

Article

Membrane Potential: The Tamagawa Experiment

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Abstract: The membrane potential or resting potential of the neuron has been the subject of many studies. Although this theory explains the generation and maintenance of the membrane potential by direct or even facilitated diffusion, there are too many contradictions to doubt that these forces are sufficient or even at work in a process whose initial conditions are of rare complexity. The aim of this article is to show that already in the past, a competing theory has been developed whose hypothesis seems more scientifically sound. To confirm this last theory, Hirohisa Tamagawa carried out an experiment of great simplicity which makes it possible to invalidate the current theory and to question the teaching and the knowledge in Biology and Biophysics.

Keywords: membrane pump theory; membrane potential; ion channel; Na/K ATPase; thermodynamics; electromagnetism

1. Introduction

This article is the third part of a series on membrane theory and its contradictions.

- Any diffusion? [1]
- The enigma of ion pumps [2]
- **The Tamagawa Experiment**
- The ignored shape
- The smoothing of math

The first article already cast reasonable doubt on the validity of the current theory. Simple diffusion or facilitated diffusion cannot be the components of membrane potential generation or maintenance.

The second part showed that the topology of ion channels in the membrane led to a total divergence in the theoretical conditions for the application of the laws of diffusion. We also showed that the electrostatic interactions at work in the ion channels prevented any soft or hard knock on. The ions are irreversibly repelled and cannot enter the pore. We also showed that the Na/K pump cannot function as described.

This third paper returns to the work of Gilbert Ling, which Hirohisa Tamagawa brings to light through simple experiments.

Ling proved experimentally, half a century ago, that the difference in ion levels between the inside and outside of the cell could be observed in a dead cell even though the Na/K pumps could not function in any way [3,4]. The cell used in Ling's experiment was not only dead, but also lacked Na/K pumps. However, his cell did have a disparity in ion levels. So there is a major problem with some of the theories of current physiology. Electromagnetism and thermodynamics are largely absent in physiology and this is where the problem lies. We will show and discuss the results of our simple experimental work in order to address physiological problems that use electromagnetism and thermodynamics.

2. Experiment

It is widely known that the disparity in cell ion levels is intimately related to the MP. We therefore focus on the characteristics of the MP. A cell contains a number of immobile charges that are bound to lipids and proteins. We will now perform some simple experiments to see what happens to the potential of an electrolyte solution in the presence or absence of immobile charges.

2.1. Potential of an electrolytic solution

A $10^{-4}M$ KCl solution was prepared in a Petri dish, and a pair of Ag/AgCl electrodes were inserted into the KCl solution as shown in Fig. 1(a). The distance between the electrode tips was 65 mm. The potential measurement was performed at time $t = 0s$ which is the start time of the potential measurement. At $t = 30 s$, a few drops of $10^{-1}M$ KCl solution were added near the indicator electrode and the potential measurement continued until $t = 60 s$.

Next, another Petri dish filled with a $10^{-4}M$ KCl solution was prepared and a pair of Ag/AgCl electrodes were inserted into the KCl solution with an electrode gap $g = 65$ mm as shown in Fig. 1(b). The potential measurement was performed by sliding the indicator electrode while the position of the reference electrode was kept fixed. The indicator electrode was moved from $g = 65$ mm to $g = 35$ mm (the dashed arrow in Fig. 1(b)) in 1 mm steps for each potential measurement, and then the indicator electrode was moved back from $g = 35$ mm to $g = 65$ mm (the dashed arrow in Fig. 2(a)) in 1 mm steps for each potential measurement again.

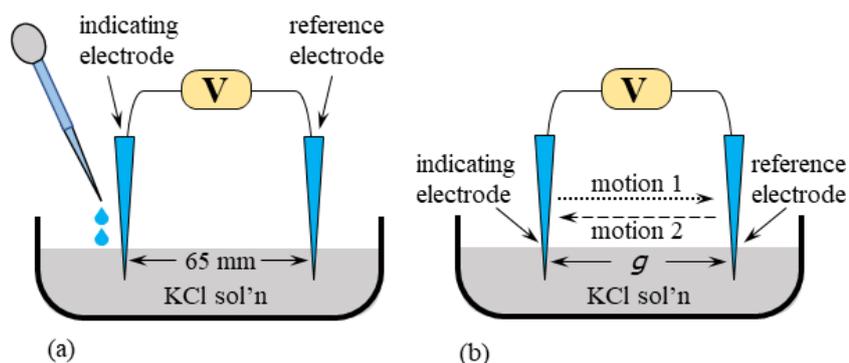


Figure 1. (a) Setup for measuring the change in potential of a $10^{-4} M$ KCl solution caused by the addition of a $10^{-1} M$ KCl solution. (b) Setup for measuring the potential of a $10^{-4} M$ KCl solution relative to the electrode spacing g .

2.2. Potential of electrolytic solution near the ion exchange resin

Next, the same experiment using the setup shown in Fig. 1(b) was carried out in the presence of a cation exchange resin as shown in Fig. 2. The cation exchange resin carries the immobile negative charges and these negative charges attract the mobile cations. The gap between the indicator electrode and the ion exchange resin was much narrower than 1 mm (see again Fig. 1(b)).

The same experiment was carried out using the anion exchange resin instead of the cation exchange resin. The anion exchange resin carries the immobile positive charges and these positive charges attract the mobile anions. When the anion exchange resin is used, the potential is easily disturbed by the movement of the indicator electrode. Therefore, after moving the indicator electrode, we waited for the potential to reach equilibrium and then recorded the equilibrium potential.

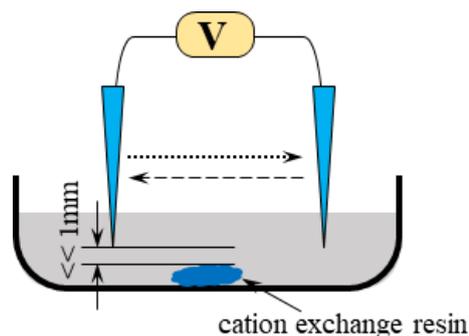


Figure 2. The setup for measuring the potential of a KCl solution in the presence of a cation exchange resin. The solution in the vessel is 10^{-4} M KCl. The same setup was used when the anion exchange resin was inserted in place of the cation exchange resin.

3. Analysis of aqueous potential in the presence of immobile charges

Figure 3 shows the experimental results obtained by the methods presented in Figure 1(a) in the section 2.1 Potential of electrolytic solution. The potential was practically zero throughout the measurement, even at the time when 10^{-1} M KCl was added at $t = 30$ s. Although there must have been a significant KCl concentration gradient between the two Ag/AgCl electrodes immediately after the addition of the 10^{-1} M KCl solution, the potential was practically maintained at zero. Thus, the mere induction of a significant ionic concentration gradient never affects the generation of a non-zero potential.

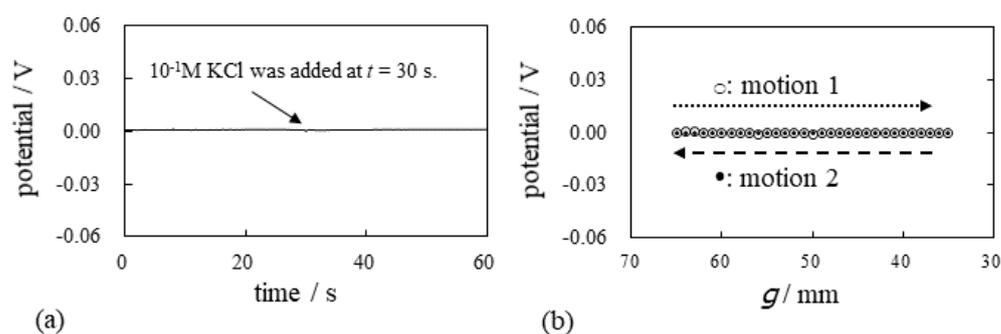


Figure 3. (a) Potential of 10^{-4} M KCl solution vs. time, a few drops of 10^{-1} M KCl solution was added nearby the indicating electrode at $t = 30$ s. (b) Potential of 10^{-4} M KCl solution vs. electrode gap g ○ and ● correspond to the potential measurement procedure “motion 1” and “motion 2” shown in Fig. 1 (b), respectively.

Figure 4 shows the experimental results performed by the methods described in the section 2.2 Potential of electrolytic solution near the ion exchange resin. We observed the negative potential and the fairly low potential, as low as ~ -0.04 V was observed at $g = \sim 55$ mm. During this potential measurement, the cation exchange resin was dispersed in the region between $g = \sim 60$ mm and $g = \sim 50$ mm at the bottom of the Petri dish (see Fig. 4). The potential profile depends purely on the g position as indicated by the quantitatively reproduced potentials measured by Motion 1 and Motion 2. Therefore, the generation of a negative potential must be due to the presence of the cation exchange resin. As the potential is very sensitive to the mechanical perturbation of the solution by the movement of the indicator electrode, the potential observed in the Motion 1 process was somewhat different from that of Motion 2. But we confirmed experimentally that the non-zero potential profile was relatively well reproducible quantitatively. In contrast to this case, the use of the anion exchange resin resulted in a positive potential as shown in Fig. 5 described in the section 2.2.

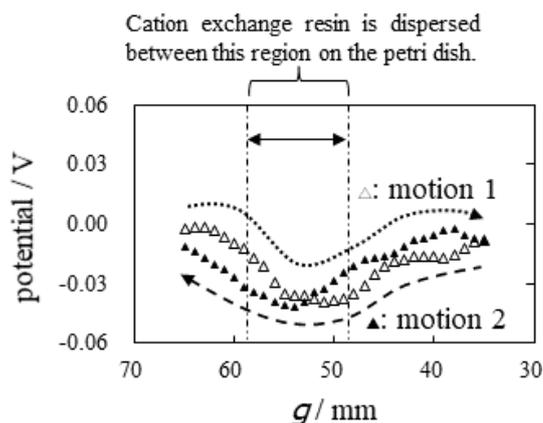


Figure 4. Potential of 10^{-4} M KCl solution vs. electrode gap g in the presence of the cation exchange resin \triangle and \blacktriangle correspond to the potential measurement procedure “motion 1” and “motion 2” shown in Fig. 1 (b), respectively.

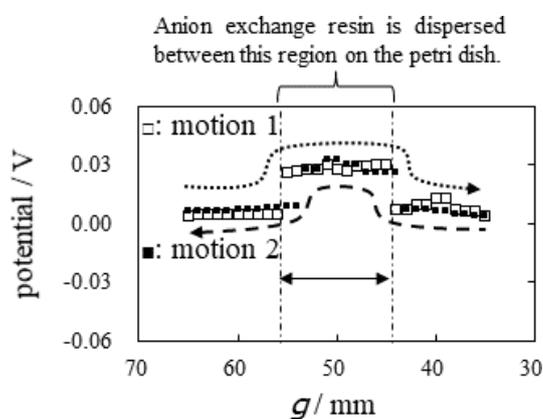


Figure 5. Potential of 10^{-4} M KCl solution vs. electrode gap g in the presence of the anion exchange resin \square and \blacksquare correspond to the potential measurement procedure “motion 1” and “motion 2” shown in Fig. 1 (b), respectively.

4. Discussion

The aim of this experiment is to demonstrate that it is possible to generate a potential in the absence of diffusion by simple attraction of ions towards a material which presents absorption sites. This experiment is easily reproducible. This type of products which are able to fix ions exist in abundance in the cell. We can therefore think that it is reproduced in the cell and around the membrane.

It is also very surprising that we do not find any simple experiment, article or video that unequivocally demonstrates the diffusion of positive ions of different types in the opposite direction as the current theory would have it [5]. Have we been unable for more than a century to set up such a simple experiment which takes up the basic hypothesis of the generation of the membrane potential?

Why are such potential profiles observed? It is merely a consequence of electromagnetism, electrostatics and thermodynamics [6]. Figure 6(a) shows the 10^{-4} M KCl solution and the dispersed cation exchange resin powder. The cation exchange resin is negatively charged and some of it traps mobile cations, K^+ as shown in Figure 6(b). Thus, the cation exchange resin is partially neutralised by the adsorbed K^+ following the mass action law of thermodynamics but still carries a large amount of immobile negative charges. These immobile negative charges generate a negative potential with respect to the infinitely remote potential of the cation exchange resin. As a result, the potential drops significantly around the cation exchange resin, as illustrated in Fig. 6(b). The potential profile shown in Fig. 4 is therefore observed, and this is simply the prediction of electromagnetism. The

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opposite potential profiles shown in Fig. 5 were observed by replacing the cation exchange resin with the anion exchange resin. Thus, all generations of zero and non-zero potentials are simply the consequences of thermodynamics and electromagnetism. Why then do we need to use the Na/K pump and ion channels to explain membrane potential generation? If Na/K pumps and ion channels are used to explain cell function, how are thermodynamics and electromagnetism incorporated into the theories that support physiology? Whether or not one wants to use thermodynamics and electromagnetism, the characteristics of the cell must obey the laws of physics and thus thermodynamics and electromagnetism.

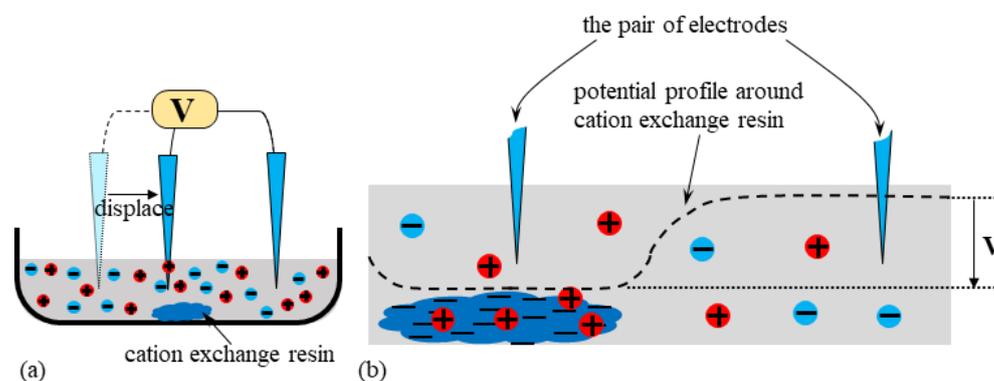


Figure 6. The expected potential profile in the presence of the cation exchange resin

A cell is full of immobile charges and nature of course follows the same laws, including thermodynamics and electromagnetism. However, these concepts based on thermodynamics and electromagnetism do not seem to be taken into consideration in the theory of membranes.

5. Conclusions

The membrane theory remains one of the foundations of current electrophysiology. Scientists try to persuade us that its validity is indestructible. This immobility regarding membrane theory is in a sense the cause of its obsolescence even though it is perhaps appropriate to say that it was the main electrophysiological topic of the last century. However, there are still some unanswered riddles in the foundation of membrane theory.

The questions "Is a Na/K pump needed or efficient?" and "Are ion channels working as thought?" sound weird. However, these are not irrational from the standpoints of electromagnetism and thermodynamics. What we have to do must be to integrate the cell theory and thermodynamics and such a process will provide the answers to all the enigmas.

The questions "Is a Na/K pump necessary or effective?" and "Do ion channels work as we think they do or are they traps that hold charges? Yet they are not irrational from the point of view of electromagnetism and thermodynamics. What we need to do is integrate cell theory and thermodynamics and such a process will provide the answers to all the puzzles.

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