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SELF-EXCITATION IN A POROUS MEMBRANE DOPED WITH SORBITAN MONOOLEATE (SPAN-80) INDUCED BY AN Na^+/K^+ CONCENTRATION GRADIENT

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The electrical potential across a fine-pore membrane doped with sorbitan monooleate (Span-80) imposed between aqueous solutions of NaCl and KCl was studied. It was found that this system showed rhythmic and sustained oscillations of electrical potential between the two aqueous solutions. These oscillations were attributed to the change of permeability of Na^+ and K^+ across the membrane, which originated from the phase transition of Span-80 molecules within the fine pores. Impedance measurement across the membrane also suggested a change in permeability. It was found that this membrane exhibited the property of differential negative resistance. In relation to this, it was shown that Na^+ and K^+ have different effects on the aggregation of Span-80 molecules. The mechanism of oscillation is discussed in relation to the ability of Span-80 molecules to behave as a dynamic channel through the membrane. This oscillatory phenomenon is interesting because in biological nervous membranes a difference between the concentrations of Na^+ and K^+ across the membranes is essential for excitability.

1. Introduction

One of the most interesting phenomena in biological systems is excitability. There is a considerable amount of literature dealing with electrical phenomena associated with excitation in biological membranes, and it has been well established [1] that differences in the compositions of inorganic cations, especially Na^+ and K^+, inside and outside the membranes are essential for excitability.

Attempts have been made to develop suitable excitable artificial membranes for studies on the mechanism of nerve excitation. Teorell [2] reported that a sintered glass filter showed excitability under an external pressure. Kobatake [3] found that a porous membrane doped with dioleyl phosphate, a so-called DOPH-Millipore membrane, showed spontaneous firing of electrical potential when placed between solutions of different KCl concentrations [4]. Mueller [5,6] observed rhythmic change of membrane potential under an external current in a bilayer membrane containing an unidentified proteinaceous material obtained from *Enterobacter cloacae.* Furthermore, Monnier [7] demonstrated that lipidic membranes exhibit electrical oscillations on application of an external voltage or electrical current.

Considerable attention has been directed to channels of excitable membranes in reconstituted systems. Under an external voltage (voltage-clamp method [1]), bi-stability or multi-stability of the electronic state has been observed in these reconstituted membranes [8–11]. Recently, we [12,13] showed that spontaneous electrochemical oscillations can be produced in a liquid membrane consisting of an oil layer, a solution of picric acid in nitrobenzene, imposed between two aqueous phases with different chemical compositions. However, there are no reports of self-excitation or spontaneous oscillation in an artificial membrane induced by an Na^+/K^+ concentration gradient with the exception of our previous report [14], which showed that rhythmic and sustained oscillations of electrical potential occurred acrosss a fine-pore membrane doped with glycerol α-monooleate and separating aqueous solutions of 0.5 M NaCl and 0.5 M KCl. As an extension of this preliminary study [14], in the present paper we wish to report that spontaneous firing occurred in porous membranes doped with Span-80 imposed between aqueous solutions of NaCl and KCl in the absence of any external stimulus such as pressure, voltage or electrical current. The spontaneous oscillations observed in our artificial membrane by the concentration difference of Na^+/K^+ are essentially the same as those in nervous membranes.

2. Materials and methods

The apparatus used in this study is shown schematically in fig. 1. Porous polytetrafluoroethylene filter paper (PF-2) of 10 μm nominal pore size and 0.5 mm thickness was obtained commercially from Toyo Roshi, Tokyo. Porous triacetylcellulose filter paper (FM-22) of 0.22 μm nominal pore size and 135 μm thicknnes was obtained from Fuji Film, Tokyo. The filter papers were soaked in Span-80 for 72 h. The quantity of Span-80 absorbed was adjusted to about 20 mg/cm^2 for the polytetrafluoroethylene filter paper and 9 mg/cm^2 for the triacetylcellulose filter paper.

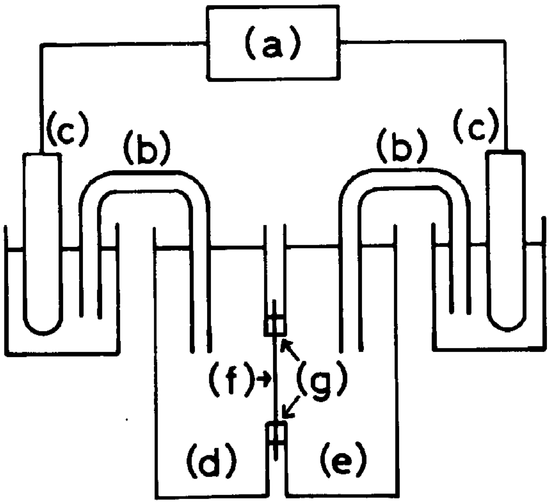

Fig. 1. Diagram of the experimental apparatus. (a) Millivoltmeter, (b) KCl salt-bridge, (c) Ag/AgCl electrode, (d) 0.5 M KCl aqueous solution (7 ml), (e) 0.5 M NaCl aqueous solution (7 ml), (f) membrane, (g) silicon rubber gasket having a bore of 8 mm diameter.

All measurements were carried out at 28 ± 1°C. The voltage across the membrane was monitored with a Hitachi-Horiba F-7 pH/mV meter connected by two salt bridges to two Ag/AgCl electrodes. All reagents were commercial products of analytical grade. Impedance of the membrane was measured with a Vector-Impedance Meter, Hewlett Packard model 4800A. The signal level was about 270 mV r.m.s. Transmittance of light at 700 nm was measured in a cell of 10 mm light path with a Hitachi 330 spectrometer at 28°C after incubation for 30 min at 60°C. PVC (polyvinyl chloride) membranes containing Span-80 were prepared as follows. A solution of 0.4 g Span-80 and 0.17 g PVC (Wako; average molecular weight, about 69000) in 6 ml tetrahydrofuran was prepared and spread on a flat glass plate. The flat membrane formed after 6 h at 20°C could be removed easily from the glass plate. Measurement of electrical current under constant d.c. voltage was performed with a picoammeter, Takeda Riken TR-8641, and a d.c. voltage-current supplier (Yokogawa, YEW type 2555).

3. Results and discussion

3.1. Oscillation across the membrane

Typical types of oscillation of the electrical potential are shown in fig. 2, where an upward change indicates an increase in the positive charge in the KCl solution. The oscillations started abruptly 10–120 min after the filter paper (polytetrafluoroehylene filter paper in fig. 2a and b) and triacetylcellulose filter paper in fig. 2c)) was placed between the solutions of NaCl and KCl. In fig. 2a the amplitude of the pulse was approx. 20 mV and the period was about 30 s. Fig. 2b shows transitions between multi-steady states, i.e., step-wise changes in potential. Fig. 2c shows that upward

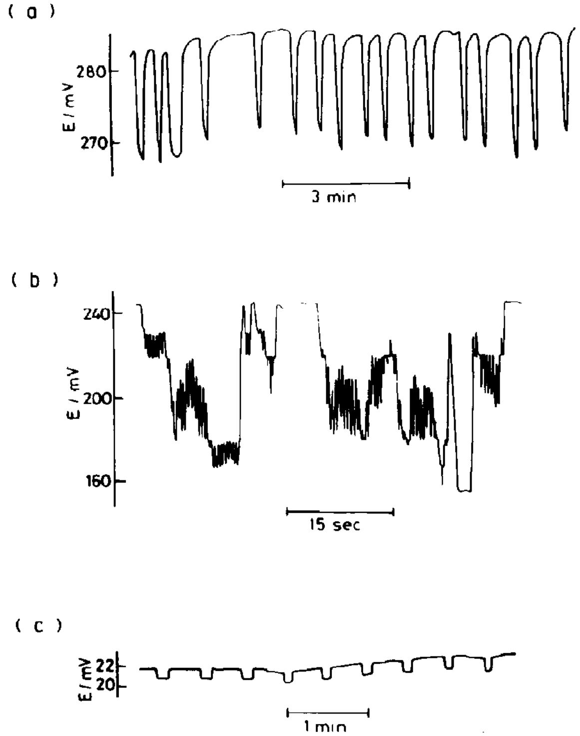

Fig. 2. Oscillations of electrical potential between the two aqueous phases. On the recording of the electrical potential, an upward change denotes an increase in the positive charge in the KCl solution. (a, b) Polytetrafluoroethylene filter paper with 10 μm nominal pore size and 0.5 mm thickness was used. The aqueous phases were 0.1 M NaCl and 0.1 M KCl solutions. (c) Triacetylcellulose filter paper with 0.22 μm nominal pore size and 135 μm thickness was used. The aqueous phases were 0.5 M NaCl and 0.5 M KCl solutions.

and downward, flip-flop, changes of the electrical potential were generated. Spike-like changes of the potential, as shown in fig. 2a and b, were observed most frequently when polytetrafluoroethylene filter paper was used. On the other hand, a flip-flop change of potential was generated on use of triacetylcellulose filter paper, in which both the pore size and the thickness are less than in polytetrafluoroethylene filter paper (see section 2). It is also noteworthy that the magnitude of the oscillations was greater at lower concentrations of 0.1 M KCl and NaCl in fig. 2a and b than of 0.5 M in fig. 2c.

These oscillations could be attributed to periodic gating, or closing, of 'channels' in the membrane. It should be stressed that these channels were formed by Span-80 molecules in the absence of any peptide or protein.

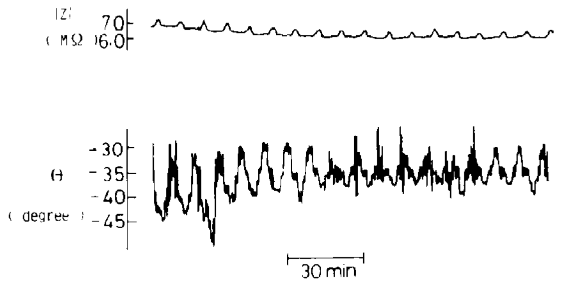

Fig. 3. Oscillations of impedance (10 kHz). Change of absolute value of impedance $|Z|$ and phase angle θ are shown. Experimental conditions were the same as in fig. 2c.

Further information on the oscillations was obtained by measurement of transmembrane impedance. Fig. 3 shows the periodic changes in the absolute value of impedance, $|Z|$, and phase angle, θ. It is noted that the impedance changed rhythmically in a manner similar to that of the electrical potential in fig. 2c. This periodic change of impedance again indicated that the porous membrane doped with Span-80 had the chracter of a 'dynamic channel'.

The electrical potential, $\Delta\psi$, between the two aqueous phases in a membrane is considered as the algebraic sum of the two phase-boundary potentials on the right and left interfaces and the diffusion potential within the organic phase. According to Gouy-Chapmann, the phase-boundary potentials are important only for a 'charged' membrane [15], but as Span-80 has no 'charged' functional group, the observed potential, $\Delta\psi$, is attributable mainly to the diffusion potential. Experimentally, the KCl solution was positive with respect to the NaCl solution throughout the measurements, as shown in fig. 2a–c. The observed potential difference should, therefore, originate mainly from the diffusion potential induced by the greater mobility of Na^+ than of K^+. In contrast, the mobility of Na^+ is known to be less than that of K^+ in aqueous solution, because Na^+ is more hydrated than K^+; in other words, Na^+ is more hydrophilic than K^+. In this context, it is interesting to note that some specificity in the permeability of monovalent cations has been observed in lipid membranes. Hopfer et al. [16] reported that a bilayer membrane made from diglycosyl diglyceride shows cation selectivity, i.e., the mobility of Na^+ is greater than that of K^+. Recently, Young et al. [17] reported that the permeability of Na^+ through a lipid membrane was increased by the addition of Tween 80, a neutral surfactant having alcoholic hydroxyl groups.

3.2. Photometric measurements of Span-80

To determine the mechanism of oscillation, or 'switching', of the membrane further, we investigated the aggregation of Span-80 molecules on change in contents of water, KCl and NaCl by photometric measurements. Fig. 4 shows the change of transmittance of light of 700 nm by Span-80 in the presence of various amounts of (a) 0.5 M NaCl aqueous solution, (b) 0.5 M KCl aqueous solution, and (c) distilled water. Decrease in transmittance represents increased emulsification of the sample. In all cases in fig. 4, the transmittance decreased abruptly when the concentration of water increased to approx. 0.8 M. The most interesting phenomenon seen in fig. 4 is the phase transition near 0.3 M water. The transmittance increased abruptly at this concentration of NaCl or KCl solution, but no increase was observed when pure water was added to Span-80. This result indicates that the manner of the phase transition is markedly affected by the presence of NaCl or KCl. Fig. 5 shows the change of transmittance of light of 700 nm by Span-80 in the presence of 0.3 M water with various amounts of NaCl and KCl. It should be noted that the phase behaviors with NaCl and KCl were different. These results suggest that the oscillation, or dynamic switching, observed in the porous membrane is closely related to the phase transition of Span-80 molecules present within the pores.

It is well established [18,19] that the nonmonotonic behavior, or nonlinearity, of a system is concerned with the mechanism of 'feedback', which is essentially important for inducing oscillatory phenomena. Thus, the rhythmic change of the potential shown in fig. 2 may be related to this nonmonotonic behavior of Span-80 molecules.

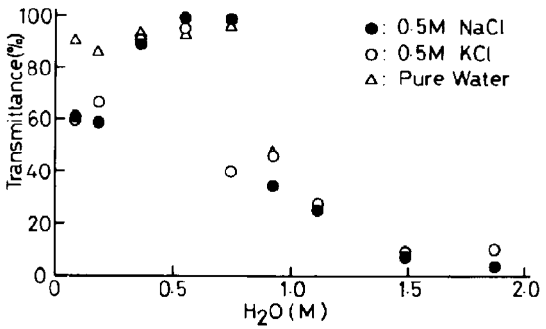

Fig. 4. Change of transmittance of 700 nm light in Span-80 with the addition of varying amounts of (a) 0.5 M NaCl aqueous soution, (b) 0.5 M KCl aqueous solution, (c) distilled water in a cell of 10 mm light path.

3.3. Mechanism of the electrical oscillation.

Based on the above results, we propose a mechanism of transition between two states with different potential levels, (I) and (II).

(I) Stage of high electrical potential with closed channels. In this stage, the Span-80 molecules fill the pores of the membrane as shown in fig. 6a, and the permeability of Na^+ is greater than that of K^+, although the permeabilities of both are low. The Na^+ gradually penetrates into the pores, inducing a diffusion potential. Simultaneously,

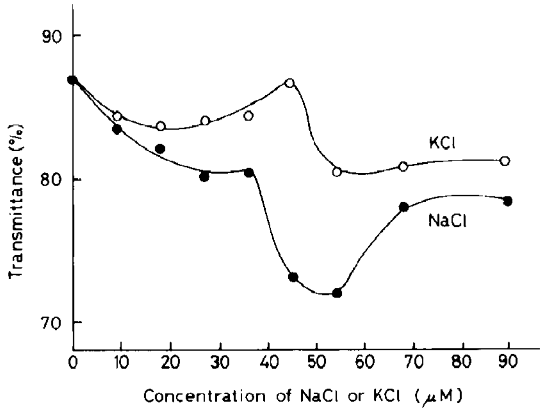

Fig. 5. Change of transmittance of 700 nm light in Span-80 in the presence of 0.3 M water with the addition of varying amounts of NaCl (●——●) and KCl (O——O) in a cell of 10 mm light path.

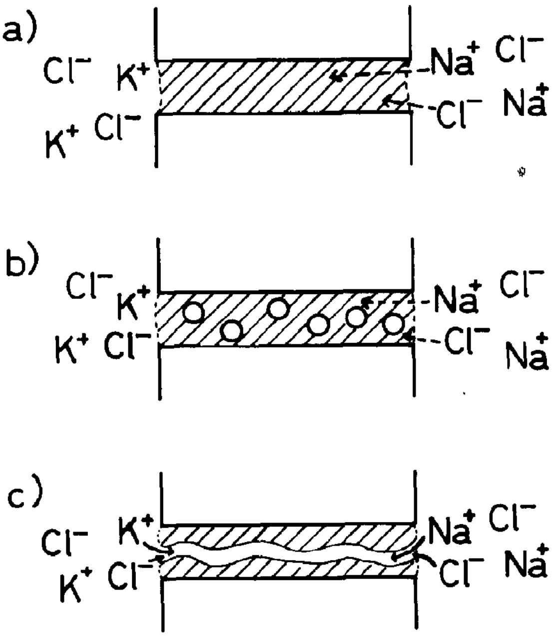

Fig. 6. Schematic representation of the mechanism of oscillation. Repeated change such as a → b → c → a, may occur.

molecules of water together with Cl^- move toward the oil phase, forming small droplets within the pores. Thus, W/O (water in oil) emulsification occurs as shown in fig. 6b.

(II) Stage of low electrical potential with open channels. When W/O emulsification reaches a critical state, the oil phase within the pore exhibits an abrupt change from b to c in fig. 6. In this stage, the permeabilities of Na^+ and K^+ increase, reducing the membrane potential. When the content of water in the pore reaches a critical value, the organic phase in the pore changes abruptly to the state in fig. 6a, excluding water molecules together with NaCl and KCl from the pore, and the permeabilities of the inorganic ions again become low. The results in figs. 4 and 5 support this mechanism. The state of Span-80 molecules in the pore thus changes repeatedly: I → II → I → II.

Phase transitions of nonionic surfactants have frequently been observed, and are called W/O–O/W transitions [20]. When the stabilities of the two states are nearly equal, a flip-flop type oscillation (fig. 2c)) may be observed, whereas when the stability of the state of high electrical potential isless than that of low electrical potential, spikes of potential may occur (fig. 2a)). When there are many pores exhibiting gating, transitions between multi-states of the potential may be seen as in fig. 2b.

3.4. Switching on of the channel

We examined the electric property of a porous membrane doped with Span-80 by monitoring change of electric current through the membrane on abrupt application of an external voltage (measurement by the 'voltage-clamp' method [1]). The change of the current when an external voltage of 500 mV was suddenly applied to the membrane is shown in fig. 7. Here the concentrations of NaCl and KCl were 0.05 M. These concentrations were chosen because when the concentrations of NaCl and KCl were decreased to 0.05 M the membrane became static, i.e., no oscillation or fluctuation of the membrane potential was observed. Fig. 7 shows that the conductance of the membrane decreased abruptly after application of the external voltage,

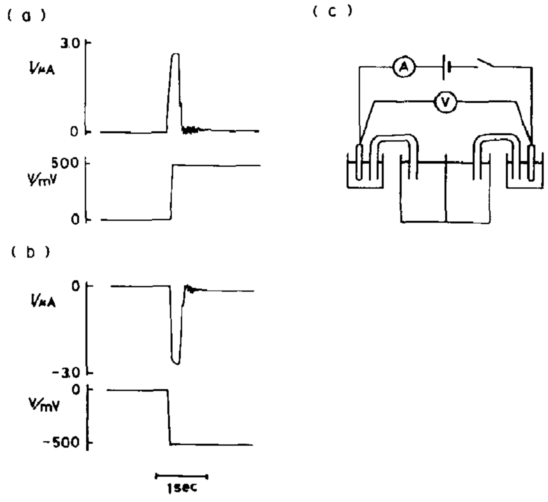

Fig. 7. Change of electrical current through a membrane with the abrupt application of an external voltage, from 0 to 500 mV. (a) +500 mV was applied to the KCl solution, (b) +500 mV was applied to the NaCl solution, (c) schematic representation of the electric circuit to measure the change of current and voltage.

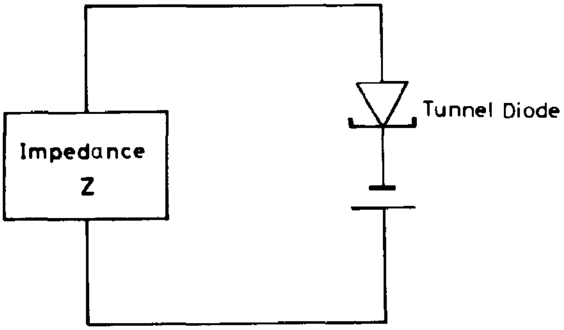

Fig. 8. Self-excitable electric circuit with a tunnel diode.

indicating that this membrane exhibits the property of "differential negative resistance'. In relation to this, it is interesting to note that an electric circuit (fig. 8) with a tunnel diode or Esaki diode, possessing the property of differential negative resistance, and with the appropriate complex impedance shows self-excitability [21]. The potential induced by an Na^+/K^+ concentration gradient in the porous membrane corresponds to the external d.c. voltage in the circuit. It should also be borne in mind that the impedance Z of the porous membrane is complex; i.e., $Z = |Z|\cos\theta + i|Z|\sin\theta$, where $|Z|\cos\theta = R$ and $|Z|\sin\theta = X$ are the resistance and reactance, respectively, as shown in fig. 3. Thus, the doped porous membrane between NaCl and KCl solutions seems to constitute an equivalent circuit as in fig. 8.

3.5. Oscillation in a PVC membrane

We also tried to develop a membrane showing self-excitability. Various types of artificial membranes such as a collodion membrane containing Span-80 and a dried oil membrane [7] were examined, and finally we found that a solid membrane of PVC containing Span-80 exhibits self-excitability. Fig. 9 shows that periodic pulses of potential across the PVC membrane were generated in the presence of an Na^+/K^+ concentration gradient, though the amplitude of the pulses was only approx. 0.1 mV. It is interesting that this oscillatory phenomenon could be observed not only in a porous membrane but also in a solid membrane on doping with Span-80.

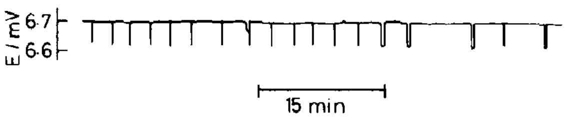

Fig. 9. Oscillations of electrical potential between 0.1 M NaCl and 0.1 M KCl aqueous solutions in a solid PVC membrane containing Span-80.

3.6. Comparison with biological excitable membranes

Rhythmic potential fluctuations and/or transient pulses, generally called excitations, have been observed in many cells ranging from algae and protozoa to nerves and muscles. Excitability in biological membranes is generated by a membrane potential that is induced by concentration gradients of inorganic ions, especially between Na^+ and K^+, across the membranes. The steady-state and kinetic relations between the membrane potential and individual ionic currents were analyzed in detail by Hodgkin and Huxley [1], and the way in which the relations lead to excitation are now well understood. However, little is known about the molecular aspects of excitation or its relation to changes in permeability of inorganic ions. It is generally thought that ions cross the membrane through channels embedded in the lipid bilayer membrane in localized regions, and that the channels are somewhat gated, i.e. opened or closed, by the membrane potential. However, details of the gating mechanism at a molecular level are unknown.

In the present study we showed that an Na^+/K^+ concentration gradient could cause excitation in an artificial membrane. This result is important in relation to the mechanism of excitation of biological systems at the molecular level and may also be significant in the field of 'membrane-mimetic chemistry' [22].

4. Conclusion

In the present study we showed that self-excitation of a porous membrane doped with Span-80 was induced by an Na^+/K^+ concentration gradient without any external stimulus such as pressure, voltage or electrical current. We deomonstrated that molecules of Span-80 behave as dynamic channels in the absence of any proteinaceous substance. This finding is important because it has been believed that proteinaceous substances, such as the acetylcholine receptor, are needed for excitability of a membrane.

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