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Transport Phenomena in a Model Membrane Accompanying a Conformational Change: Transient Processes in Response to External Stimuli

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Summary. A model membrane composed of a filter paper and dioleylphosphate was studied by applying various kinds of external stimuli. When the concentration in the external solution was varied successively, the physico-chemical properties of the membrane changed drastically at a certain value C_t. The relationships between the electrical response and the external stimuli studied are as follows: (1) The membrane potential oscillates spontaneously in a spikelike fashion when the concentration of the external solution is suddenly changed. (2) The current through the membrane oscillates in spikelike fashion for a duration of about 50 msec when the constant external voltage, V larger than a certain value V_c, is applied across the membrane. (3) The electric resistance sharply decreases, and a kind of action potential similar to that observed in living tissues is produced when a short rectangular electric stimulus, whose magnitude is higher than a critical value V_p, is applied. (4) If a hydrostatic pressure difference across the membrane is applied with appropriate salt conditions, the value of the membrane potential varies with time, as in the case of (3). The observed changes in emf and electric resistance are discussed in connection with the conformational change of DOPH molecules in the membrane.

A series of papers is concerned with experimental studies of transport processes in a model system, in which a change of molecular conformation in the membrane takes place under various conditions of environmental salt condition or due to an external stimulus. This paper attempts to clarify the correlations between the transmembrane phenomena and the conformational change of molecules composing the membrane.

In previous papers [4, 9], it was shown that dioleylphosphate DOPH (used as a synthetic lipid analogue), impregnated in a Millipore filter paper,

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changed its conformation at a certain critical concentration of the external aqueous solution of a 1:1 type electrolyte. The Millipore-DOPH membrane showed an appreciable difference in its characteristics above and below the critical concentration. The values of membrane potential, ion permeability, electric resistance and of the electric capacitance in the steady state were found to be changed gradually with the external salt concentration, but drastically at a critical concentration [8]. It has been shown that the Millipore-DOPH membrane has two distinct states. The membrane is hydrophobic in a solution of low salt concentration, while hydrophilic in a higher concentration. The transition between them, however, shows hysteresis phenomena against the change in the salt concentration. In the hysteresis region, the transition is easily induced by a slight external perturbation. If the results obtained are similar to those in a living membrane, this may afford a hopeful clue to the understanding of living membrane; e.g., production of action potential, specific electrical response observed in sensory cells, etc., are able to be produced by the transition between two states of the present model membrane, which is triggered by an external stimulus. This paper describes electrical transient phenomena observed in the Millipore-DOPH membrane in response to external electrical and hydrostatic pressure stimuli applied across the membrane.

Two States of a Millipore-DOPH Membrane

To clarify the present viewpoint, the results of previous papers are referred to in this article. These are the dependence of the membrane potential, impedance, and ion permeability of the Millipore-DOPH membrane with a variety of external salt concentrations [4, 8, 9]. Fig. 1 shows a typical example of the membrane potential observed with a Millipore-DOPH membrane that adsorbed quantity of DOPH, $Q=2.18$ mg/cm^2 in various concentrations of KCl solution. Here, the observed membrane potential was plotted against log C_2 under the condition that the concentration of KCl in one side of the membrane C_1 was fixed at 5 mM, and that in the other compartment C_2 was changed successively. When C_2 was lower than C_t (50 mM for this specific membrane), the slope of $\Delta\varphi$ *vs.* log C_2 relation gave approximately the ideal value; i.e., 59 mV per 10-fold variation of C_2. However, when C_2 arrived at C_t, $\Delta\varphi$ decreased drastically, and further increase of C_2 caused a continuous decrease of the observed $\Delta\varphi$, as illustrated by arrow ③ in the figure. If C_2 was decreased progressively from this stage, $\Delta\varphi$ increased smoothly, as shown by the arrow marked ④,

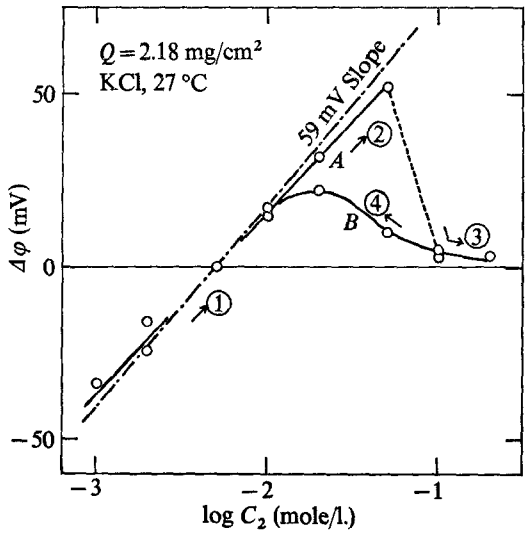

Fig. 1. Relation between membrane potential, $\Delta\varphi$ and salt concentration. C_1, the concentration of KCl in a cell is fixed at 5 mM, and C_2, that in another cell is changed successively as shown by the arrows, $\rightarrow$ ① $\rightarrow$ ② $\rightarrow$. $-\cdot-\cdot$: theoretical value of $\Delta\varphi$ for an ideal permselective negative membrane. Branch A has a hydrophobic character and B has a hydrophilic character

and manifested a hysteresis loop as seen in the figure. For the sake of illustration, the upper state of the membrane potential, i.e. curves ① and ②, is referred to as state A, while the lower state of $\Delta\varphi$, i.e. curves ③ and ④ is referred to as state B.

Simultaneous measurements of the membrane potential and of the impedance of the membrane revealed that the electric resistance in the A-state is independent of the external salt concentration, being on an order of $10^6\ \Omega\,\mathrm{cm}^2$. Furthermore, no permeation of ions was detected in this state [8]. From these and other characteristics of the membrane, it is considered that the membrane in state A is an oil membrane, since DOPH forms like oil droplets on the filter paper. On the other hand, state B is considered to be that of a charged membrane, since the concentration dependence of the membrane potential is represented quantitatively by the fixed-charge theory of the membrane potential, and since both ion permeability and d-c conductance are roughly proportional to the salt concentration in the external solution in this state. The electrical resistance of state B is $1/2 \sim 1/10$ of that of state A, depending on the salt condition. These results may be interpreted as follows: A fraction of the oil membrane in state A changes to bilayer leaflets, some of which make a leaky and

charged pore in the filter paper when the salt concentration in the external solution arrives at critical salt concentration C_t. This inference is consistent with the discrete change in the electric capacitance of the membrane at C_t with successive change of KCl concentration. It also is in accord with the ability of bilayer formation of DOPH with cholesterol in KCl solution[1].

In the next place we must decide which state is more stable in the region of concentration with hysteresis. In dilute solutions, where both C_1 and C_2 are lower than C_t, the stable steady state of the Millipore-DOPH membrane is state *A*. The membrane potential in state *B* increases very slowly toward *A* when the membrane is allowed to stand in this salt condition (C_1, $C_2 < C_t$) for several hours. However, state *B* is stable with respect to small external perturbation. In this respect, state *B* is considered to be a metastable state in the thermodynamic sense. Under the condition that C_1, $C_2 > C_t$, state *B* is more stable than state *A*. In the intermediate case, $C_1 < C_t < C_2$, the situation is very delicate and the stable phase is easily shifted from one to the other by slight external perturbations. Such phenomena are studied in the following sections.

Experimental

Membranes used were prepared by the same method as described in the previous papers [8, 9]; namely a Millipore filter paper composed of cellulose ester, with a nominal pore size of 5 μ, was soaked in a benzene solution of DOPH with an appropriate concentration, and the adsorbed quantity of DOPH in the membrane Q was adjusted between 2 and 3 mg/cm^2. Under these conditions, critical salt concentration C_t was about 40 mM of KCl solution. The soaked membrane was thoroughly dried in air, and then immersed overnight in an electrolyte solution of 125 mM KCl. Then the membrane was immersed in 1 mM KCl solution for at least 2 hr and was mounted in the cell shown in Fig. 2. Concentrations of KCl solution on both sides of the membrane were maintained at C_1 and C_2, respectively. The cell contained two pairs of electrodes; one pair was Ag–AgCl plate electrodes (*A* & *B*) for current supply, and the other was Ag–AgCl wire electrodes (*C* & *D*) for potential measurements. A couple of glass tubes (*E* & *F*), were attached to each compartment as shown in Fig. 2, through which was introduced the pressure difference applied across the membrane. Compressed air was used to produce the pressure difference, which was measured by a manometer.

A block diagram of the circuit used for measurements of electrical properties of the membrane is also shown in Fig. 2. The potentiometer was adjusted to provide a desired voltage difference across the membrane. The voltage amplifier for the potential measurements was a follower type, and the input impedance of the amplifiers was sufficiently high in comparison with that of the membrane. Both voltage and current amplifier outputs were recorded by an X-Y recorder and stored in a memory scope.

The electrolyte used was KCl, which was purified by repeated crystallization. The water used as solvent was prepared by redistillation of ion-exchanged water. The pH value of the salt solution was 5.5 throughout the present study. All measurements were carried out in an air chamber regulated at 27 ± 1 °C.

1 S. Morita, *private communication.*

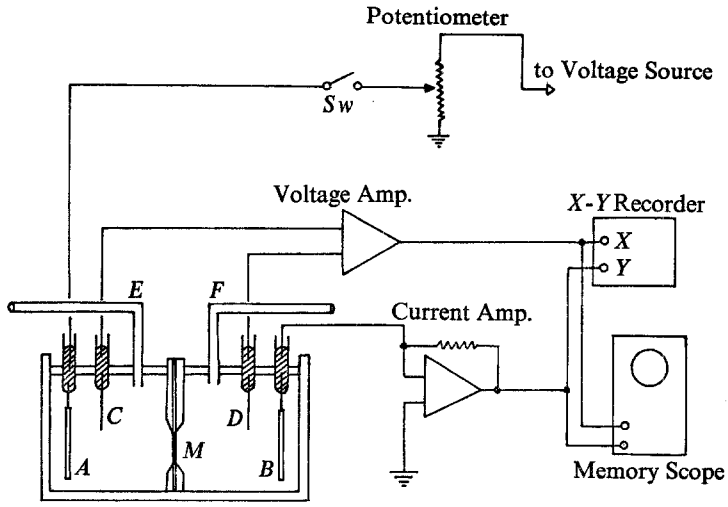

Fig. 2. A block diagram of the measuring circuit and the cell used. *A* and *B*: Ag–AgCl plate electrode for current supply; *C* and *D*: Ag–AgCl wire electrode for measuring the potential difference across the membrane; *M*: membrane; *E* and *F*: glass tubes through which compressed air pulses are sent to provide the pressure difference across the membrane as necessary; Potentiometer: Helipot precision potentiometer, 50 kΩ (Beckman-Toshiba, Ltd., Tokyo) to provide a desired voltage across the membrane

Results and Discussions

Potential Response when the Concentration of the External Solution is Suddenly Changed

First, a sample membrane was immersed in a 1-mM KCl solution and then the membrane was placed between two cells into which solutions of 7.8 mM and 62.5 mM KCl were poured. The results in Fig. 3 show

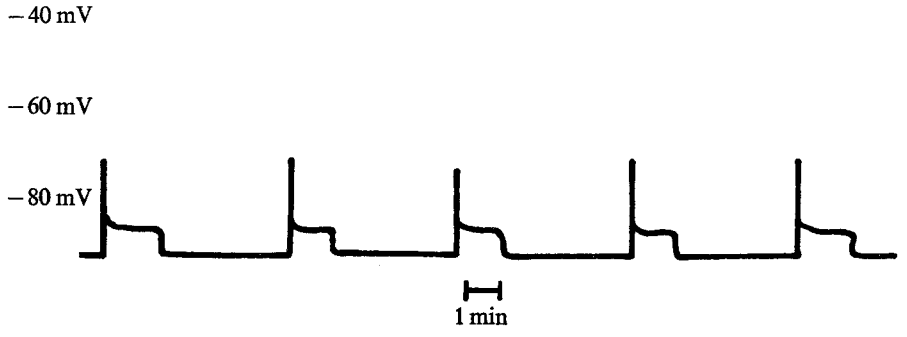

Fig. 3. Effect of the sudden change of the salt concentration. Spontaneous firing of the emf across the membrane. Initial salt concentration, 1 mM/1 mM KCl. Final salt concentration, 7.8 mM/62.5 mM KCl

spontaneous firing of spikelike variations of the emf (at zero current) across the membrane. In this case, $Q=2.78$ mg/cm^2, the spike magnitude is about 20 mV, and the duration of each spike from peak value through the saddle to zero extends for about 1 min. The duration and the height of the spike depend on the magnitude of the variation of the concentration, while the profile of the spikes does not. Such oscillation occurs only under conditions of $C_1 < C_t < C_2$. It is evident that the oscillation of the membrane potential comes from the change in the conformation of the membrane. For comparison with the succeeding sections, studies in the case of pulse-like change of the salt concentration are desirable. This process, however, is very difficult and not carried out.

Current Response when External Voltage is Applied Across the Membrane

In the next step, the membrane was placed between the cells with the same salt concentration of 15.6 mM, which is slightly lower than the C_t value of 40 mM. Then the effect of the applied voltage was studied. Fig. 4 shows an example of the oscillation of the electric current observed under a fixed applied voltage where a $Q=2.69$ mg/cm^2 membrane had been placed between the cell with the conditions described above. When the external

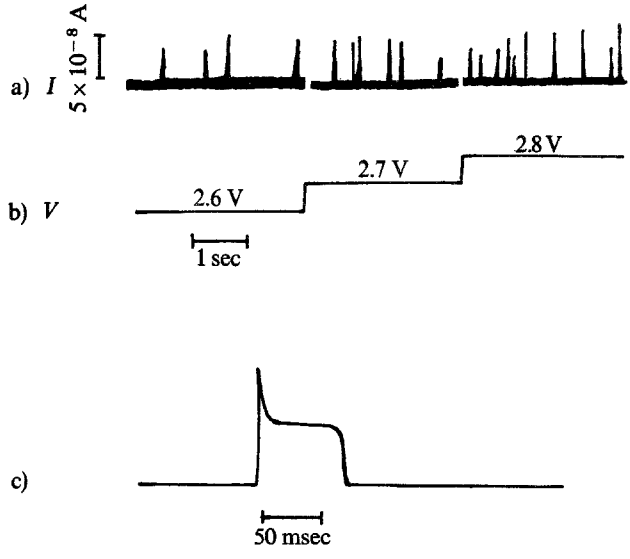

Fig. 4. Oscillation of the electric current observed in the DOPH/Millipore filter membrane. (*a*) Current response to the stepwise applied voltage. (*b*) Form of applied voltage. (*c*) Detailed form of the response pulse. Membrane whose $Q=2.69$ mg/cm^2 is immersed in a 15.6 mM KCl solution

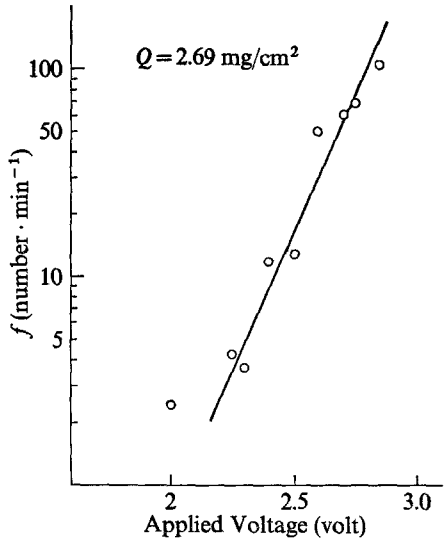

Fig. 5. Relation between the number of current spikes per unit time f, and applied voltage V where f is plotted on the logarithmic scale. Membrane whose $Q=2.69$ mg/cm^2 is immersed in 15.6 mM KCl

voltage was increased to a certain value (2.0 V for this specific case), spontaneous firing of the current spikes was observed. The critical voltage, above which the current spikes appeared, is denoted by V_c. The form of each current spike is shown in the lower half of Fig. 4. As seen in the figure, the form of a current spike is the same as that found in Fig. 3, but the duration of the pulse is only about 50 msec. compared with about 1 min. With an increase of the external voltage, the number of spikes occurring in unit time f is increased. Fig. 5 shows the relation between $\log f$ and V for the system of Fig. 4.

More unstable states of the membrane under the condition $C_1 < C_t < C_2$, were studied. Relations between voltage and current were measured. Fig. 6 shows an example of I-V relation for a membrane where $Q=2.26$ mg/cm^2 was placed between 125 mM and 1 mM KCl solutions. A similar I-V relation was observed for the other combination of salt concentration and a membrane with a different Q. In Fig. 6, the positive direction of the applied voltage is such that the electric current is passed through the membrane from the concentrated to dilute solution side. As seen in Fig. 6, when V is smaller than V_c, current I is porportional to V, and the electric resistance is calculated to be $9.3 \times 10^6\ \Omega\,\text{cm}^2$, which is equal to that of state A in Fig. 1. When V was increased to V_c, 2.1 volts in this specific membrane, many current spikes were observed. If V rises to a certain value V_b (3.3 V

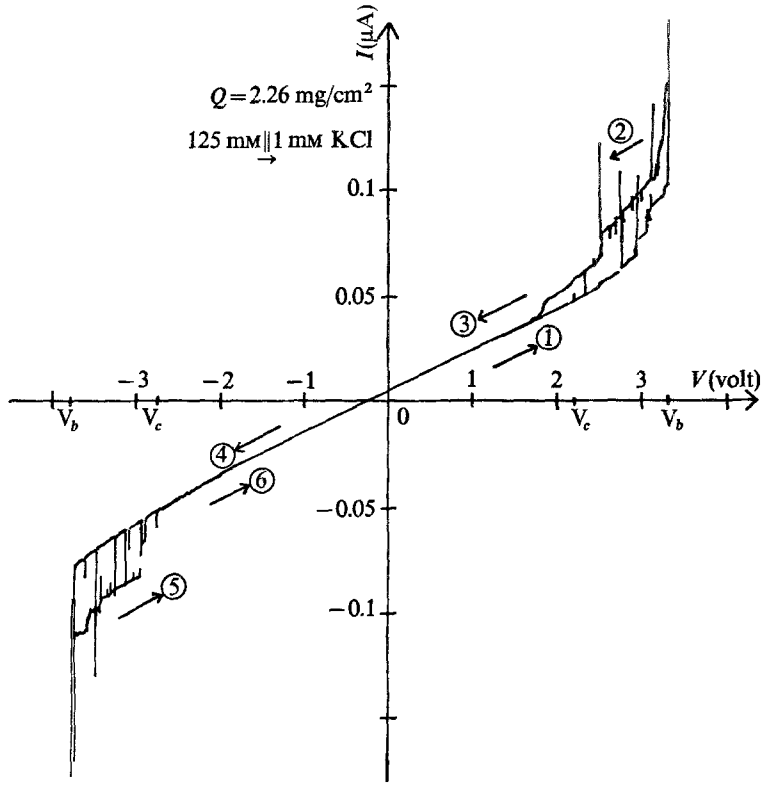

Fig. 6. I-V relation for a membrane whose $Q=2.26$ mg/cm^2 was placed between 125 mM and 1 mM KCl solutions. The applied voltage was changed successively following ① → ② ⋯ → ⑥. The significance of V_c and V_b are referred to in the text

for the system presented in Fig. 6), current I increases indefinitely, just as subunits in the membrane burst. If the applied voltage is decreased from this value, subunits are reformed and current decreases with V at a low electric resistance and shows an appreciable hysteresis loop. As seen in the figure, the current shows negative spikes in the case of a membrane having low resistance; i.e., the current decreases transiently in spikelike fashion. However, the external voltage V is approximately decreased to V_c and the system returns to the original high-resistance state, where the oscillation of current ceases. A similar hysteresis loop in the I-V relation is also observed when the direction of the field of the external voltage is reversed. Critical voltage V_c and the number of current spikes per unit time in the negative field differ from those observed in positive V. However, the shape of the current spike depicted in Fig. 3 is not altered.

It may be reasonable to consider that the generation of current spikes during a constant applied voltage is related to the variation of DOPH conformation in the Millipore filter. As is well known, an applied electric field accelerates the thinning process in the formation of a phospholipid bilayer leaflet [3]. The thinning process due to external electric field depends strongly on the external salt concentration. Therefore, it is said that the current spikes observed in Figs. 4 and 6 may be attributed to the conformational change of DOPH molecules due to the applied voltage in the membrane.

Discrete Variation of the emf after an External Electric Stimulus

The hysteresis loop in I-V relation observed in Fig. 6 indicates that the membrane is transformed to a low resistance state when the membrane is subjected to a high external voltage $V(>V_b)$, and stays in that state until the applied voltage is diminished to less than V_c. This fact implies that the membrane returns from the metastable state to the original state with a relaxation time when the external voltage $V(>V_b)$ is cut off. Fig. 7 shows some typical examples of the potential variation in response to an external electrical stimulus. Fig. 7*a* shows the case where the external voltage $V(>V_b)$ is applied across the membrane from the concentrated to the dilute solution side for a membrane where $Q=2.85\ \mathrm{mg/cm^2}$ is placed between 1 mM and 125 mM KCl solutions. Different combinations of membranes with other Q values and salt concentrations show essentially a similar potential response, as long as the voltage exceeds V_b. Fig. 7*b* shows the case in which a membrane whose $Q=2.38\ \mathrm{mg/cm^2}$ is placed between 1 mM and 15.6 mM KCl solutions and where the external voltage is applied from the dilute to the concentrated solution side. It is noted that the polarities of the membrane potential generated are the same in both Fig. 7*a* and *b*, irrespective of the direction of the applied field. As seen in Fig. 7*a* and *b*, the height of the peaks of the generated potentials are about 100 and 40 mV, respectively. These emf variations correspond to the difference in the membrane potential of two steady states, *A* and *B*, observed in Fig. 1 for the respective combination of concentrations, indicating that the membrane is transiently transformed from state *A* to *B* by the external electrical stimulus.

Fig. 7*c* shows the time course for the voltage response when applied voltage V is smaller than V_b. It is noted that the potential decays exponentially when the external voltage is turned off. The time constant of this decay curve is calculated to be about 1 sec.

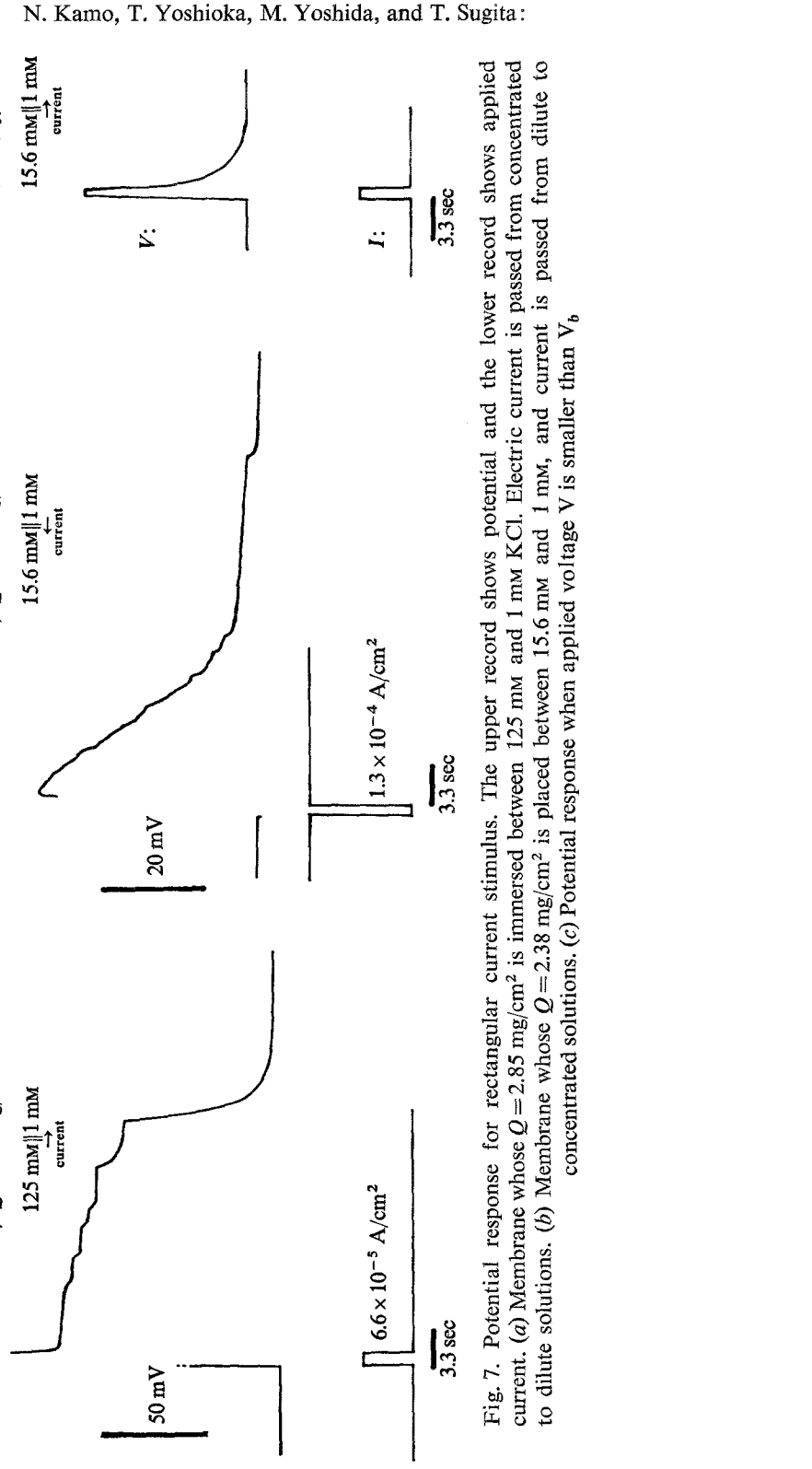

Fig. 7. Potential response for rectangular current stimulus. The upper record shows potential and the lower record shows applied current. (*a*) Membrane whose $Q = 2.85$ mg/cm^2 is immersed between 125 mM and 1 mM KCl. Electric current is passed from concentrated to dilute solutions. (*b*) Membrane whose $Q = 2.38$ mg/cm^2 is placed between 15.6 mM and 1 mM, and current is passed from dilute to concentrated solutions. (*c*) Potential response when applied voltage V is smaller than V_b

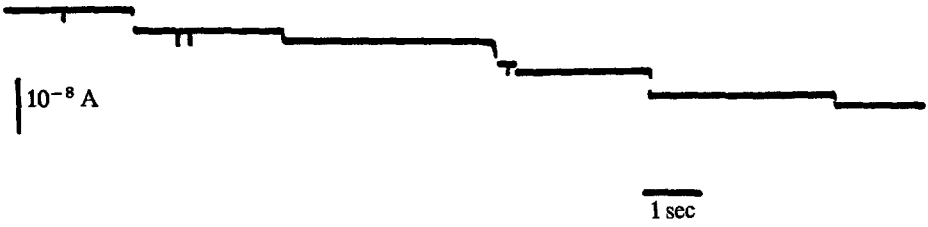

Fig. 8. After a current stimulus depicted in Fig. 7*a* was applied, electrode *A* in Fig. 2 was connected to the ground and step-wise variation of emf obtained in Fig. 7*a* was observed as the variation of current which was passing through the membrane. These observations were carried out to shorten the time constant of measuring circuits

As seen in Fig. 7*a* and *b*, the emf generated by an external stimulus shows a stepwise decrease with time. When the time scale is enlarged, Fig. 8 is obtained. The depth of each decrease of the potential is almost a given quantum or an integral multiple of it (*cf.* Fig. 8). A similar discrete decrement of the electric potential or current was observed by Bean, Shepherd, Chan and Eichner [1] and by Ehrenstein, Harold and Ralph [2] in the system of a lipid bilayer membrane with a small amount of excitability inducing material (EIM) being added on one side of the membrane. This fact suggests that the emf and/or the electric resistance of the subunit in the membrane changes independently. The observed membrane potential reflects the discrete event occurring in the small domain. In the case of the present Millipore-DOPH membrane, the subunit is considered to be the small DOPH membrane formed in the void space in the filter paper. It is worthwhile to point out that the discrete variation of the electrical properties is not specific for black lipid membrane with EIM.

Transient Variation of the Membrane Potential due to Pressure Stimulus

Consider a system in which a Millipore-DOPH membrane separates two electrolyte solutions of 125 and 1 mM KCl. The steady membrane potential was -100 mV, which is the largest negative emf observable for the external salt condition under consideration. When a rectangular hydrostatic pressure difference is applied across the membrane, so that the concentrated solution flows into the membrane, the membrane potential increases and reaches about 0 mV after a small time lag. Then the membrane potential recovers gradually to the original value, as shown in Fig. 9. The membrane potential change due to the stimuli (about 100 mV) is equal to that observed in Fig. 7*a*, where the external salt concentrations are the same as shown. It is noted that the variation of the emf decreases

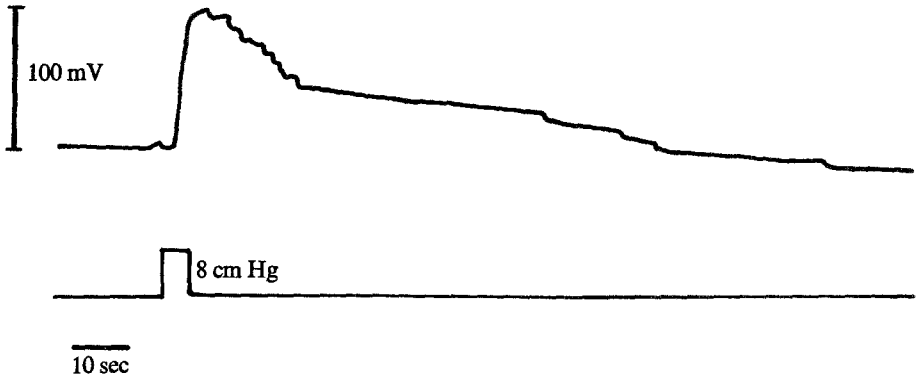

Fig. 9. Generation of action potential due to hydrostatic pressure stimuli. Membrane whose $Q = 2.34$ mg/cm^2 was placed between 125 mM and 1 mM KCl. Hydrostatic pressure was applied across the membrane so that the concentrated solution flowed into the membrane (lower line). The upper line shows the emf time duration. Initial membrane potential was -100 mV

stepwise or in discrete manner with time. No transient emf variation can be observed when the concentrations of both external solutions are lower than C_t. Therefore, the change in the emf for the external pressure stimulus is apparently due to the transformation of the conformation of DOPH molecules in the filter paper. The result is in harmony with the variation of the membrane potential due to salt concentration observed in Fig. 1. The reason for the discrete variation of the emf shown in Fig. 9 may be the same as the reasons, discussed in connection with Fig. 7, that the transformation of DOPH occurring independently in a small domain is responsible for the discrete variation of the potentials.

Concluding Remarks

At present, the detailed molecular mechanism for the oscillatory response of current and voltage in the Millipore-DOPH membrane is not clear, but it is feasible that these transient phenomena come from the movement of DOPH molecules or from a change of their conformation in the filter paper. If appropriate external and boundary conditions are selected, it is not very difficult to produce various functions of a biological membrane using this relatively simple model membrane. The conformational change of lipid molecules in the membrane may be considered to be sufficient conditions for the characteristic electrical stimulus-response relationship observed in living tissues.

Similar phenomena to those reported here have already been described with various model membranes; e.g., lipid bilayer membrane with EIM [2, 6], with linseed oil spread on the surface of an oxidizing solution [5] and Teorell's electrohydraulic model [7]. The molecular mechanisms of these different membranes may differ from each other. It is certainly an interesting future work, however, to seek the underlying physical principle which governs these transient processes observed in various model systems, as well as in living tissues.

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