in muscle cell volume regulation. Eur J Appl Physiol 2022;122:541-559.

36   Lindinger MI, Leung M, Trajcevski KE, Hawke TJ: Volume regulation in mammalian skeletal muscle: the role of sodium-potassium-chloride cotransporters during exposure to hypertonic solutions. J Physiol (Lond) 2011;589:2887-2899.

37   Vereninova SA, Romanenko GI: [The potassium and sodium content of muscles equilibrated with high potassium concentrated solutions]. Tsitologiia 1964;6:478-481.

38   Vereninov IA, Yurinskaya VE, Model MA, Vereninov AA: Unidirectional Flux Balance of Monovalent Ions in Cells with Na/Na and Li/Na Exchange: Experimental and Computational Studies on Lymphoid U937 Cells. PloS one 2016;11:e0153284.

39   Yurinskaya VE, Vereninov AA: Cation-Chloride Cotransporters, Na/K Pump, and Channels in Cell Water and Ion Regulation: *In silico* and Experimental Studies of the U937 Cells Under Stopping the Pump and During Regulatory Volume Decrease. Front Cell Dev Biol 2021;9:736488.

40   Yurinskaya VE, Vereninov AA: Cation-Chloride Cotransporters, Na/K Pump, and Channels in Cell Water/ Ionic Balance Regulation Under Hyperosmolar Conditions: *In Silico* and Experimental Studies of Opposite RVI and AVD Responses of U937 Cells to Hyperosmolar Media. Front Cell Dev Biol 2022;9:830563.

41   Vereninov AA: Computation of Pump-Leak Flux Balance in Animal Cells, [Cited 2022 Dec 5]. URL: https://vereninov.com/cellionfluxes/.

42   Harris EJ, Steinbach HB: The extraction of ions from muscle by water and sugar solutions with a study of the degree of exchange with tracer of the sodium and potassium in the extracts. J Physiol (Lond) 1956;133:385-401.

43   Martirosov SM, Lev AA: [Membrane potential of muscle fibers in reduced intracellular potassium content]. Tsitologiia 1965;7:181-188.

44   Vereninov AA, Vinogradova TA, Toropova FV: [The "anomalous" relationship between the concentration of potassium in the medium and the membrane potential of muscle fibers with a decreased intracellular potassium concentration. I. Movement of potassium, accompanying ions and water]. Tsitologiia 1976;18:58-65.

45   Vereninov AA, Vinogradova TA, Toropova FV: [The "anomalous" relationship between the concentration of potassium in the medium and the membrane potential of muscle fibers with a decreased intracellular potassium concentration. III. Change in the membrane potential during prolonged muscle incubation in saccharose-sulfate media containing 2.5 or 75 mM of potassium]. Tsitologiia 1976;18:195-202.

46   Simons TJ: Potassium: potassium exchange catalysed by the sodium pump in human red cells. J Physiol (Lond) 1974;237:123-155.

47   Bechtel W, Abrahamsen A: Explanation: a mechanist alternative. Stud Hist Philos Biol Biomed Sci 2005;36:421-441.

48   Hodgkin AL, Huxley AF: A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol (Lond) 1952;117:500-544.

49   Zook E, Pan YE, Wipplinger A, Kerschbaum HH, Clements RJ, Ritter M, Stauber T, Model M: Delayed vacuolation in mammalian cells caused by hypotonicity and ion loss. Sci Rep 2024;14:29354.