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# STUDIES OF EXCITABLE MEMBRANE FORMED ON THE SURFACE OF PROTOPLASMIC DROPS ISOLATED FROM *NITELLA*

## IV. EXCITABILITY OF THE DROP MEMBRANE IN VARIOUS COMPOSITIONS OF THE EXTERNAL SALT SOLUTION

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SUMMARY

A protoplasmic drop isolated from an internodal cell of *Nitella* became electrically excitable in a solution composed of 0.5 mM $NaNO_3$, 0.5 mM $KNO_3$, 1 mM $Ca(NO_3)_2$ and 2 mM $Mg(NO_3)_2$. This solution is referred to as the "testing solution". The electrophysiological properties of the drop membrane were examined in various ionic compositions, in which each salt species was changed while the other salt composition was kept the same as that in the testing solution. The results are summarized as follows.

(1) The $Na^+$ concentration could be changed between zero and about 20 mM with no loss of excitability. The resting potential changed from −90 mV to −70 mV gradually, while the membrane resistance remained about 2 $k\Omega \cdot cm^2$ in this $Na^+$ concentration range. When the $Na^+$ concentration exceeded 20 mM, the intracellular potential was depolarized discontinuously by more than 50 mV, and the resistance was reduced to 0.2–0.5 $k\Omega \cdot cm^2$. In the depolarized state, the drop membrane showed a hyperpolarizing response.

(2) The concentration of $K^+$ also could be changed between zero and 20 mM preserving the excitability of the drops. The dependence of the electrical properties of the drop membrane on the $K^+$ concentration were almost the same as those for $Na^+$, except that a discrete change in the membrane potential was not observed at 20 mM of $K^+$ concentration.

(3) The $Ca^{2+}$ concentration could be changed between 0.1 mM and 10 mM without loss of excitability, where the intracellular potential stayed at approximately −90 mV. Below and above this $Ca^{2+}$ concentration range, the intracellular potential was reduced to about −20 mV.

(4) The excitability was retained in the range of $Mg^{2+}$ concentration between zero and about 20 mM. Higher than 20 mM of $Mg^{2+}$, the membrane potential changed sharply with increase of $Mg^{2+}$.

(5) The electrical properties of the drop membrane were not varied by replacing $NO_3^-$ with $Cl^-$ in the external solution.

(6) All the properties of the drop membrane described above were changed reversibly with the variation of salt compositions.

The implications of the experimental results are discussed in connection with the physical properties of the drop membrane.

## INTRODUCTION

In this series of papers we intend to study the structure and properties of an excitable membrane using protoplasmic drops isolated from an internodal cell of *Nitella*, anticipating that the molecular mechanisms underlying the process of excitation of living tissues may be clarified.

Study of protoplasmic drops was initiated by Kamiya and Kuroda in 1958[1]. They found that the protoplasm isolated from an internodal cell of *Nitella* formed a sessile drop in an aqueous salt solution, and suggested that a new thin membrane was formed on the surface of the drop[2]. In 1971, Inoue *et al.*[3] found that the protoplasmic drop was able to produce an action potential in response to an electrical stimulus when the external salt concentration was diluted appropriately. The semi-artificial excitable cell thus obtained has great advantages for studying the molecular mechanism of excitation in comparison with intact excitable tissues, since the functional surface membrane of the protoplasmic drop is contiguous to the external solution.

In Part I of this series, we discussed the structure of the surface membrane measuring the refractive index of the drop and studying enzyme actions[4]. It was suggested that formation of a lipid–protein complex in the membrane is essential for the appearance of the excitability. The measurement of the surface tension indicated that the structure of the membrane was affected very much by the ionic composition in the external solution (Part II)[5].

This paper describes the results of the electrophysiological properties of the membrane, *i.e.* the membrane potential, the membrane resistance and excitability are examined as functions of ionic composition in the external solution. The relation between electrophysiological or electrochemical properties of the drops and the structure of the surface membrane is discussed in the light of membrane functions.

## EXPERIMENTAL

### *Materials and drop formation*

Internodal cells of *Nitella flexilis* used in the present study and all procedures for isolation of the protoplasm were essentially the same as those employed throughout in this series of papers[4–6]. When the lower end of an internodal cell held vertically was amputated in a solution containing 80 mM $KNO_3$, 40 mM $NaNO_3$, and 5 mM $CaCl_2$, the protoplasm flowed down from the opening of the cell. Hereafter we shall refer to the salt solution described above as the "initial solution". The effused protoplasm formed a sessile drop of about 300 $\mu$m in diameter on the bottom of the vessel. After two or three drops were isolated from a cell, the external solution was replaced with a solution containing 0.5 mM $KNO_3$, 0.5 mM $NaNO_3$, 1 mM $Ca(NO_3)_2$ and 2 mM $Mg(NO_3)_2$, pH 6.4–6.8, adjusted by 1 mM Tris–acetate buffer. The osmotic pressure of the solution was kept isotonic with that of the intact internodal cell of *Nitella* by adding mannitol[4]. We shall call the second solution described above as the "excitability testing solution" or the "testing solution".

*Change of the salt composition in the external solution*

As was reported in Part I[1], the membrane potential and the electric resistance changed with time in the testing solution and approached their respective steady values within 1 h. After the membrane potential of the drop reached the steady value in the testing solution, the external salt composition was changed successively by perfusing the solution having a different ionic composition. When a denser solution was replaced by a lighter one, the latter solution entered the vessel from the upper inlet and flowed out from the lower outlet of the vessel, or *vice versa* (see Part II)[5]. The flow rate of the perfusion of the solution was approx. 2 ml/min. With this flow rate, the exchange of the whole external solution was completed within 1 or 2 min.

All solutions were kept isotonic by adding mannitol. The mannitol used was recrystallized from hot ethanol. Water used as solvent was distilled twice with pyrex receptacles.

*Measurements of electrical properties*

Two glass microcapillary electrodes filled with 3 M KCl were used as internal electrodes. One was used for recording the transmembrane potential and the other for current supply. Two microelectrodes were inserted into a drop by means of micromanipulators. For measurements and recordings of the transmembrane potential and the applied current the following instruments were used: a high input impedance pre-amplifier (Nihon Kohden, Type MZ-3B), a two-beams oscilloscope (Iwatsu, Type SS-5527), an oscilloscope camera (Nihon Kohden, Type PC-2B), a pen-writing recorder (Nihon Kohden, Type WI-260), and a home made feedback amplifier for current clamp (gain 60 db). For the external reference electrode, a calomel electrode with a salt bridge was used. A silver–silverchloride wire electrode (100 $\mu$m in diameter) was used for the external current supply. These external electrodes were placed as close as possible to the outlet of the perfusing fluid in order to eliminate the contamination of $Ag^+$ and of leaked KCl from the salt bridge (see Part I).

All measurements were carried out in an air chamber regulated at $20 \pm 1$ °C.

## RESULTS

The steady membrane potential of the drop in the testing solution stays either about $-50$ mV or $-90$ mV, the inside of the drop being negative. As pointed out in Part I, the state of $-90$ mV of the intracellular potential is more stable than that of $-50$ mV, and in most cases the membrane potential approached finally to $-90$ mV level. In the present studies we examined only the drops whose membrane potential reached to about $-90$ mV. The membrane resistances at the steady state in the testing solution were scattered between 1 and 5 $k\Omega \cdot cm^2$.

The concentration dependences of the properties of drop membrane were examined by changing the salt composition of the testing solution, where only one kind of cation species changed progressively with the other salt composition being kept as it stands in the testing solution, and the electrical properties, *e.g.* the membrane potential, electric resistance, and electrical excitability, were examined.

*Effects of $Na^+$ and $K^+$ concentrations*

It has been observed in various excitable living tissues such as intact *Nitella* internodes and nerves[7,8] that either an addition of $K^+$ or a decrease in $Ca^{2+}$ concen-

tration in the external solution induces a depolarization of the membrane potential, *i.e.* the intracellular potential is reduced from its resting value. The depolarization of the membrane potential by means of the external salt composition was frequently observed in an abrupt manner when the concentration changed successively[9,10]. Especially, in squid giant axons under internal perfusion with dilute Na or Cs salts, an addition of $Na^+$ in the external medium led to an abrupt transition of the membrane potential from the resting to the depolarized (or excited) state at a certain Na concentration[10,11]. An abrupt depolarization is also observed in the protoplasmic drops isolated from *Nitella*, and the results obtained are well compared with those of the excitable living cells.

Fig. 1 shows the experimental results, where the membrane potential and the membrane resistance are plotted as functions of the external $Na^+$ concentration. Here, as noted above, the other salt compositions except $NaNO_3$ were kept at the same level as in the testing solution. Different symbols in the figure refer to different drops examined. Both membrane potential and membrane resistance were not affected appreciably by the change in the external Na concentration from zero to about 10 mM (State A in the figure); that is, the membrane potentials stayed between $-90$ mV and $-70$ mV, and the resistances were 2–4 $k\Omega \cdot cm^2$, respectively. When the external Na concentration was raised to 10–20 mM, the membrane potential fluctuated appreciably and followed an abrupt depolarization by about 50 mV. At the same time, the membrane resistance decreased discontinuously about a factor of 10, *i.e.* the resistance became about 0.2 $k\Omega \cdot cm^2$. Fig. 2a shows a time course of an abrupt variation of the membrane potential when the external Na concentration changed from 10 mM to 20 mM. A further increase in the external Na concentration did not induce any further discrete change in the membrane potential. In State B in Fig. 1, the membrane potential changed about 20 mV with a 10-fold variation of Na concentration, and the membrane resistance stayed approximately constant between 0.2–0.5 $k\Omega \cdot cm^2$.

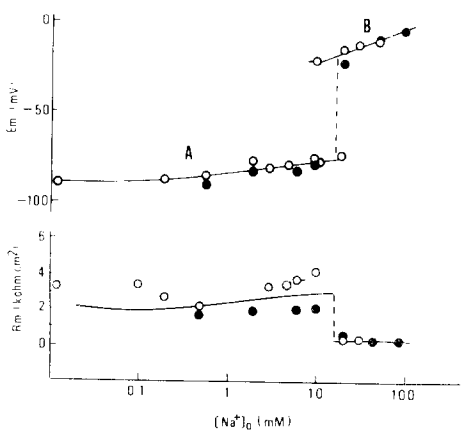


Fig. 1. Dependence of the membrane potential, $E_m$ (mV), and membrane resistance, $R_m$ ($k\Omega \cdot cm^2$), on $Na^+$ concentration in the external solution. Ionic composition other than $Na^+$ is the same as that in the testing solution (see text). Both $E_m$ and $R_m$ changed discontinuously at about 20 mM $[Na^+]_o$. Different symbols refer to different drop specimens. A and B in the figure indicate the resting and the depolarized states, respectively.

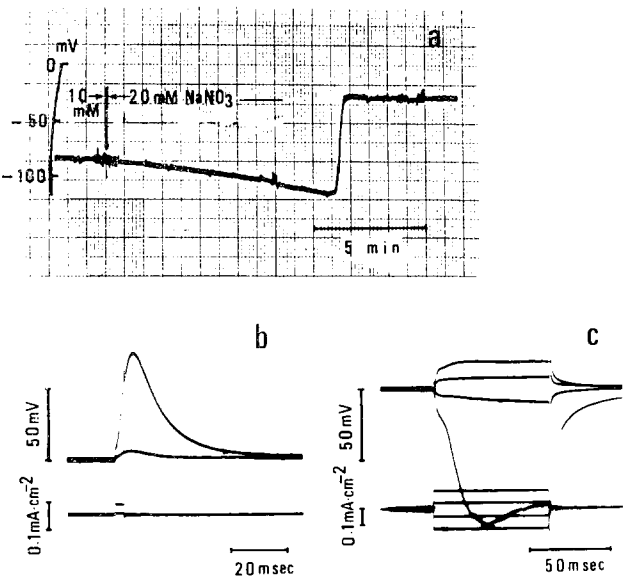


Fig. 2. (a) The intracellular potential depolarized abruptly within several minutes after the concentration of $NaNO_3$ was changed from 10 mM to 20 mM. Fluctuations of $E_m$ are observed in the figure before and after the abrupt depolarization. (b) Action potential induced by a brief outwardly directed current pulse in State A (Fig. 1). The salt composition is the original testing solution (see text). (c) Hyperpolarizing response observed in State B induced by a constant inward current. Salt composition is 40 mM of $NaNO_3$, and the other salts are the same as in the testing solution.

State A in Fig. 1 was found to be excitable in response to an outwardly directed current pulse. In State B, on the other hand, no action potential was produced by an outwardly directed current pulse, but inwardly directed current with a fixed strength led a hyperpolarizing response when the current strength exceeded a certain value, *i.e.* the transmembrane potential varied ohmically at first, and then induced another change and approached a more negative level of transmembrane potential. A photographic record of an action potential elicited by a brief outward current is shown in Fig. 2b, where the external solution is the testing solution. A hyperpolarizing response in State B is demonstrated in Fig. 2c, where the external salt composition is 40 mM $NaNO_3$, 0.5 mM $KNO_3$, 1 mM $Ca(NO_3)_2$, and 2 mM $Mg(NO_3)_2$.

From the results illustrated above, State A is regarded as the resting state, while State B may be considered as the depolarized or excited state of the drop membrane. The transition between the resting and the depolarized states was also induced by successive variation of $K^+$ concentration in the external solution.

The membrane potential as well as the electric resistance are shown in Fig. 3 as functions of the $K^+$ concentration, where the salt composition other than $KNO_3$ is fixed at the same level as in the testing solution. Different symbols in the figure refer to different drop specimens. As seen in the figure, the intracellular potential increased gradually with increase in the external $K^+$ concentration when $K^+$ was lower than 15 mM. The membrane resistance was not affected in this concentration range of $K^+$, and remained at about 2 $k\Omega \cdot cm^2$. At about 30 mM $KNO_3$, an abrupt

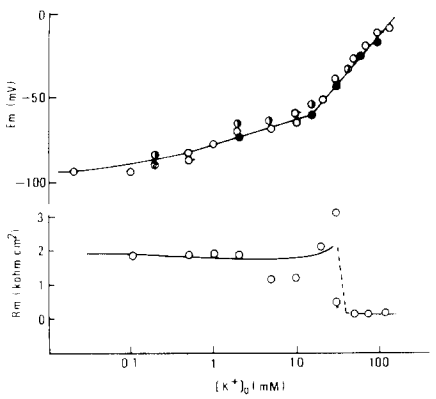


Fig. 3. Dependence of the membrane potential, $E_m$, and the membrane resistance, $R_m$, on $K^+$ concentration. Different symbols indicate data for different drops. Note that the slope of $E_m$ *vs* $[K^+]_o$ changes discontinuously at about 20 mM of $[K^+]_o$, and $R_m$ decreases abruptly at about 30 mM $K^+$. The action potential and the hyperpolarizing response were observed below and above the critical concentration (20–30 mM of $K^+$), respectively.

decrease in the membrane resistance was observed. The intracellular potential was not changed discretely at this $K^+$ concentration. When the external $K^+$ concentration was increased further, the membrane potential varied linearly with the logarithm of the $K^+$ concentration; the membrane potential changed approximately 50 mV with 10-fold variation of K concentration. In this region, the membrane resistance was 0.2–0.5 $k\Omega \cdot cm^2$. The excitability of the drop got lost at about 20 mM of K concentration when $KNO_3$ was increased progressively. In this state, a hyperpolarizing response similar to Fig. 2c was observed in response to the inwardly directed current. It is important to note that the variation of the intracellular potential and the membrane resistance with successive change in $K^+$ or $Na^+$ concentrations are reversible unless the drop is immersed in high concentration media for a few hours.

A substitution of anion species (from $NO_3^-$ to $Cl^-$) of the salts in the external solution did not lead to any appreciable change in the electrical properties of the drops. These results are the same as those observed for squid giant axons[10,12,13], and suggest that the membrane formed on the protoplasmic drops is charged negatively. This point will be discussed precisely in a subsequent paper in connection with the ion permeability of the drop membrane[14].

*Effects of $Ca^{2+}$ concentration*

$Ca^{2+}$ is known to be indispensable for maintaining the excitability of nervous tissues[10,12]. $Ca^{2+}$ plays an essential role, not only for nerve activity, but also for various regulation mechanisms of living organisms such as receptor cells, muscles, *etc.* It is interesting to note that the physical and electrical properties of the protoplasmic drops investigated here are also drastically affected by $Ca^{2+}$ concentration in the external solution. As was reported in the preceding papers[4,5], complete removal of $Ca^{2+}$ from the external solution led to the immediate loss of excitability, and following disruption of the drop itself[4]. On the other hand, too great a concentration of $Ca^{2+}$ in the medium also suppressed the excitability of the drops[5].

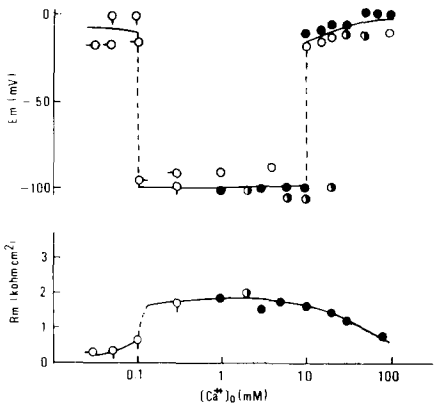


Fig. 4. Dependence of $E_m$ and $R_m$ on $Ca^{2+}$ concentration in the external solution, in which the salt composition other than $Ca^{2+}$ is the same as in the testing solution. Abrupt depolarizations of $E_m$ are seen at about 0.1 and 10 mM $Ca^{2+}$, whereas $R_m$ decreased sharply only at about 0.1 mM $Ca^{2+}$.

Fig. 4 shows the experimental results of the intracellular potential and the membrane resistance of drops as functions of the concentration of $Ca^{2+}$. Again, the different symbols in the figure show the results obtained for different drops. The membrane potential as well as the membrane resistance was not affected appreciably in the concentration range between 0.1 and 10 mM $Ca^{2+}$, where the drops were found to be electrically excitable. The membrane potential and the membrane resistance in this region were about $-90$ mV and approximately 2 $k\Omega \cdot cm^2$, respectively. A decrease in $Ca^{2+}$ concentration induced a discontinuous change in the intracellular potential for about 80 mV at 0.1 mM Ca, which was accompanied by an abrupt decrease in the membrane resistance. When the external Ca concentration was reduced to around 0.01 mM, the drop became very fragile and was frequently disrupted by a slight mechanical agitation. Moreover, the depolarized state of the drop in a medium of low $Ca^{2+}$ concentration, showed some rectified response by either outwardly or inwardly directed current. Typical examples of oscilloscope records are shown in Fig. 5a. It is noted that no clear threshold value of the applied current

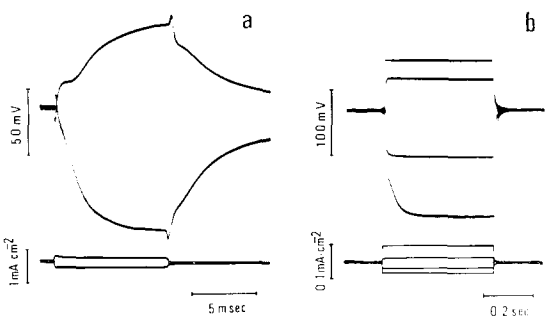


Fig. 5. Electrical responses in the depolarized states of a drop both in low and high $Ca^{2+}$ concentrations. (a) 0.03 mM $Ca^{2+}$, and (b) 80 mM $Ca^{2+}$. The other salt composition is the same as that of the testing solution.

strength was observed in these responses. Fig. 5a referred the case where the drop was placed in a medium containing 0.03 mM of $Ca(NO_3)_2$ with the other ionic composition being fixed as it stands in the testing solution.

As seen in Fig. 4, an increase in the external Ca concentration also brought about a discrete change in the membrane potential for about 80 mV at approx. 10 mM $Ca^{2+}$. This abrupt depolarization, however, was not accompanied by a discrete change in the membrane resistance. A further increase in $Ca^{2+}$ concentration led to a gradual decrease in the membrane resistance. At this depolarized state, no excitability was elicited by an outwardly directed current pulse, but a hyperpolarizing response due to inwardly directed current was observed. Examples of hyperpolarizing responses under constant current strength are shown in Fig. 5b, where the external Ca concentration is fixed at 80 mM, and the other salt composition in the external medium are the same as that in the testing solution. It is noted that an intact internodal cell of *Nitella* is also depolarized in high $Ca^{2+}$ concentration media[15].

*Effect of $Mg^{2+}$*

Fig. 6 illustrates the effect of $Mg^{2+}$ concentration in the external medium on the membrane potential and the membrane resistance, where the salt composition other than $Mg(NO_3)_2$ is fixed at the same level as that in the testing solution, *i.e.* 0.5 mM $NaNO_3$, 0.5 mM $KNO_3$, and 1 mM $Ca(NO_3)_2$. Different symbols in the figure refer to different drop specimens. Appreciable variation of the intracellular potential was not observed when $Mg^{2+}$ was varied between 0.01 mM and about 20 mM. In this Mg concentration range, the drop was found to be electrically excitable. When the concentration of external $Mg^{2+}$ was greater than 20 mM, the intracellular potential changed its value sharply with increase in Mg concentration. The excitability was suppressed in this region. Removal of $Mg^{2+}$ from the external medium led to a depolarization of the membrane potential by about 30 mV. The membrane resistance was, however, not changed appreciably in Mg-free solution. The membrane resistance decreased proportionally with increase in Mg-concen-

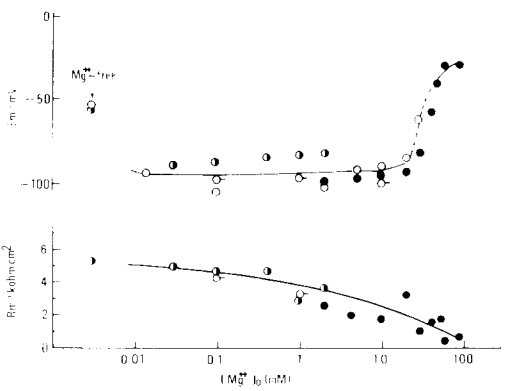


Fig. 6. Dependence of the membrane potential, $E_m$, and the membrane resistance, $R_m$, on $Mg^{2+}$ concentrations.

tration, from about 5 to 0.5 $k\Omega \cdot cm^2$. One may notice that the effects of $Mg^{2+}$ on the membrane properties described above are different from those of $Ca^{2+}$. It is clear that $Ca^{2+}$ seems to play an essential role for the formation of structure of the membrane and maintenance of the excitability of the drop membrane. On the other hand, $Mg^{2+}$ is dispensable for preserving the excitability of the drop, although the presence of $Mg^{2+}$ in the medium seems to be favorable for the occurrence of excitation.

*Effects of $Ca^{2+}$ and $Na^+$ ratio on the transition point*

In the study of internally perfused squid giant axons, it is well known that the critical concentration of $Ca^{2+}$ where the abrupt depolarization takes place varies with the species and the concentration of univalent cations in the external solution[11,13,16]. Similarly, the critical concentration of depolarization due to variation of univalent cations depends on the concentration of $Ca^{2+}$ in the media. It has been suggested that the ratio of concentrations of divalent and univalent cations is essential for the maintenance of the membrane functions for the squid giant axons[10,12,17]. The similar phenomenon is observed in the protoplasmic drops in question. A typical example is shown in Fig. 7, where the $Ca^{2+}$ concentration in the external medium was fixed at 0.3 mM instead of 1 mM $Ca^{2+}$ in the testing solution, and the $Na^+$ concentration was increased progressively. The membrane potential did not change appreciably when $Na^+$ was increased to 1 mM. When the $Na^+$ concentration rose to 3 mM, however, the intracellular potential increased abruptly by about 20 mV and followed the gradual increase in the potential as seen in the figure. In this specific $Ca^{2+}$ concentration, the critical concentration of $Na^+$ was 3 mM, as compared with 20 mM in the testing solution (see Fig. 1). In other words, the concentration range in which the drop membrane retains its excitability depends both on the concentration of $Ca^{2+}$ and univalent cations in the medium. The results described above show that the ratio of $Ca^{2+}$ and $Na^+$ (or $K^+$) concentration plays a role for the process of excitation in low ionic strength media.

The drop membrane which was depolarized by a high concentration of univalent cations (higher than 20 mM of $Na^+$ or $K^+$) did not flip-back to the resting state by an addition of any amount of $Ca^{2+}$.

These observations imply that a low ionic strength of the external solution is a necessary condition for maintaining the excitability of the drop membrane, and

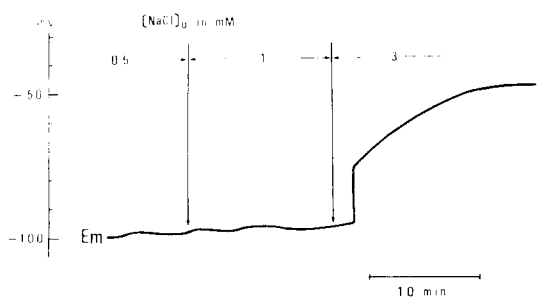


Fig. 7. Effects of $Ca^{2+}$ and $Na^+$ concentrations on the transition point. Abrupt depolarization was induced at 3 mM of $Na^+$ in a solution of 0.3 mM $Ca^{2+}$, which compared with 20 mM observed in Fig. 2a in a solution of 1 mM $Ca^{2+}$.

are consistent with our previous suggestions derived from the measurements of physical properties of the drop membrane, *i.e.* the surface tension and the refractive index. In the low ionic strength region, the $Ca^{2+}$ and univalent cations seem to act antagonistically for the development of the process of excitation, as has been observed in squid giant axons[10–12].

## DISCUSSION

From an accumulation of the experimental results on the electrophysiological and electrochemical measurements carried out with squid giant axons under internal perfusion, it becomes increasingly clear that the process of excitation of nervous tissues is accompanied by a conformational change in membrane macromolecules. As reported in this series of papers, various physicochemical and physiological properties obtained with the excitable protoplasmic drops isolated from *Nitella* support this notion.

The experimental results shown here indicate that the excitability of the protoplasmic drop membrane appears in the limited range of ionic composition in the external solution. Outside this region of salt composition, the surface membrane of the drops undergoes a depolarization and loses its excitability. Fig. 7 shows that $Ca^{2+}$ and univalent cations act antagonistically in the excitable range of salt composition for the transition between the resting and depolarized (excited) states. When the external ionic concentration or ionic strength exceeds a certain limit, the membrane stays in the depolarized state indefinitely even when the ratio of uni- and divalent cations is varied over a wide range.

As seen in Fig. 3, the membrane potential in the depolarized state seems to be $K^+$ sensitive. It is important to note, however, that the logarithmic dependence of the membrane potential on $K^+$ does not imply that the membrane is permeable only for $K^+$. Actually, in a low membrane resistance state of the drop, the permeability of $K^+$ determined from flux measurements is not very different from that of $Na^+$ (ref. 14).

It is important to note that the physical properties of the drop surface membrane, *e.g.* the surface tension, and refractive index, are varied remarkably by the external salt composition, and that these physical properties of the membrane are closely related to the electrophysiological or electrochemical properties of the drop membrane. For the sake of comparison, various properties thus far obtained for the drop membrane are summarized in Table I. One may notice that either the resting state or the depolarized state is characterized both by electrical and/or by physical properties of the membrane. For example, the refractive index and the surface tension of the drop at the resting state are 1.42 and 0.1 dyne/cm, respectively. These values are changed to 1.5–1.6 and 0.001–0.01 dyne/cm, respectively, when the surface membrane is depolarized. These results imply that the structures of the surface membrane are very different between the resting and the depolarized states. Hence the transition between these two states must be accompanied with a change in the membrane structure. The production of an action potential in response to external stimulus seems to be an electrical reflection of the transient variation of the membrane structure.

The membrane state in the high Ca concentration medium is considered to be a kind of depolarized state. The membrane potential in high $Ca^{2+}$ media is almost

TABLE I

PHYSICAL AND ELECTRICAL PROPERTIES OF PROTOPLASMIC DROP MEMBRANE IN THE RESTING AND THE DEPOLARIZED STATES

| | *Resting state* | *Depolarized state* |
|---|---|---|
| Electrical functions | Action potential (by outward current pulse) | Hyperpolarizing response (by inward constant current) |
| Membrane potentials (mV) | $-60 \sim -100$ | $-10 \sim -30$ Higher than $-50$ mV in high $K^+$ media |
| Membrane resistance ($k\Omega \cdot cm^2$) | $1 \sim 5$ | $0.2 \sim 0.5$ |
| Surface tension (dyne/cm) | 0.1 | $0.001 \sim 0.01$ Larger than 0.1 in high $Ca^{2+}$ media |
| Refractive index | 1.42 | $1.5 \sim 1.6$ |

at the same level with that in the depolarized state in the low Ca concentration range (see Fig. 5). In these states, a hyperpolarizing response is induced by an inwardly directed current. It is noted that not only the membrane potential but also physical properties in high Ca concentration media (*i.e.* the surface tension and refractive index) seem to fall on the same continuous line with those in the depolarized state in low $Ca^{2+}$ media[5]. The molecular mechanism for the appearance of the same depolarized states both in high and low $Ca^+$ media is not clear to us at present.

In conclusion, the experimental results presented here imply that the conformation of the membrane macromolecules is easily changed by a small external perturbation, *i.e.* a slight change in the ionic composition of the external media and/or a small electrical stimulus. This lability of the membrane structure is essentially responsible for the production of the action potential or excitability as stressed repeatedly.

As will be shown in a subsequent paper[14] the ionic strength in the protoplasmic drops is about 150 mM with $K^+$ and $Na^+$ salts. Therefore, the range of ionic composition in the external media where the drops are excitable, seems very much lower in comparison with that in the drops. This fact suggests that an appropriate difference in salt concentrations should exist between the inside and outside of the membrane for maintaining the excitability. In other words, the energy dissipation which stems from the difference in free energy on two sides of the membrane seems to be responsible for the formation and maintenance of various functions of the membrane[5,18].

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