Electrical Characteristics and Activation Potential of *Bufo* Eggs

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ABSTRACT Electrical characteristics and their changes during activation were studied with the microelectrodes on the oocytes and eggs of the toad, *Bufo vulgaris formosus* Boulenger.

In young oocytes, the membrane characteristics had some similarities to those of nerve and muscle, except for a relatively large resistance of 25 KΩcm.^{2} and an absence of the action potential in the former. After maturation, however, the membrane characteristics became entirely different from those of oocytes and other excitable tissues. In the mature eggs the membrane resistance was measured to be as high as 200 KΩcm.^{2}, and no specific permeability of the membrane to potassium ions was observable.

A slow monophasic change in the membrane potential was recorded in every activation produced by mechanical stimulation, and termed "activation potential." In fresh water, its amplitude was as large as 80 to 90 mv. with an overshoot of about 50 mv. The activation potential might be comparable to the action potential of nerve and muscle, but was fundamentally different in ionic mechanism from the latter, since the former was caused by a marked increase in permeability to chloride ions.

INTRODUCTION

Concerning the primary process of activation of eggs, it was suggested recently by Yamamoto (1944 *a*, *b*, 1949, and 1956) that insemination or mechanical, chemical, and sometimes electrical stimulations gives rise to a kind of conducting impulse or wave which causes the breakdown of cortical alveoli and the elevation of the fertilization membrane. This was termed the "fertilization wave or impulse."

In excitable cells, especially in nerve and muscle, the process of excitation has been studied electrophysiologically in detail; it is found that a transient increase in membrane conductivity is responsible for the generation of the

139

action potential and that the inward current during the action potential is carried by sodium ions.

If activation of the egg is a kind of excitation, similar electrical phenomenon might be expected. Péterfi and Rothschild (1935) reported a potential change in activation of frog eggs. More recently, a potential change through fertilization was recorded on echinoderm eggs (Scheer *et al.*, 1954; Tyler *et al.*, 1956; Hiramoto, 1959) and also on fish eggs (Maéno *et al.*, 1956; Hori, 1958). On the other hand, Kao (1956) could observe neither a resting potential nor potential change during fertilization of *Fundulus* eggs.

Although many electrical measurements have been carried out on various eggs, most of the electrical properties of eggs are still unknown. In this paper, membrane characteristics of oocytes and unfertilized eggs and their changes during activation are studied. The presence of an "activation potential" is confirmed, which may be comparable to the action potential, though entirely different in nature.

Materials and Methods

Materials used were oocytes and unfertilized eggs of the toad, *Bufo vulgaris formosus* Boulenger. The ovary was dissected from the female animal, and oocytes were isolated after carefully removing the surrounding tissues with fine tweezers under a binocular microscope. Mature unfertilized eggs were obtained by artificial ovulation as follows. The dissected ovary was hung in the beaker containing "hypophysis-suspended" Ringer solution (one hypophysis of *Bufo* per 100 cc.). After completing the maturation process within 20 to 30 hours at a temperature of around 15°C., mature unfertilized eggs were freed from the enveloping tissues and dropped onto the bottom of the beaker. The eggs thus obtained survived about 40 hours at room temperatures of 10–15°C. Various commercial products of the hypophysis were tested for artificial ovulation but the best results were obtained with a fresh or acetone-treated hypophysis of *Bufo vulgaris*.

The apparatus and method for potential measurement were similar to those described by Tyler *et al.* (1956) and Kao (1956) in most respects. The microelectrodes were drawn by machine, and 3 M KCl solution was filled into the microelectrodes according to the method of Tasaki *et al.* (1954). The resistance of the electrodes used ranged from 10 to 20 MΩ. The preamplifier was a 12AU7 cathode follower. Observation of the activation potential required an oscilloscope with a longer time base than usual. The author constructed a modified type of the oscilloscope described by Ferguson (1951) with a two-stage direct coupling amplifier. For the measurement of membrane resistance a dual beam oscilloscope with three-stage direct coupling amplifiers was used.

The sign of the intracellular potential was taken with reference to the external medium. No allowance was made for the junction potential. The transmembrane current of inward direction was termed anodic current, and that of outward direction cathodic.

Throughout the experiments a solution of the constitution, NaCl 110.3, KCl 1.3, and $CaCl_2$ 2.2 mM, was used as the normal environmental medium and termed normal Ringer. In order to investigate the effects of various species of ions on the membrane potential, the ionic constitution of the bathing solution was changed in various ways. When one of the ionic components of normal Ringer was changed, the test solution was described as Ringer-labelled with the altered ions expressed in mM concentration. For example, 50 mM K-Ringer was the solution containing 50 mM KCl, 110.3 mM NaCl, and 2.2 mM $CaCl_2$. For investigation of the effect of sodium ions on the membrane potential, sodium chloride was added to the normal Ringer or was replaced by isotonic glucose. Concentrations of potassium and calcium were varied by subtracting from or adding to the normal Ringer. In some cases the effects of potassium and chloride ions on the membrane potential were studied with a series of potassium chloride solutions containing an amount of calcium chloride equivalent to normal Ringer, and this was called the K-solution series. Isotonicity was also maintained by glucose. The effect of anions was tested with solutions of Na- and Cl-Ringer or K-solution series. In the Cl-Ringer series chloride ions were replaced with sulfate ions which were regarded as non-penetrating anions, and Na-Ringer and K-solution series also could serve as the test solutions for the effect of chloride ions.

RESULTS

I. Electrical Properties of the Oocytes

The resting potential of the *Bufo* oocytes differed widely depending on their degree of maturity as was shown in *Oryzias* oocytes by Maéno *et al.* (1956). Oocytes obtained from an individual animal, however, had relatively uniform resting potentials. On the other hand, the membrane potential of the isolated oocytes, which was initially as high as -70 mv., decreased gradually with the time after isolation, and at room temperature it declined to about -30 mv. within 12 hours. Because of these facts, isolated oocytes were kept in the ice box prior to the experiments, and a series of experiments was conducted on the oocytes isolated from an individual animal. It was considered convenient to represent the degree of maturity by the membrane potential, because there should be a correlation between maturation stage and membrane potential in the oocytes. The stage of maturity of the oocytes was expressed by a rounded figure of the mean resting potential in normal Ringer, and the effects of various ions on the resting potential were compared with the stages under consideration.

The membrane of the oocytes was relatively hard to penetrate with the microelectrode, though still easier than in mature eggs. The surface of the oocyte invaginated as the electrode was pushed forward. However, on allowing it to stand in this condition for a while or when (*cf.* Tyler *et al.*, 1956) the experimental table was tapped lightly, there appeared a sudden change in the

potential, of 50 to 70 mv. Vigorous pricking with the microelectrode invariably resulted in low membrane potential but it increased gradually to normal value within a few minutes.

EFFECTS OF VARIOUS IONS ON THE RESTING POTENTIAL OF THE OOCYTES

The effects of sodium, potassium, and calcium ions on the resting potential were studied with oocytes of around −70 and −40 mv. stages. Results are given in Figs. 1, 2, and 3. It is shown in Fig. 1 that a change in concentration of sodium ions has no appreciable effect on the resting potential in both −60 and −40 mv. stages. However, in some cases a slight decrement of the membrane potential was observed when the external sodium concentration was reduced. The effect of potassium ions on the membrane potential of the oo-
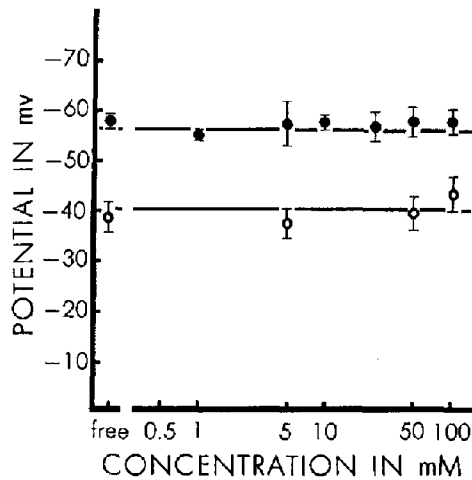

Figure 1.   Effect of external sodium ions on the resting potential of oocytes. ●, −60 mv. stage; O, −40 mv. stage. In this and all other figures of the concentration-voltage curve, each symbol represents the mean value of the resting potential and the vertical line indicates the standard error of the mean.
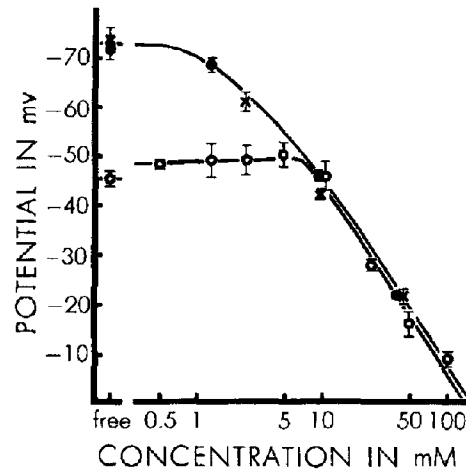
Figure 2.   Effect of external potassium ions on the resting potential of oocytes. ●, −70 mv. stage in K-Ringer; O, −50 mv. stage in K-Ringer; ×, −70 mv. stage in K-solution series.

cytes was similar to that on nerve and muscle as already reported by many authors (cf. Hodgkin, 1951). The membrane potential had a linear relationship to the logarithm of the potassium concentration above 5 mM in both −50 and −70 mv. stages. The relationship was somewhat different when the concentration of external potassium ions was lower than 5 mM. In the −70 mv. stage a linear relationship of the membrane potential to the logarithm of the external potassium concentration was maintained even when a concentration

as low as 1 mM was used, whereas in the −50 mv. stage the membrane potential showed a slight decrease with a potassium concentration of less than 5 mM. Calcium ions had a relatively marked effect on the resting potential of oocytes compared with the effect on nerve and muscle (Jenerick and Gerard, 1953; Weidmann, 1955; and Frankenhaeuser, 1957). The membrane potential had a relatively linear relationship with the concentration of calcium ions over the range of 0.1 to 1.0 mM, and the results obtained on both −50 and −60 mv. stages fell on a single curve with calcium concentrations lower than 1 mM. The resting potential of the −60 mv. stage increased to −65 mv. in calcium-rich Ringer, whereas that of the −50 mv. stage remained unchanged. In Ca-free Ringer no difference in membrane potential was observed between the intact oocytes and those treated with sodium oxalate.
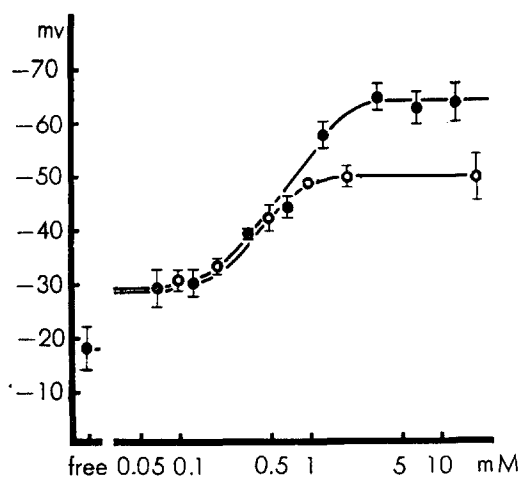

FIGURE 3. Effect of external calcium ions on the resting potential of oocytes. ●, −60 mv. stage; ○, −50 mv. stage.
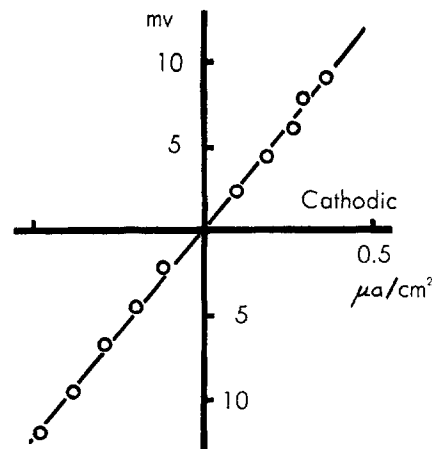
FIGURE 4. Current-voltage relationship of the oocyte in normal Ringer. −60 mv. stage.

MEMBRANE RESISTANCE OF THE OOCYTES

Membrane resistance was studied with two microelectrodes. Typical results are given in Fig. 4. In the normal Ringer, the membrane had the resistance of 20 to 30 KΩcm.^{2} (mean 25 KΩcm.^{2}) and the capacitance of 6 to 11 μf./cm.^{2} (mean 8.6 μf./cm.^{2}). No rectifier property of the membrane was observed in the oocytes.

The measurements of the membrane resistance on narcotized eggs indicated that some narcotizing agents affected the membrane in such a way as to increase its permeability to ions. The same effect was confirmed also on oocytes in 3 per cent urethane Ringer in which the membrane resistance was de-
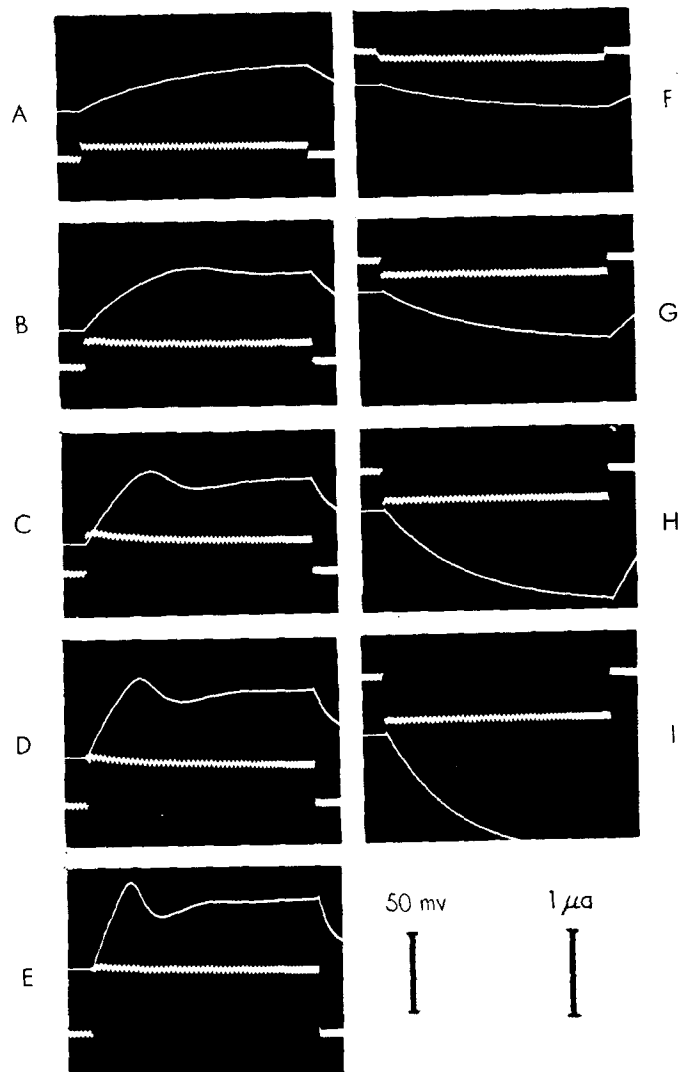
FIGURE 5.   Photographs showing the potential change generated by a transmembrane current pulse. Oocyte of −60 mv. stage in normal Ringer. Cathodic current is increased from A to E, and anodic pulse from F to I. 60 c. p. s. is superimposed on the current records of each photograph.

creased to about a quarter of the normal value (6 KΩcm.²) though the resting potential was not so remarkably changed. On the other hand 0.1 per cent cocaine had little effect on the membrane resistance of the oocytes.

RESPONSE OF THE OOCYTES TO ELECTRICAL STIMULATION

In the ovarian eggs of *Rana esculenta*, Umrath (1954) claimed to have observed
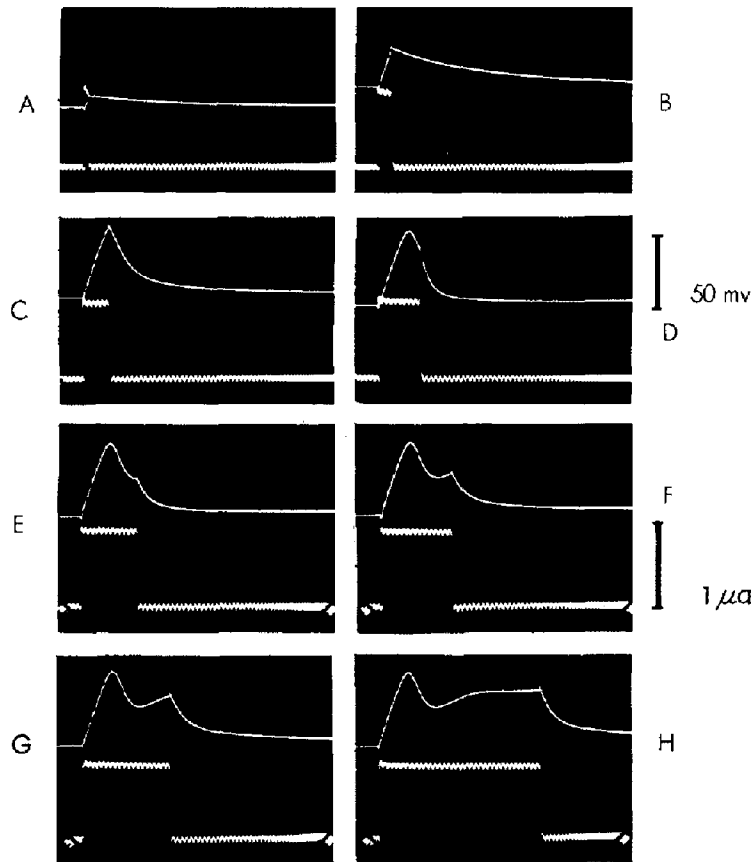
FIGURE 6. Photographs showing the potential change generated by cathodic current pulse of different durations. Oocytes of −60 mv. stage in normal Ringer.

an action potential generated by electrical shock. In the present experiments such an action potential was not observed but a different kind of response to the large cathodic current pulse was noted. When a weak cathodic current was applied through the membrane, there appeared only a typical electrotonic depolarization. As the current increased, the electrotonic potential gave rise to a hump, shown in Fig. 5 B to E. No such response was observed with an anodic current (Fig. 5 F to I). It must be emphasized here that the height of potential during the hump was relatively independent of the current strength,

suggesting that this potential may be a kind of equilibrium potential. After cessation of the current, the membrane potential returned to the resting level in a rather exponential manner, and the time constant of decay varied with duration of the current (Fig. 6).

II. Electrical Properties of the Eggs

Recent studies on marine eggs (Lundberg, 1955; Tyler et al., 1956) had revealed that insertion of the microelectrode into the egg is very difficult. This was true also in the present material. Though the microelectrode was observed under the microscope to be inserted into the egg deeply, no potential difference was recorded. The cortex of the egg became plastic during the maturation process and usually became invaginated as the electrode advanced. Sometimes, light tapping of the experimental table or application of an electrical pulse was useful to allow the electrode held in contact with the egg surface to penetrate into the egg just as in the oocyte (above). In this way the resting potential in the normal Ringer was measured to be about −12 mv. However, it was usually followed by the activation potential. In order to prevent activation after electrode insertion, the eggs were narcotized with 0.1 per cent cocaine or 3 per cent urethane Ringer prior to the experiments for about 10 minutes, and most of the measurements of the resting potential were made on the narcotized eggs.

ELECTRICAL PROPERTIES OF THE VITELLINE MEMBRANE OR CHORION

On the eggs of Fundulus (Sumwalt, 1929, 1933), and on the unfertilized eggs of salmon (Oikawa, 1952) a potential difference was observed across the vitelline membrane or chorion, which depended on the external ionic concentration. In the present experiments no potential difference across the chorion was recorded in normal Ringer. When the eggs equilibrated with normal Ringer were put into solutions of low ionic concentration, however, the potential at the inside of the chorion became slightly negative. In Na-free Ringer it amounted to −10 mv., and had a linear relationship with the concentration of the external monovalent cations, showing a 4 mv. shift per tenfold alteration of concentration. No difference was observed between the effects of sodium and potassium ions (Fig. 7). Change in calcium concentration had no effect. Application of a current pulse showed that the chorion of the unfertilized egg was very permeable to all ions and served as a very weak diffusional barrier to monovalent cations.

EFFECTS OF VARIOUS IONS ON THE RESTING POTENTIAL OF THE EGGS

The effects of sodium, potassium, and calcium ions are shown in Figs. 8 and 9. The resting potential of the eggs had a linear relationship to the logarithm

of the concentration of sodium ions, whereas it showed no appreciable change in the K-Ringer series in which the concentration of sodium chloride was high and constant in every solution. On the other hand, the effect of potassium ions
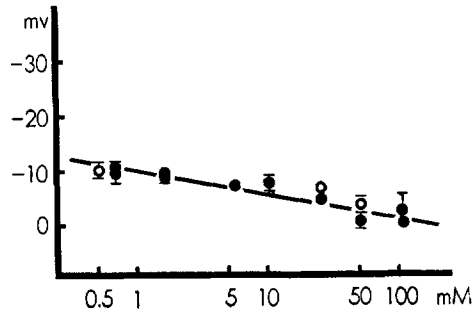
FIGURE 7. Effect of external monovalent cations on the potential difference across the chorion of the eggs. o, in Na-Ringer; •, in K-solution series.
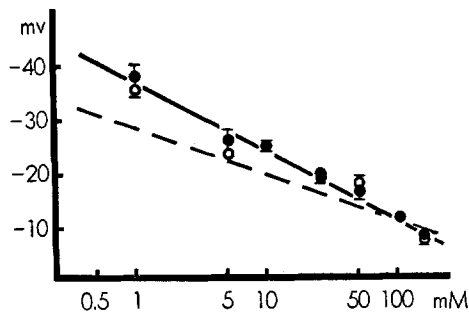
FIGURE 8. Effect of external monovalent cations on the resting potential of the eggs. •, in Na-Ringer; o, in K-solution series. Broken line is obtained by correcting the potential difference across the chorion.
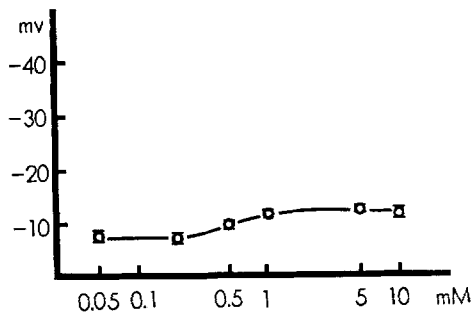
FIGURE 9. Effect of external calcium ions on the resting potential of the eggs.

was tested with the K-solution series which contained no sodium ions, and dependence of the membrane potential on the concentration of external potassium ions was proved (Fig. 8). These results indicated that after maturation the selective permeability of the oocytes to potassium ions was lost and transformed into non-selective permeability to monovalent cations. No difference in effect was observed between sodium and potassium ions.

The effect of calcium ions on the membrane potential of the eggs was not so remarkable as on the oocytes. The resting potential of the eggs decreased slightly in Ca-deficient Ringer as shown in Fig. 9. In order to investigate the effect of chloride ions, the membrane potential was measured in Cl-Ringer series in which chloride ions were replaced with sulfate ions. In Cl-Ringer series the membrane potential depolarized to zero or reversed its sign as the
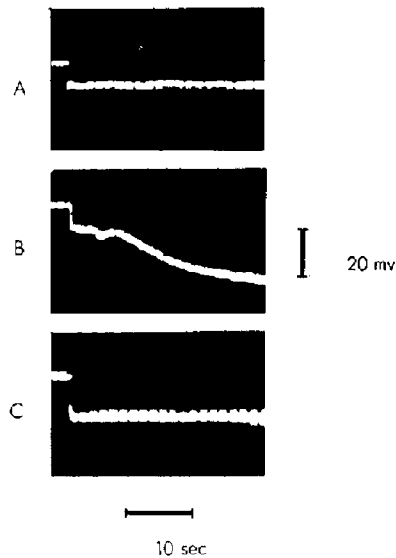

FIGURE 10.   Time course of the membrane potential in normal Ringer after impalement with the electrode. Electrode insertion without activation is shown in A. Activation is observed in B. Electrode is inserted into an activated egg in C.

concentration of chloride ions was decreased; however, no linear relationship between the membrane potential and the logarithm of the concentration of external chloride ions was observed. This finding is attributed to the depolarizing effect of sulfate ions and it was also observed on unfertilized trout eggs (Pumphrey, 1931).

ACTIVATION POTENTIAL

Following the puncture of the egg with the microelectrode, activation was observed inevitably unless extreme care was taken. Activation of the egg was optically recognizable by elevation of the vitelline membrane or chorion (Motomura, 1952), and electrically by a monophasic potential change invariably observed prior to the elevation of the membrane, which was defined as activation potential. In Fig. 10 A, the time course of the membrane potential without activation is shown. The resting potential appeared suddenly when the microelectrode was inserted into the egg, then remained constant during an entire sweep. On the contrary, when activation took place the membrane potential changed usually within 30 seconds (Fig. 10 B). No such poten-

tial change was recorded on insertion of the electrode into the activated egg proving that the observed potential change was not an artifact produced by electrode insertion (Fig. 10 C). The activation potential was recorded as a hyperpolarizing potential change in normal Ringer. With reduction of external ionic concentration, the activation potential decreased and finally reversed its sign. In isotonic glucose or tap water the activation potential was as high as 80 to 90 mv., and had an overshoot of about 50 mv. The time course
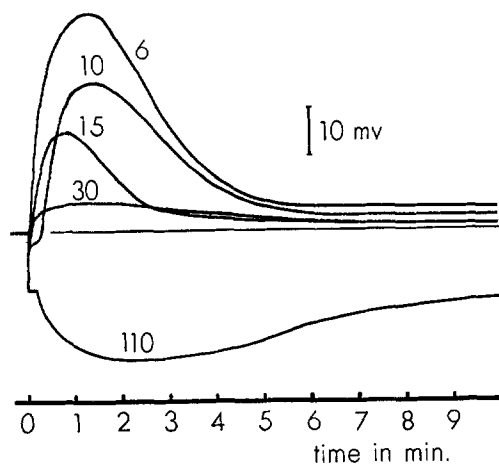

FIGURE 11. Time course of the activation potential obtained in Cl-Ringer series. Numerals by each curve denote concentrations of external chloride ions in millimols.
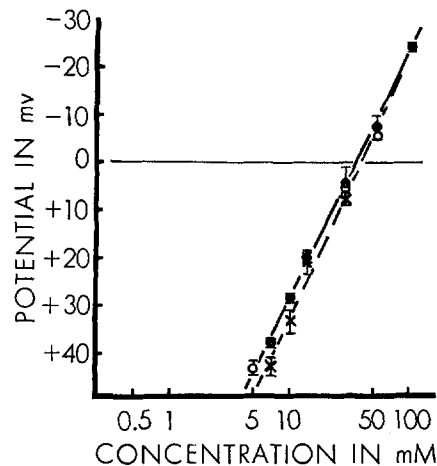
FIGURE 12. Effect of the external chloride ions on the crest level of the activation potential. ●, in Na-Ringer; o, in K-solution; ×, in Cl-Ringer series. Broken line is obtained by correcting the potential difference across the chorion in Na-Ringer and K-solution series. It agrees well with the results obtained in Cl-Ringer series.

of the activation potential in various chloride concentrations is shown in Fig. 11. In this figure it is seen that the potential attained a maximum value within 1 to 2 minutes and then declined rather slowly, usually taking over 5 minutes before reaching the steady state. The potential declined to zero in Cl-deficient solution and never repolarized to around − 15 mv. as was the case in normal Ringer (110 mM Cl-Ringer) probably because of the depolarizing action of sulfate ions on the resting membrane. Increase in permeability during the activation potential was confirmed, as is described later.

The effects of various ions on the activation potential were also studied. Measurements in the K- and Ca-Ringer series showed that neither of these two ions had any appreciable effect on the potential at the crest of the activa-

tion potential, whereas Na- and Cl-Ringer or K-solution series caused a re-
markable change in the amplitude of the activation potential (Figs. 11 and
12). A plot of the crest level of the activation potential obtained in these three
series falls upon a single straight line if correction is made for the potential dif-
ference across the chorion in Na-Ringer and K-solution series (Fig. 12, broken
line). This shows an inverse relationship to the effect of sodium ions on nerve
and muscle in the active state. The effect of the external solution on the activa-
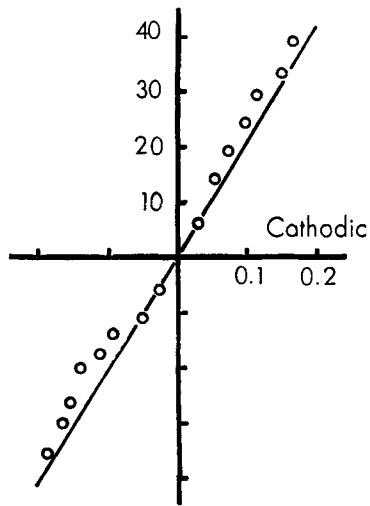

Figure 13. Current-voltage re-
lationship of the intact egg in
normal Ringer. Abscissa, current
density in μa./cm.^{2}; ordinate,
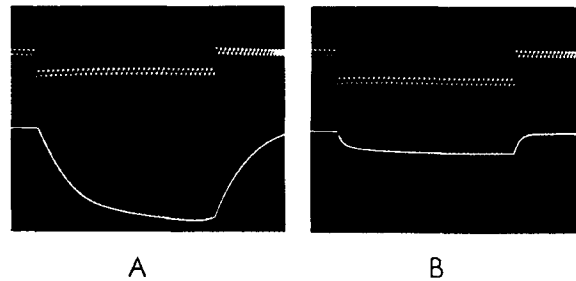electrotonic potential in milli-
volts.

Figure 14. Photographs showing increase in permea-
bility during activation. A, before and B, during activa-
tion. Upper record, transmembrane current; lower rec-
ord, intracellular potential. See the text.

tion potential is well explained if the membrane of the egg becomes infinitely
permeable to chloride ions so that the flux of other ions makes only a negligible
contribution. The shift of the peak level caused by a tenfold increase in the
concentration of chloride ions was about 56 mv. and agreed well with the ex-
pected value derived from the equation of the chloride equilibrium potential,

$$E_{Cl} = -\frac{RT}{F} \log_e \frac{[Cl]_o}{[Cl]_i}.$$

MEMBRANE RESISTANCE AND ITS CHANGE ON ACTIVATION

Measurement of the membrane resistance with two electrodes on unactivated
eggs was very difficult and extraordinary care was required, for the egg was

liable to be activated by the insertion of the electrodes. Typical results are given in Fig. 13. In this figure, it is shown that the membrane resistance of the unactivated egg was about 8 times larger (200 KΩcm.^{2}) than that of the oocyte. These results indicated that the normal egg cortex becomes increasingly less permeable to all ions. On the contrary, the membrane capacitance of the egg decreased to about one-sixth that of the oocyte (1.5 μf./cm.^{2}), though this determination was only approximate.
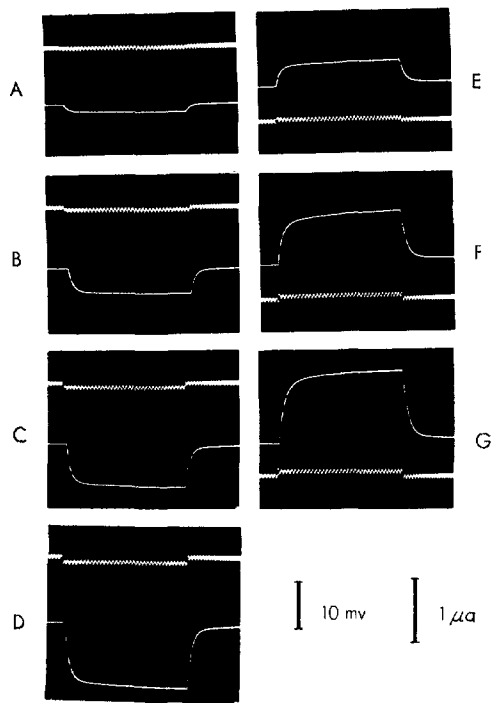

Figure 15. Photographs showing current-voltage relationship of the egg treated with 0.1 per cent cocaine Ringer. Anodic pulse is increased from A to D, and cathodic pulse from E to G.
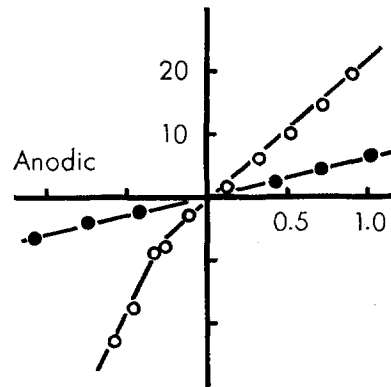
Figure 16. Current-voltage relationship of the egg treated with 1 per cent urethane Ringer. o, before and •, during activation. Abscissa, current density in μa./cm.^{2}; ordinate, electrotonic potential in millivolts.

Application of mechanical shock or large current pulses across the egg cortex resulted in activation of the egg. It was noted that activation always gave rise to a decrease in membrane resistance to the order of 10 KΩcm.^{2} in the initial phase of the activation potential and to an increase again in the recovery phase. In Fig. 14 A, a current pulse was applied to the intact egg immediately after impalement by the electrodes and a sign of decrease in membrane resistance was recorded near the end of the pulse. The photograph in Fig. 14 B was recorded about 10 seconds later and shows a more marked decrease in the

resistance. There was no appreciable change in membrane capacitance on activation, in spite of the reports that membrane capacitance increased on fertilization in marine eggs (cf. Rothschild, 1956; Hiramoto, 1959).

The resting potential of the egg in normal and various test solutions was determined after the application of narcotics. In order to confirm whether the narcotics change the permeability of the membrane or not, membrane resistance was also measured on narcotized eggs. The results are given in Figs. 15 and 16. It was found that narcotizing agents decreased the membrane resistance of the egg markedly. In some cases the partially narcotized eggs treated with 1 per cent urethane were activated by insertion of the electrodes, and a further decrease in membrane resistance was observed (Fig. 16).

DISCUSSION

I. Resting Potential of the Oocytes

The resting potential of the oocyte in normal Ringer is as high as −70 mv. in the early stage, but declines gradually as the oocyte develops. Considering the following facts that (1) little change in the resting potential is observed in Na-Ringer series, and that (2) on the oocytes of the same stage the resting potentials measured in both K-Ringer and K-solution series fall on a single curve (see Fig. 2, −70 mv. stage), it is clear that the resting potential of the oocyte is determined mainly by a mechanism similar to that of nerve and muscle. Providing that the membrane of the oocyte is exclusively permeable to potassium ions, the resting potential is determined by the concentration difference of potassium ions across the membrane as defined by Nernst's equation:

$$E = -\frac{RT}{F} \log_e \frac{[K]_i}{[K]_o}.$$

In the present experiments the decrease of membrane potential per tenfold increase in potassium concentration of the external solution is about 35 mv., which is considerably lower than the expected value. Measurement of the membrane resistance revealed that the resistance of Bufo oocytes was 10 to 20 times larger than usually reported values on excitable cells. The disagreement with Nernst's equation might be ascribed to low relative permeability of the membrane to potassium ions, and contributions of sodium and chloride ions to the membrane potential should be considered (cf. Hodgkin, 1951).

It has been mentioned already that the membrane characteristics have some similarities to those of nerve and muscle. However, these characteristics changed considerably as the maturation process proceeded as seen in Figs. 2, 3, and 8. The effect of potassium ions on the membrane potential in the oo-

cytes of the −70 mv. stage can be plotted in a single curve, while in the −50 mv. stage it is represented by two straight lines. It is much less dependent on potassium ions when the concentration of potassium ions in the Ringer solution is less than 5 mM, but an identical effect is still observed in a Ringer solution whose concentration of potassium ions is more than 5 mM (Fig. 2). Thus, dependence of the membrane potential on potassium ions is related to the degree of maturation of the oocytes and finally no specific effect of potassium ions on the membrane potential is observed in the mature eggs, but only a nonspecific effect of monovalent cations. The situation is the same in the effect of calcium ions on the membrane potential. The membrane potential decreases to a level close to that measured in the Ca-free Ringer as the oocytes develop and also becomes less dependent on calcium ions. The membrane potential of the oocytes has a definite maximal value according to the stage as seen in Figs. 2 and 3, and never exceeds this value, irrespective of the external solution. It is highly probably that calcium ions play some important role in determining this definite value and also in altering the membrane characteristics during the maturation process.

II. Unactivated Eggs

The present experiments confirm that the electrical characteristics of *Bufo* eggs are different from those of the oocytes or excitable cells. The membrane of the former shows no specific response to potassium. It seems to have a low relative permeability to monovalent cations, since the resulting potential decreased only 9 mv. for a tenfold increment of the external monovalent cations when correction is made for the potential difference across the chorion (Fig. 8, broken line). The same kind of results have been obtained on the brown trout, in which Pumphrey (1931) reported that the membrane potential of the unfertilized egg was dependent on the external concentration of both sodium and potassium.

The disappearance of selective permeability to potassium ions after maturation is accompanied by a decrease of total ionic permeability, the membrane resistance increasing to 200 KΩcm.[2] It is clearly evident that some changes in the egg cortex occur during the maturation process. Migration of the cortical granules in frog eggs (Motomura, 1952) and also the appearance of birefringence in sea urchin eggs (Monroy, 1948) during the maturation process indicate some kind of rearrangement of the egg cortex. The increased membrane resistance is probably caused by reorganization of the molecular structure of the membrane in such a way as to prevent ions from diffusing through it. Since during maturation the membrane capacitance decreases until in the mature egg it is only one-sixth that of the original oocyte, the increased membrane resistance may result mainly from membrane thickening.

Cole and Guttman (1942) measured the membrane resistance of the unfertilized frog egg with an alternating current bridge method and obtained a value of 170 $\Omega cm.^2$, a low figure on the basis of the present results. This discrepancy may be due to differences in technique. More recently, the membrane resistance has been studied with two microelectrodes and the following values were obtained on unactivated eggs; 3.1 $K\Omega cm.^2$ on starfish (Tyler *et al.*, 1956), 3.5 $K\Omega cm.^2$ on *Fundulus* (Kao, 1956), and 3.9 $K\Omega cm.^2$ on sea urchin (Hiramoto, 1959). In the present experiments the membrane resistance of the unactivated *Bufo* egg is about 100 times larger than the values reported on the marine eggs. The disagreement is not due to difference in technique because the same method was adopted by all authors. Kao (1956) reported that *Fundulus* eggs were liable to be activated by penetration of the electrodes, and that during activation the membrane resistance increased markedly while the membrane capacitance remained unchanged. On the other hand, decrease in membrane resistance during activation was shown on sea urchin (Hiramoto, 1959) and on *Bufo* eggs. The discrepancy in these results is not easy to explain. Because membrane resistance as high as 200 $K\Omega cm.^2$ was obtained on the fresh water alga (Gaffey and Mullins, 1958), high membrane resistance may be a characteristic of the cells in fresh water.

The current-voltage relationship presented in Fig. 13 is determined on the normal egg whereas the concentration-voltage curves shown in Figs. 9 and 10 are obtained on the narcotized eggs. The membrane resistance of the egg decreases with application of narcotics (Figs. 13, 15, and 16). It might be thought that the concentration-voltage curves of normal eggs differ from those of narcotized eggs, and also that the disappearance of selective permeability to potassium ions in the unfertilized eggs is due to the application of the narcotic agent. However, no significant difference is observed in the membrane potentials of normal and narcotized eggs in normal Ringer. In oocytes, moreover, selective permeability to potassium ions under narcotics is still observed even though the membrane resistance of oocytes decreases markedly in 3 per cent urethane Ringer.

III. Activation Potential

The existence of a slow monophasic potential change is confirmed in the activation of the *Bufo* egg caused by mechanical stimulation, and is termed the activation potential. It is a kind of irreversible response and is never recorded in the cases of oocytes or already activated eggs. It seems comparable to an action potential, because its amplitude is as large as 80 to 90 mv. and has an overshoot of about 50 mv. in fresh water, which is regarded as the normal environment of the *Bufo* eggs. However, the time course is about 300,000 times slower than that of the action potential in nerve, and moreover it must be em-

phasized that the activation potential is an irreversible phenomenon and that the permeability of the membrane to the ions increases exclusively with chloride but not with sodium ions during the activation potential. Assuming that activation is an excitatory process of the egg (Yamamoto, 1944 *a*, *b*, 1949, 1956), the activation potential may be regarded as a different and unfamiliar type of action potential recorded on the *Bufo* eggs. Though electrical activities have been studied on various excitable cells especially on nerve and muscle and it is well known that the action potential has its origin in increased permeability to sodium ions in response to stimulation, no report is available at present on excitatory phenomena in relation to increased permeability to chloride ions, except for the action potential reported on the fresh water alga, *Chara globularis* (Gaffey and Mullins, 1958). The action potential of *Chara* involves a transient increase in the permeability of the membrane to chloride ions and the fact that the depolarization produced by chloride efflux is followed by an increased potassium efflux which returns the membrane potential to resting levels. It may not be reasonable to make a generalization from these results only. However, a specific feature of the electrical activity in relation to increased chloride permeability is that it is observable in the cells whose normal environment is fresh water, and that it is a rather slow process lasting from several seconds to minutes.

Although the occurrence of an activation potential is confirmed in unfertilized eggs during activation produced by mechanical stimulation, it is still not clear whether an activation potential is observable in normal fertilization. Efforts have been made to decide this point, but without success, because of the failure of fertilization in eggs without a jelly coat (Kambara, 1953). Péterfi and Rothschild (1935) reported in the frog egg an electrical response without recovery phase, in both fertilization and mechanical activation. Since their measurements were made with a condenser coupling amplifier, the activation potential, whose time course is much longer than the time constant of the amplifier circuit, might not be recorded at all, or perhaps only observed as a potential change without recovery. Tyler *et al.* (1956) reported a slow potential change on fertilization of starfish eggs, but Kao (1956) could not record any potential change in activation of *Fundulus* eggs. Maéno *et al.* (1956) and Hori (1958) observed a transient potential change on fertilization of Japanese killifish eggs. However, further investigations revealed that this potential change was not an activation potential, but that it probably originated in the cortical change like the breakdown of cortical alveoli (Maéno, unpublished data).

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