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Excitatory potential of artificial membrane in response to current pulse stimulation

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Artificial membrane; Excitatory potential response; Current stimulation

An excitatory potential generated in response to stimulation by a current pulse has been studied on an artificial membrane composed of a Nuclepore filter impregnated with sorbitan monooleate. An excitatory potential response accompanying a fall in the resistance of the membrane was found to occur above a threshold value of the pulse height for current stimulation. The relation between the pulse width of current stimulation and the threshold for the generation of an excitatory potential response has also been examined.

1. Introduction

Biological membranes are electrically excitable owing to the presence of voltage-sensitive ion channels which are selective for Na^+ and K^+. The channels are considered to open and to close depending on the membrane voltage and to allow the rapid passing on an ion current into or out of the cell, thereby depolarizing or hyperpolarizing the membrane. These notions are based on the Hodgkin-Huxley equations which have explained a large number of experimental results on the electrical excitability of biological membranes, however, at the microscopic level, the mechanism underlying the on/off response process of channels remains to be fully understood.

On the other hand, similar excitatory phenomena have also been observed for various kinds of artificial membrane systems. Teorell [1] demonstrated self-sustained oscillations in a system consisting of salt solutions at different concentrations separated by a porous glass membrane, through which a constant current was passed.

A more controllable system comprises a Millipore filter impregnated with dioleyl phosphate (DOPH). A DOPH-Millipore membrane immersed in KCl solution displays a rapid increase in electrical conductance on attaining a certain threshold concentration c_t, with increasing concentration [2]. If the salt concentration of one side of the membrane is taken to be higher than c_t and that of the other side lower, self-sustained potential spikes with a long period of duration are observed across the membrane [3,4]. Further, the spike-like potential changes into a continuous oscillation with a short period, in the case where a d.c. current is passed through the membrane from the more concentrated to the dilute side [3,5].

The change in conductance of the DOPH-Millipore membrane at a critical concentration of the environmental solution was interpreted in terms of a change in adsorptive reaction rate at the interface which was derived from an autocatalytic model; the model also accounted for the self-sus-

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tained oscillation of this system [6]. Kamo et al. [7] carried out measurements of the variation in potential in response to stimulation of a DOPH-Millipore filter by an external electrical pulse and observed a potential response when the pulse height exceeded a particular threshold value; its response was similar to that observed in the case of an action potential on excitation of biological membranes.

Oscillatory phenomena have not necessarily been observed only for DOPH membranes. Yoshikawa et al. [8] reported the occurrence of oscillatory behavior for Millipore filters which were impregnated with sorbitan monooleate and placed between aqueous solutions of NaCl and KCl.

In the present investigation, an excitatory potential generated in response to stimulation by a current pulse has been studied, using an artificial membrane consisting of Nuclepore filter impregnated with sorbitan monooleate. We have examined the dependence of the potential form on the magnitude of stimulation, the decrease in membrane impedance at the onset of excitation, the relation between pulse height and pulse width of the current stimulation necessary for the occurrence of an excitatory potential and finally the effect of reversing the direction of the stimulation current.

2. Experimental methods

The artificial membrane used in the present work comprised a Nuclepore filter made of polyester sheet with a thickness of 8 μm. The filter has 10^5 pores/cm^2; each pore penetrates through the filter and is 10 μm in diameter. The filter was impregnated with a mixture of benzene and sorbitan monooleate, a kind of surfactant, in the ratio 3 : 1 (w/w), then dried in air and immersed for 1 day in an aqueous solution of 100 mM KCl. This process was essential for stabilizing the resting potential which appeared when placing the filter between salt solutions of different concentrations. Sorbitan monooleate is a dark-yellow, oily, nonionic surfactant and is soluble in organic solvents such as benzene and hexane but only slightly soluble in water. The surfactant used in this work was a commercial product of practical grade (Kanto Chemical Co., Tokyo).

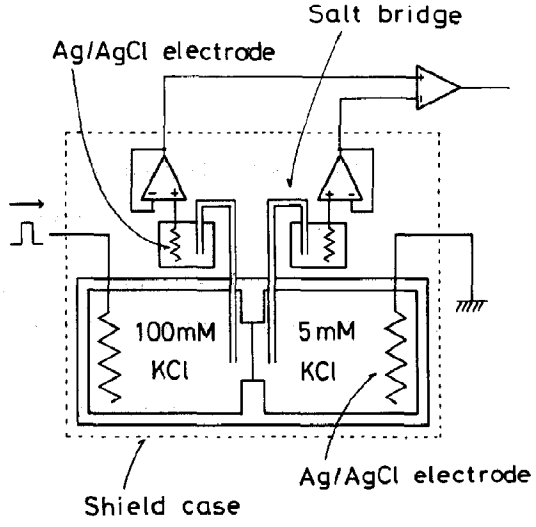

Fig. 1. Schematic diagram of the experimental cell.

The experimental cell is represented in fig. 1. The sample membrane fabricated as described above was washed for a few seconds in distilled water and held between two silicon rubber gaskets which had a circular window of 8 mm diameter. After sandwiching the sample between two cells of acrylic, we carefully poured aqueous solutions of 100 and 5 mM KCl into the cells on both sides of the membrane.

The membrane potential was measured with a differential amplifier through impedance converters connected to Ag/AgCl electrodes which were coupled by KCl salt bridges to the aqueous solutions in the two cells. The potential for the solution of low concentration (5 mM KCl) was used as a reference. Stimulation by pulsed current was effected with a home-made current-clamp circuit through Ag/AgCl electrodes. The response signal to current stimulation was simultaneously measured on two different time scales; one was of the order of 1 ms during the initial stages which was of the same order as the width of the pulse stimulation, the other being of the order of 1–10 s which covers the whole form of a response signal.

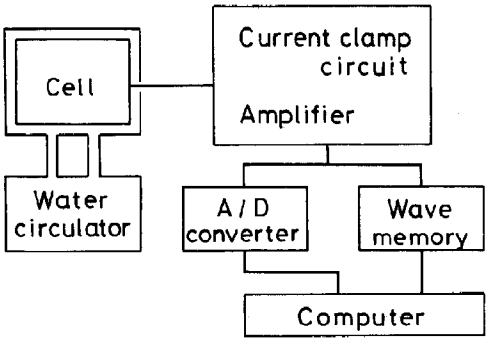

Fig. 2. Schematic diagram of the system employed for measuring the potential response.

The initial fine structure of the response signal was recorded in a disk through a wave memory and the global form of the response signal was recorded through an A/D converter (fig. 2). The experimental cell, salt bridges, electrodes and impedance converters were placed within a shield case in order to eliminate external noise. The shield case was placed into a water bath maintained at 25 °C by means of a water circulator.

3. Results

All measurements were carried out at 25 °C. When aqueous solutions of KCl were poured into the right and left cells, the potential across the membrane approached a resting potential in a few minutes. The value of the resting potential was determined as about −60 mV, although some scattering was observed depending on the samples. The resting potential decreased by a few millivolts over several hours but could be regarded as remaining practically constant during the experiment.

We measured the potential response to various values of stimulatory current of rectangular wave form passing through the membrane from the high- to low-concentration solution. At low values of current stimulation, the membrane potential was rapidly enhanced and decreased exponentially to the resting potential as shown in fig. 3. This can be interpreted in terms of an equivalent circuit consisting of a resistor and condensor; we designated this type as a passive potential response. Above a certain threshold of current stimulation, a potential response displaying a specific form was generated as shown in fig. 4a. The response, characterized by a plateau following the exponential phase, is presumed to be due to electrochemical activity of the membrane, and hence is termed an excitatory potential response. In the following figures, we present only the excitatory potential response (fig. 4b) obtained by subtracting the passive potential response from that observed.

Figs 5–8 illustrate the results of a series of experiments carried out with stimulation currents of various pulse heights but of pulse width fixed at 1 ms. Current stimulation was applied after the membrane potential had attained a steady value about 1 h after pouring the aqueous solutions into the cells. The membrane impedance was 138.2 $k\Omega/cm^2$ for this sample. Below the threshold value (13 $\mu A/cm^2$), only the passive potential response appeared, whereas above it, an excitatory potential response occurred as shown in fig. 5. The potential response was found to be generated up to 5 mV in the direction of depolarization and to vanish rapidly, within 1 s. With further increase in current stimulation (15.56 $\mu A/cm^2$), a two-step relaxation process was observed for the potential response as shown in fig. 6. The potential response was first enhanced to 10 mV, then decreased step-

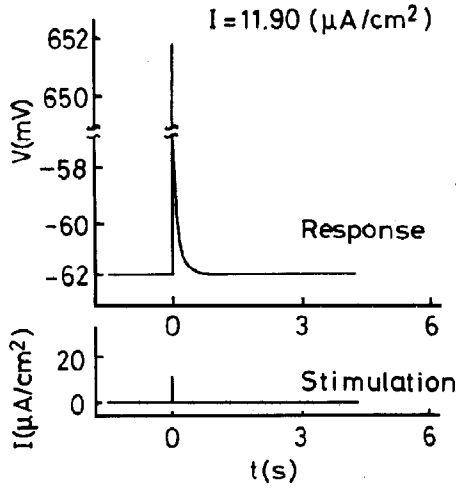

Fig. 3. Passive potential response on a stimulation by low current. Pulse width of current stimulation: 1 ms.

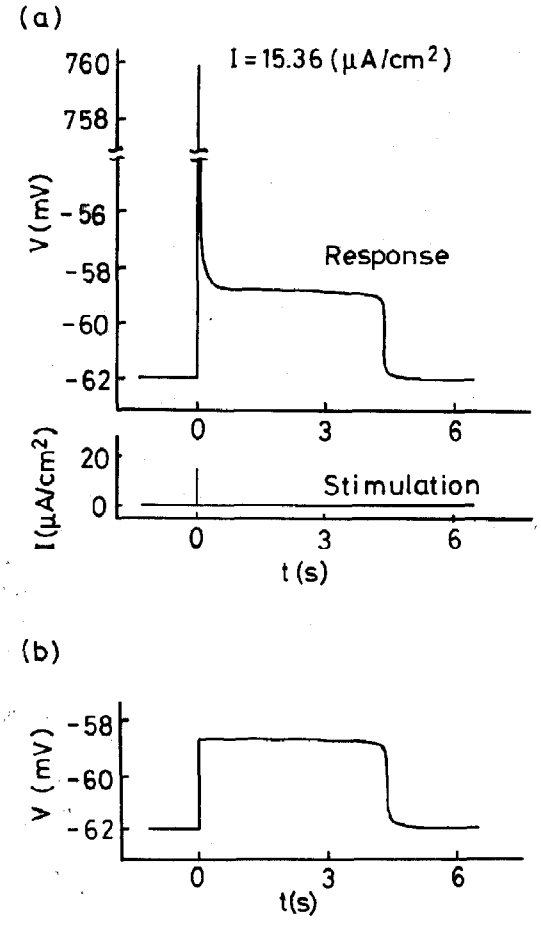

Fig. 4. (a) Observed potential response on current stimulation above threshold. Pulse width of current stimulation: 1 ms. (b) Excitatory potential response obtained by subtracting the passive response from that observed.

wise to 5 mV after 1 s and finally vanished after 2 s. Fig. 7 shows the response behavior for current stimulation at 16.63 $\mu A/cm^2$. In this case, the membrane potential was depolarized rapidly by 15 mV, decreased stepwise and relaxed to the resting potential after 20 s. The heights of the steps were almost identical, whereas the time of duration increased step by step. Fig. 8 shows that the relaxation to the resting potential took place over a greater number of steps at higher values of current stimulation (28.05 $\mu A/cm^2$). The same kind of experiment was carried out about 20 times for different samples of resistance varying between 50 and 500 $k\Omega/cm^2$, and qualitatively the same result as illustrated in figs 5–8 was obtained for samples with a resistance within the range 100–300 $k\Omega/cm^2$. However, for samples with a

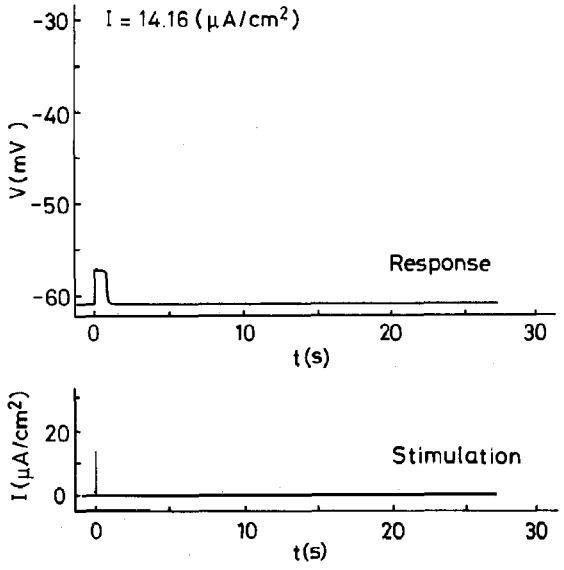

Fig. 5. Excitatory potential response for a stimulation current of pulse height 14.16 $\mu A/cm^2$ and pulse width 1 ms.

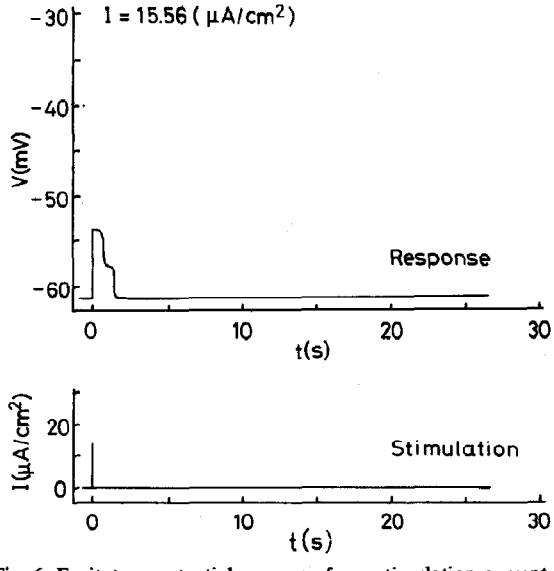

Fig. 6. Excitatory potential response for a stimulation current of pulse height 15.56 $\mu A/cm^2$ and pulse width 1 ms.

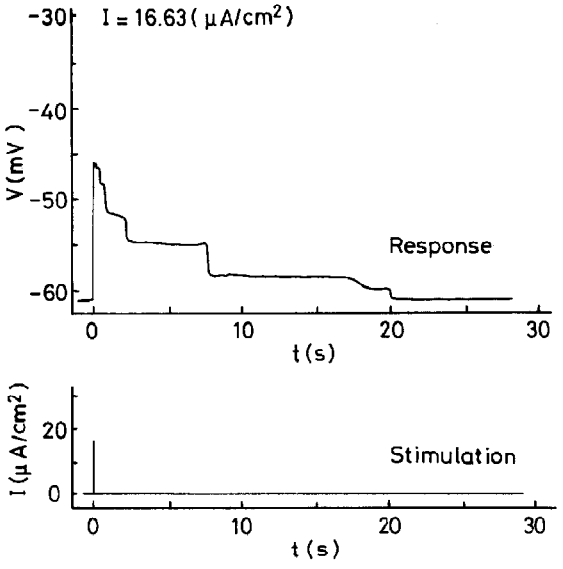

Fig. 7. Excitatory potential response for a stimulation current of pulse height 16.63 $\mu A/cm^2$ and pulse width 1 ms.

higher or lower resistance, an excitatory potential response was not readily observed.

We examined the initial stages of the response in more detail by expanding the time scale and obtained the signal form shown in fig. 9. Fig. 9a

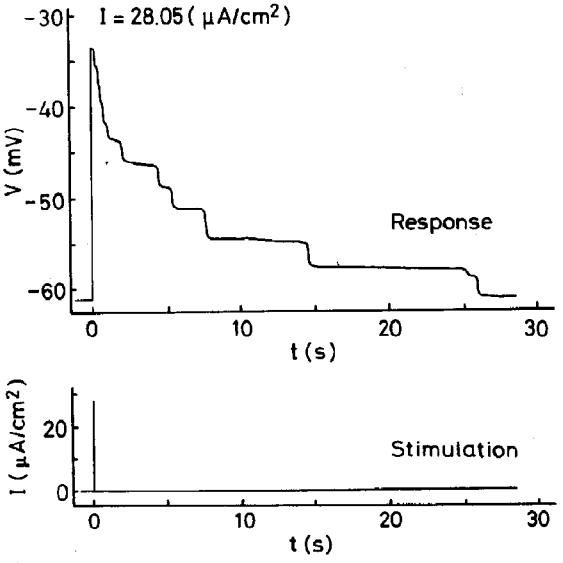

Fig. 8. Excitatory potential response for a stimulation current of pulse height 28.05 $\mu A/cm^2$ and pulse width 1 ms.

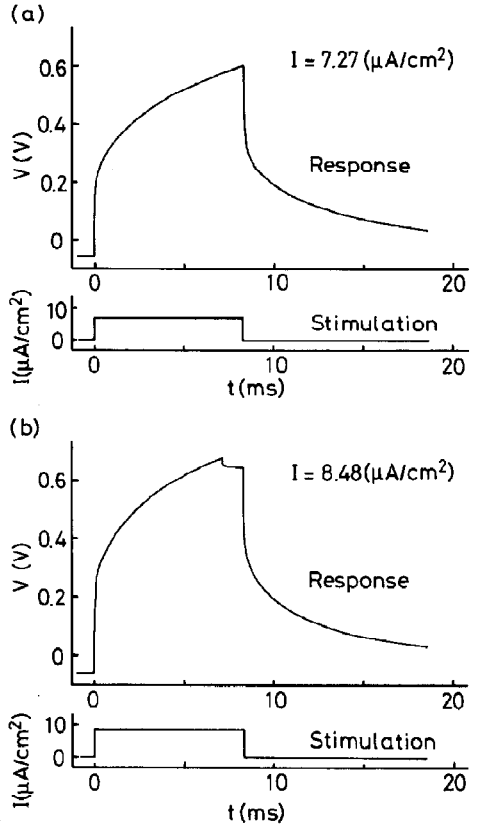

Fig. 9. Forms of the signals for the potential response during the initial stages. (a) Current stimulation below threshold. Pulse width: 8.3 ms. (b) Current stimulation above threshold.

shows the results in the case of passive response corresponding to fig. 3, fig. 9b displaying those of the initial stage of the excitatory response corresponding to fig. 4a. We can see in fig. 9b that a shoulder in the potential response curve is absent at the end of the duration of stimulation, indicating a decrease in membrane impedance. The relation between the pulse width and pulse height of the stimulatory current necessary for generating an excitatory potential response was investigated. Fig. 10 shows potential responses for various time widths of current pulses. One observes that the threshold value of current stimulation decreases with increasing values of pulse width. The lack of a shoulder in the potential response curve signifying a fall in resistance of the membrane reflects the generation of an excitatory potential response,

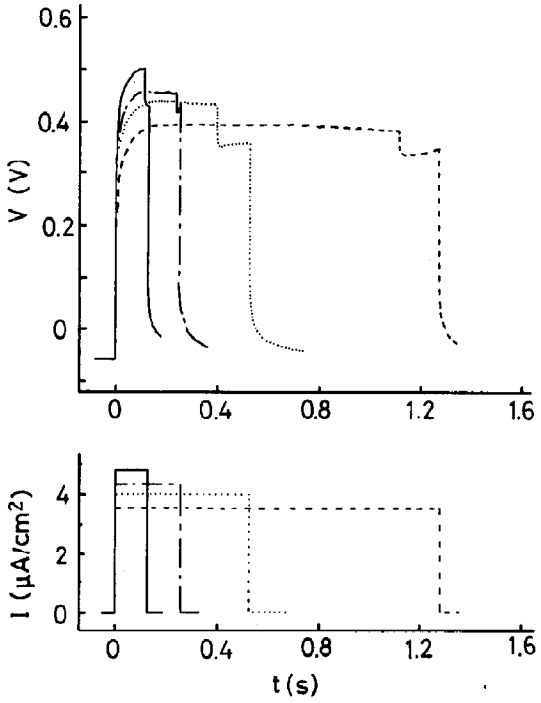

Fig. 10. Potential responses for current stimulation with various pulse widths.

and therefore we used the duration of time between the beginning of current stimulation and the occurrence of the lack of shoulder as a measure of the pulse width of the current stimulation required for generating an excitatory potential. Fig. 11 shows the pulse width dependence of threshold current stimulation.

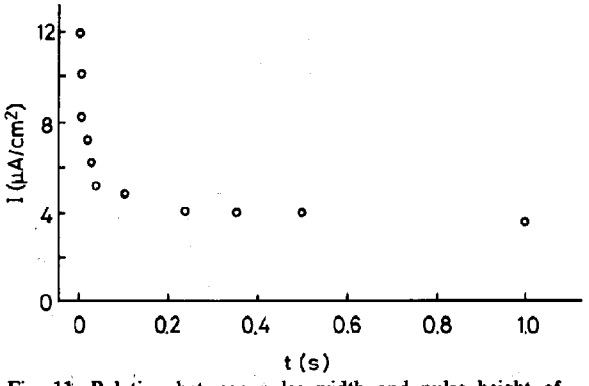

Fig. 11. Relation between pulse width and pulse height of current stimulation necessary for the generation of an excitatory potential response.

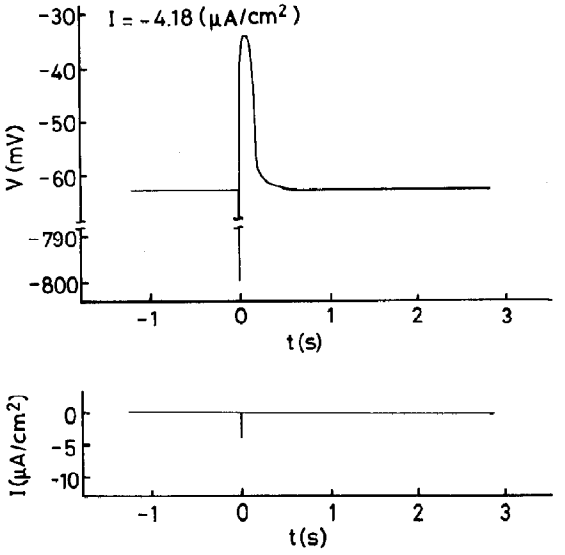

Fig. 12. Potential response on current stimulation (pulse height, 4.18 $\mu A/cm^2$; pulse width, 1 ms) applied from the low- to high-concentration solution.

We also determined the potential response for the application of current stimulation from the low- to high-concentration solution. The impedance of the membrane for the sample used in this

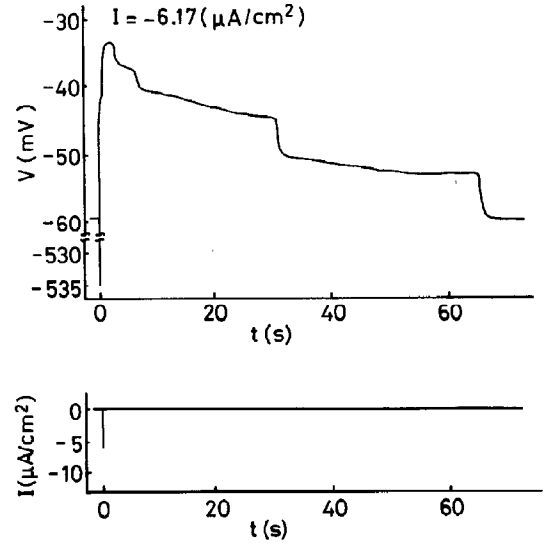

Fig. 13. Potential response on current stimulation (pulse height, 6.17 $\mu A/cm^2$; pulse width, 27.7 ms) applied from the low- to high-concentration solution.

experiment was 260.8 $k\Omega/cm^2$. For current stimulation (pulse width 1 ms) below the threshold value, the variation in potential showed only a passive response polarized in the direction of hyperpolarization, whereas for higher current stimulation ($-4.18\ \mu A/cm^2$) above threshold an excitatory response did occur (fig. 12). As shown in fig. 12, a passive potential response appeared in a downward direction, then an excitatory potential response was generated upward in the direction of depolarization. It is noticeable that the excitatory potential response was always generated in the direction of depolarization regardless of the direction of current stimulation. A relaxation response behaving in stepwise fashion was also observed for the inverse stimulation current. Fig. 13 shows an example, in which an inverse current of pulse height $-6.17\ \mu A/cm^2$ and width 27.7 ms was passed.

4. Concluding remarks

Nuclepore membranes impregnated with sorbitan monooleate showed an excitatory potential response to stimulation by rectangular current pulses when the height of the current pulse exceeded a particular threshold determined for each value of the pulse width. At high values of current stimulation, the potential response was found to relax in a stepwise manner to zero level. The same behavior for relaxation of the potential response has been reported in the case of Millipore membranes impregnated with dioleyl phosphate [7]. It is significant that the excitatory potential generated in response to current stimulation is polarized in the direction of depolarization irrespective of the direction of the current. This is a well-known observation for biological membranes [9] and has also been reported for Millipore membranes [7]. Thus, the behavior of the potential response observed on the Nuclepore membranes is a universal phenomenon and should be interpreted from a physicochemical point of view. The electrical potential across a membrane system is very complicated but reflects at least the dynamic behavior of ionic current passing through the membrane or the concentration of ions in the solutions on both sides of the membrane. Therefore, real time measurements of the concentrations of K^+ and Cl^- during the appearance of the excitatory potential would be desirable.

Acknowledgement

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