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The Anomalous Rectification and Cation Selectivity of the Membrane of a Starfish Egg Cell

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Summary. The cation selectivity and its relation to the inward-going rectification of the immature egg cell membrane of a starfish, *Nordora punctiformis*, were studied and the following results were obtained. (1) When the external saline contains usual ion species the cell membrane at rest is predominantly permeable to K ions. The K chord conductance g_k depends on the electrochemical potential of K ions, $\Delta V = V - E_K$, and the external K concentration $[K^+]_o$ by

$$g_k = A\left[1+\exp\left(\frac{\Delta V-\Delta V_h}{v}\right)\right]^{-1}([K^+]_o)^{\frac{1}{2}}$$

($\Delta V_h \fallingdotseq -15$ mV, $v \fallingdotseq 7$ mV). (2) The permeability sequence of monovalent cations and the permeability ratios P_x/P_K of the cell membrane at rest obtained with membrane tpotential measurements are, Tl(1.5) > K(1.0) > Rb(0.3 to 0.4) > NH_4(0.03 to 0.04) > Na, Cs. (3) Current-voltage relations obtained when the external solution contains Rb^+, Cs^+ or Tl^+ in addition to K^+ show: (a) Rb^+ and Cs^+ decrease the K conductance and the rate of decrease becomes greater with an increasing hyperpolarization, thereby the inward-going rectification is reduced; (b) the membrane conductance shows an 'anomalous mole fraction dependence' in Tl-K media, i.e., the conductance becomes minimum at a certain mole fraction; and (c) the current-voltage relation often shows a transition-type behavior suggesting that the membrane undergoes metastable states during an ncrease of hyperpolarization.

Katz (1949) studied the electrical properties of frog twitch muscle fibers in an isotonic K-sulfate solution. In this solution the K concentration was almost identical on either side of the cell membrane. Under such symmetrical conditions he found a much higher membrane conductance for inward current than for outward current. This phenomenon has been referred to as "inward-going" or "anomalous" rectification and its electrophysiological properties have been studied by a number of workers (Hodgkin & Horowicz, 1959; Adrian & Freygang, 1962*a*, *b*; Nakajima, Iwasaki & Obata, 1962;

Adrian, 1964, 1970; Horowicz, Gage & Eisenberg, 1968; Adrian, Chandler & Hodgkin, 1970; Almers, 1971). However, a detailed study of the ion selectivity of this system has not yet been reported. Recently, a similar "inward-going" rectification was found in the egg cell membrane of a tunicate (Takahashi, Miyazaki & Kidokoro, 1971; Miyazaki, Takahashi, Tsuda & Yoshii, 1974) and a starfish (Miyazaki, Oomori & Sasaki, *personal communication*). The experimental analyses show a striking similarity between the rectification in muscle fiber membranes and egg cell membranes. In both cases the K conductance of the membrane shows inward-going rectification and depends on the electrochemical potential of K ions rather than on the absolute membrane potential (Hodgkin & Horowicz, 1959). Replacement of K with Rb results in a marked reduction of the rectification of muscle membranes (Adrian, 1964; Adrian *et al.*, 1970) as well as egg cell membranes (Miyazaki *et al.*, 1974). A study of membrane properties of tunicate cells at various stages of development from egg cell to mature muscle cell shows that the "inward-going" rectification of the egg cell membrane remains almost unaltered during differentiation (Kidokoro, Miyazaki, Takahashi & Tsuda, *in preparation*). This suggests that the inward-going rectification in the egg cell and striated muscle cell operate by similar molecular mechanisms.

A certain species of starfish, *Nordora punciformis*, in the Great Barrier Reef of Australia has egg cells of relatively large size (300 to 400 μm in diameter) making it easy to insert multiple intracellular pipettes. In twitch muscle fibers the relatively large resting Cl permeability overshadows the inward-going rectification and it is, therefore, necessary to eliminate the Cl permeability. In contrast, the resting Cl-permeability in the egg cell of a starfish is negligible (Miyazaki, Oomori & Sasaki, *personal communication*). Therefore, the egg cell membrane of *Nordora punciformis* provides an excellent preparation in which to study inward-going rectification.

Materials and Methods

Materials

Specimens of starfish, *Nordora punciformis*, were collected in shallow lagoons of the Great Barrier Reef of Australia. The experiments were performed in the laboratory vessel, ALPHA HELIX. Specimens were kept in running sea water at 27 °C, the temperature of the water in the lagoon where they were found. Ovarii were isolated from female specimens and kept in sea water. Immature egg cells spontaneously spawned from the ovarii were collected and used for the experiment.

Methods

Eggs were kept on the floor of a Lucite chamber in the mesh of a piece of gauze. The solution in the chamber was continuously perfused and complete exchange of the solution in the chamber required about 2 min. The temperature of the solution was kept at 19 to 20 °C by a thermoelectric device. The compositions of the salines used are listed in Table 1. The basic solution was standard saline (STD saline), the composition of which was very similar to that of the artificial sea water. Monovalent cation species examined were K, Rb, Cs, NH_4 and Tl. All species except Tl were introduced as chloride salts. Because of low solubility of TlCl, all chloride salts in the solutions were replaced with nitrate salts to study the effects of Tl. Desired concentrations of the ion species examined were obtained by mixing the K-Na-free saline, 400 K saline and 400 *X* salines in appropriate proportions. The tonicity of the solution was kept equal to that of the STD saline with Tris-Cl.

Glass micropipettes filled with 3 M KCl containing 0.1 M K salt of EGTA (ethyleneglycol *bis* (aminoethylether)-N, N′-tetra-acetic acid) were used, one for recording membrane potential and the other for applying membrane current. The techniques and precautions for micropipette insertion were the same as described elsewhere (Takahashi *et al.*, 1971). The two micropipettes were inserted into the cell with an angle of 60° between their axes (Eisenberg & Engel, 1970). A large chlorided silver plate in the saline served as an indifferent electrode. A third KCl-filled micropipette was introduced into the external saline and the potential of the external saline was kept at ground level by a feed-back system. The membrane potential was recorded through a cathode follower (ELSA 5, Electronics for Life Sciences). The external solution was altered while the two intracellular electrodes were kept inside the cell. The effect of the test solution upon the electrical properties of the membrane reached a steady state within two minutes which was required for the exchange of the solution in the chamber. Since no significant modification of the internal ionic composition should have occurred in this period the liquid junction potential between the internal electrode and internal solution can be considered unaltered. The liquid junction potential between the external 3 M KCl-filled micropipette and the external solution may introduce an error in the measurement of the change in the membrane potential for the change of the external solution. Calculation from the Henderson-Planck equation indicates that the possible error in the present experiment is at most 2 mV and, therefore, it was neglected. The current electrode was connected to an M4A precision electrometer (WP Instruments) and current pulses were applied through the bridge circuit of the electrometer. The potential at the tip of the current pipette was also continuously observed to monitor if the tip was actually inside the cell. The intensity of the applied current was recorded from the current monitor

Table 1. Composition of salines (mM)

Saline	NaCl	KCl	$CaCl_2$	$MgCl_2$	Tris-OH	HCl	XCl
STD saline	400	10	10	50	84	56	—
Artificial sea water	470	10	10	50	—	—	—
K-Na-free saline	—	—	10	50	576	384	—
400 K saline	—	400	10	50	96	64	—
400 *X* saline	—	—	10	50	96	64	400

Tris-OH, Trizma base. X=Rb, Cs and NH_4. For X=Tl, Cl in the above solutions was replaced with NO_3. The pH of the solution was adjusted to 7.7 with 10 mM Tris-HCl buffer. For Tl experiments, 10 mM Tris-HNO_3 was used.

output of the electrometer together with the membrane potential by means of a Moseley dual ink writer (Model 7100 BM). The time constant of the recording system was about 0.1 sec; sufficiently fast for the present analysis.

Results

Behavior of the Cell Membrane in K Media

The resting membrane potential of an immature egg cell in standard saline ranged between −85 and −82 mV. It did not change significantly during the transfer of the cell into the artificial sea water or when the NaCl from the standard saline was replaced with isosmolar Tris chloride. These solutions all contained 10 mM K. Thus, the difference between the permeabilities of Na^+ and $Tris^+$ is not large enough to produce a significant potential change in the presence of 10 mM K. In contrast, an alteration of the K concentration in the external saline led to a marked change in the resting potential. Records in Fig. 1*A* were obtained when $[K^+]_o$ was increased first from 10 to 25 mM and then to 100 mM in Tris media. The membrane potential attained a steady level for each K concentration in the 1 to 2 min required for the exchange of the solution in the chamber. The steady level of the membrane potential was recorded at varying $[K^+]_o$ in several different cells and the results are summarized in Fig. 2*A* (continuous line, K). Different symbols on the graph represent data obtained

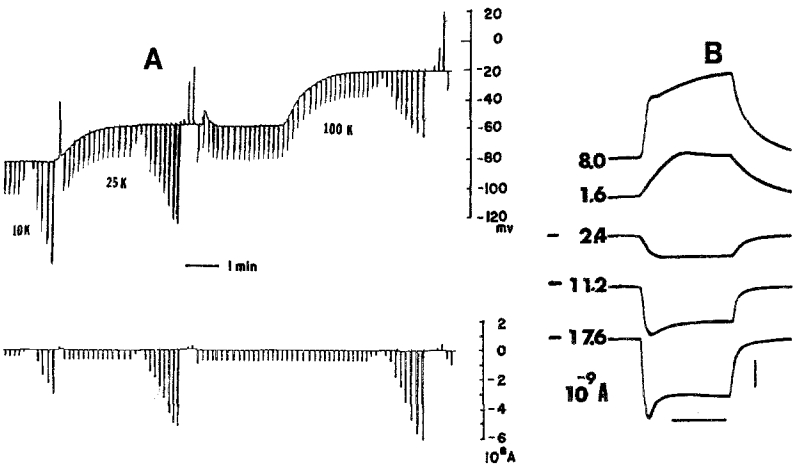

Fig. 1. Potential changes of the egg cell membrane to constant current pulses during the increase in the external K concentration in Tris media (*A*) and in the STD saline (*B*). The top and bottom traces in *A* are membrane potential and applied membrane current, respectively. Vertical and horizontal bars in *B* correspond to 20 mV and 1 sec. The resting potential in *B*, −84 mV. Cell diameter, 300 μm in *A* and 320 μm in *B*

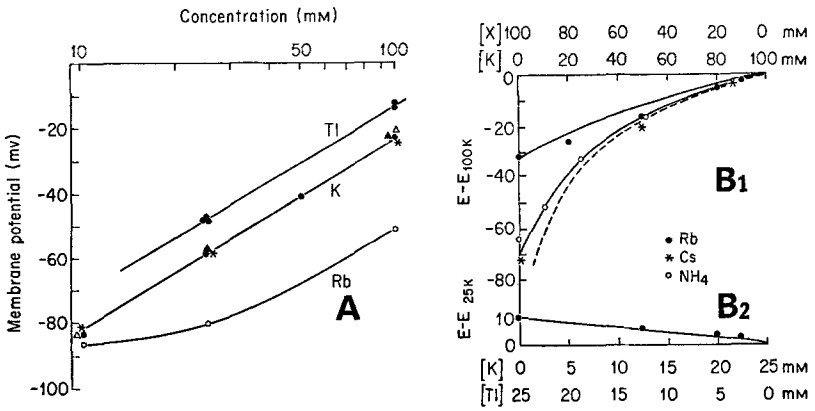

Fig. 2. (*A*) Relations between the membrane potential and the concentration of K^+, Rb^+ or Tl^+ in Tris media. (*B*) Membrane potential changes obtained by replacing a varying fraction of 100 mM (B_1) or 25 mM (B_2) K^+ with Rb^+, Cs^+, NH_4^+ or Tl^+. The membrane potential in the original K solution was taken as the reference potential. Continuous lines were drawn from the Goldman-Hodgkin-Katz equation with $P_{Rb}/P_K = 0.3$, $P_{NH_4}/P_K = 0.04$ and $P_{Tl}/P_K = 1.49$. The broken line in B_1 represents the relation obtained when KCl was replaced with Tris-Cl

from different cells. The results show: (1) membrane potential for a given $[K^+]_o$ is remarkably constant in different cells and (2) the membrane potential $-\log [K^+]_o$ plot is linear with a slope of 58 mV for a tenfold change in $[K^+]_o$. The resting cell membrane behaves as a K electrode, at least for the range of $[K^+]_o$ larger than 10 mM.

Fig. 1*A* shows that there is a marked inward-going rectification in the egg cell membrane. Constant current pulses of different polarities and intensities (shown by the lower trace) were applied to the cell membrane at three different K concentrations in Tris media. The amplitude of potential changes produced by an outward current is substantially greater than that produced by an inward current of a comparable intensity. The time course of the potential change for each current pulse is shown in Fig. 1*B*. The amplitude of potential change to an inward current reached a peak in about 0.3 sec and declined to a steady level in the following 0.5 sec. When the intensity of the outward current pulse was increased, the potential change became more complicated (top trace) and this was probably due to a potential dependent increase of Na or Ca permeabilities of the membrane (Miyazaki, Oomori & Sasaki, *personal communication*).

Fig. 1*A* shows that the amplitude of potential change produced by an inward current of a given intensity decreases as the external K concentration increases. In Fig. 3*A* the membrane potential at the steady state was plotted

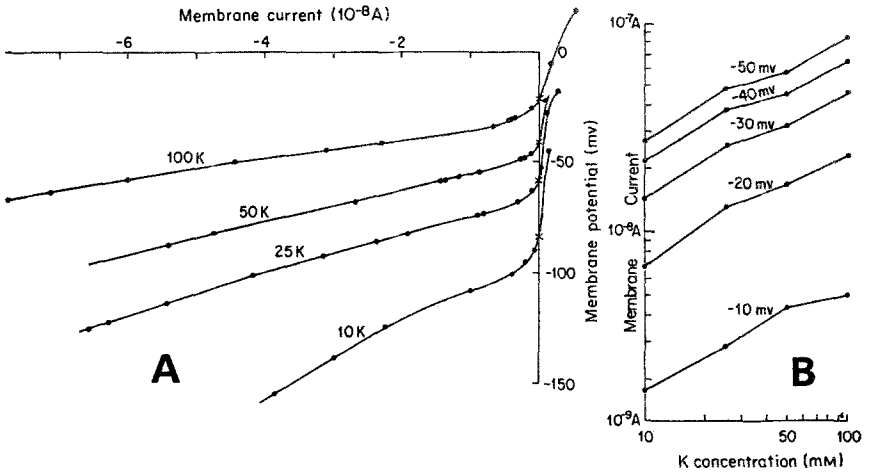

Fig. 3. (*A*) Steady-state current-voltage relations of the membrane of the same cell at $[KCl]_o = 10$, 25, 50 and 100 mM in Tris media. (*B*) The intensity of inward current necessary to produce a hyperpolarization of a given amplitude 10, 20, 30, 40 or 50 mV is plotted against $[K^+]_o$. For further explanation, *see text*. *A* and *B* from the same cell. Cell diameter, 300 μm

against the intensity of the applied current pulse at $[K^+]_o = 10$, 25, 50 and 100 mM. Although a similar set of relations can be obtained for the membrane potential at the initial peak, the behavior of the membrane at the peak will not be discussed in the present paper. The relations show an inward-going rectification and their curvatures show a characteristic change with change in the K concentration. The slope conductance increased with an increasing hyperpolarization. At large hyperpolarizations the relation tended to approach a straight line, the extension of which intersected the potential axis at the zero current membrane potential. The reciprocal of the slope of this straight line will be referred to as the *limiting slope conductance*. At 25 mM K in the case of Fig. 3*A*, the slope conductance estimated at the resting potential was about 0.045 μmho and the limiting conductance was 1.0 μmho. This shows that the slope conductance increases by a factor of 22 during the inward-going rectification. As described already, the resting membrane potential changes with $[K^+]_o$ in the Tris media according to a Nernst slope at least at K concentrations above 10 mM. Since the major species of permeable cations inside the cell is likely to be K^+, the resting potential E_o should approximate the K equilibrium potential E_K. If one assumes that the inward current is carried by K ions, the current-voltage relation can be expressed by:

$$I = g_k \cdot (V - E_K) = g_k \cdot (V - E_o) = g_k \cdot \Delta V. \quad (1)$$

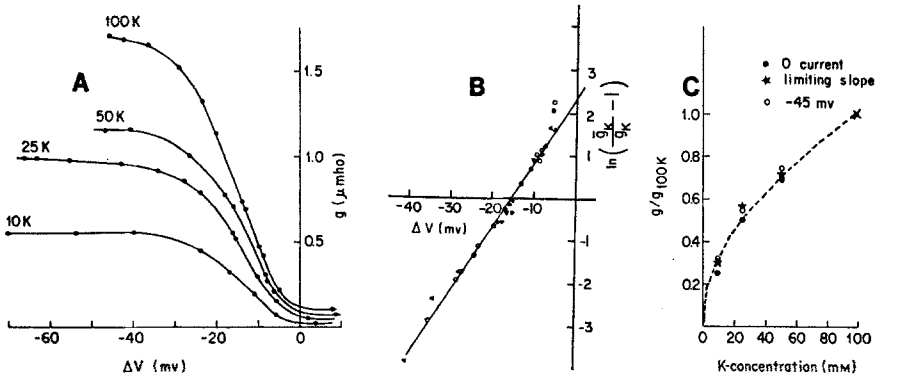

Fig. 4. (*A*) Chord conductance defined by $I=g(V-E_o)$ is plotted against $(V-E_o)=\Delta V$, for different $[K^+]_o$. All relations were obtained from the same cell membrane. (*B*) $\ln\left(\frac{\bar{g}_K}{g_K}-1\right)$ is plotted against ΔV at different K concentrations. Filled circles, 10 mM K^+; open triangles, 25 mM K^+; filled triangle, 50 mM K^+, and open circles, 100 mM K^+. (*C*) The membrane conductance at zero current (filled circles), chord conductance at $\Delta V=-45$ mV (open circles) and limiting slope conductance (filled stars) were plotted against $[K^+]_o$. The conductance at 100 mM K^+ is taken as the unity in each cell. A broken line in the figure is drawn from the assumption that the relative conductance is proportional to the square root of $[K^+]_o$. Cell diameter, 300 μm in *A*

Here g_k represents the K chord conductance and is a function of ΔV and $[K^+]_o$. In order to analyze the form of this function, log I for a given ΔV was plotted against log $[K^+]_o$ in Fig. 3*B*. The five curves represent plots for $\Delta V=-10$, -20, -30, -40 and -50 mV, respectively. The results show: (1) curves for different ΔV's are all parallel, and (2) each plot approximates a straight line of a slope of approximately 1/2. These findings indicate that g_k can be expressed by a product of two factors, one depending exclusively on ΔV and the other exclusively on $[K^+]_o$:

$$g_k=g'_K(\Delta V)\cdot g''_K([K^+]_o). \tag{2}$$

Chord conductances at different $[K^+]_o$ were calculated from the current-voltage relations in Fig. 3*A* and plotted against ΔV in Fig. 4*A*. The conductance shows an S-shaped increase with an increasing hyperpolarization and reaches a saturation level when $\Delta V \fallingdotseq -40$ mV. If $g'_K(\Delta V)$ is defined in such a way that it becomes unity at the saturation level, the shape of the curve suggests that $g'_K(\Delta V)$ is expressed by

$$g'_K(\Delta V)=\frac{1}{1+\exp\left(\frac{\Delta V-\Delta V_h}{v}\right)}. \tag{3}$$

Here ΔV_h represents the ΔV at which g'_K becomes 1/2 and v is a constant which characterizes the curvature of the relationship. From Eqs. (2) and (3), for a given $[K^+]_o$,

$$\ln\left(\frac{\bar{g}_K}{g_K}-1\right)=\frac{\Delta V}{v}-\frac{\Delta V_h}{v} \tag{4}$$

$\bar{g}_K$ being g_K at the saturation level. If the right-hand side of Eq. (4) is calculated and plotted against ΔV, a straight line should be obtained. The result of this calculation is shown in Fig. 4*B*. Four different symbols represent data obtained at four different K concentrations. The plot approximates a single straight line for the range of $\Delta V > 0$. It shows that ΔV_h is about -15.5 mV and v is 6.7 mV.

The fact that the slope of the $\log I - \log [K^+]_o$ line for a fixed ΔV in Fig. 3*B* is approximately 1/2 indicates that g''_K is roughly proportional to the square root of $[K^+]_o$. To analyze this point in detail, the *conductance at zero current* and the *limiting slope conductance* were calculated. The former is g_K at $\Delta V = 0$ or $V = E_o$ and this should, by definition, coincide with the slope conductance at E_o. As mentioned already, the chord conductance g_K approaches a saturation value $\bar{g}_K$ at large hyperpolarizations. This corresponds to the fact that the current-voltage relation approaches a straight line, the extension of which intercepts the V axis at $V = E_o$. In other words, the *limiting slope conductance* of the egg cell membrane in K media coincides with the K chord conductance at the saturation level. When the external saline contains other cations such as Rb^+, Cs^+ or Tl^+, the limiting slope conductance may no longer represent $\bar{g}_K$ and simply represents the slope conductance at large hyperpolarizations. Even under such conditions this conductance is useful in describing the membrane properties during the inward-going rectification. The two kinds of conductances obtained at different $[K^+]_o$ were plotted against $[K^+]_o$ in Fig. 4*C* together with the chord conductance g_K at $\Delta V = -45$ mV. Each type of conductance has been normalized by taking the respective value of $[K^+]_o = 100$ mM as unity. The broken line in the figure was drawn from the assumption that the conductance is proportional to the square root of $[K^+]_o$. The result shows that the three sets of plots all coincide with the calculated curve. Thus,

$$g''_K([K^+]_o) \doteqdot A\sqrt{[K^+]_o} \tag{5}$$

A being a constant.

The specific values of the K chord conductance at saturation level in the cases shown in Fig. 4*A* were 1.9, 3.5, 4.2 and 6.2×10^{-4} mho/cm^2 at $[K^+]_o = 10$, 25, 50 and 100 mM, respectively, since the cell had a spherical shape of 300 μm diameter.

Permeability Sequence among Monovalent Cations

Fig. 2*A* shows changes in the resting potential when the concentration of Tl^+, K^+ or Rb^+ was altered in Tris media. Relations for Tl^+ and K^+ both show a Nernst slope and the difference in the membrane potential for a given concentration indicates $P_{Tl}/P_K \doteqdot 1.5$. The egg cell membrane is more permeable to Tl^+ than K^+. A similar result has been found for the membrane of a squid giant axon (Hagiwara, Eaton, Stuart & Rosenthal, 1972) as well as that of the Ranvier nodes of frog myelinated fibers (Hille, 1972, 1973). The slope of the curve for Rb^+ became significantly smaller when the Rb concentration was low. P_{Rb}/P_K calculated from the difference of the membrane potential at 100 mM was 0.35. Smaller slopes at lower Rb concentrations were probably due to a shunt introduced by the penetration of micropipettes. The membrane conductance became very small at low concentrations of Rb^+ and consequently even a slight shunt may shift the membrane potential significantly away from the one determined by the Rb concentration. The permeability of Cs^+, NH_4^+ or Na^+ is much smaller than that of Rb^+. The following three observations show that only NH_4^+ has a significant permeability: (a) Replacement of 400 mM NaCl in the STD saline containing 10 mM K with Tris-Cl resulted in no appreciable change in the resting potential. (b) Replacement of NH_4Cl in the solution containing 90 mM NH_4 and 10 mM K with Tris-Cl resulted in a hyperpolarization of 6 to 8 mV. (c) The resting potential obtained in 100 mM CsCl was significantly more negative (10 to 15 mV) than that obtained in 100 mM NH_4Cl. Other monovalent cations in these solutions were Tris ions. P_{NH_4}/P_K calculated from observation (b) is $0.03 \sim 0.04$. Thus, the permeability sequence in the resting cell membrane is:

$$Tl\ (1.5) > K(1.0) > Rb(0.3 \text{ to } 0.4) > NH_4(0.03 \text{ to } 0.04) > Na,\ Cs,\ Tris.$$

The present experimental method was not capable of determining differences among permeabilities of Na^+, Cs^+ and $Tris^+$.

Fig. 2*B* shows changes in the resting potential observed when a varying part of either 100 or 25 mM K^+ in Tris media was replaced with Rb^+, Cs^+, NH_4^+ or Tl^+. The resting potential in the original K saline (100 or 25 mM K) was taken as the reference potential. A broken line in the figure represents the result obtained when the K was replaced with Tris. Three continuous lines were calculated by the Goldman-Hodgkin-Katz equation with $P_{Tl}/P_K = 1.5$, $P_{NH_4}/P_K = 0.04$ and $P_{Rb}/P_K = 0.3$, respectively. Although some deviation was seen for Rb^+, one can conclude that the calculated curve agrees

with the observed potential change satisfactorily. In other words, the ratio is a true constant and clearly independent of the concentration and also the membrane potential under these experimental conditions.

Current-Voltage Relations in Other Cation Media

Rubidium. The current-voltage relations illustrated in Fig. 5 were obtained when variable fractions of 100 mM K^+ in Tris media were replaced with Rb^+. Fig. 5*B* was drawn with an expanded current scale to illustrate in detail the relationship in the region of zero current. These relations are very different from those obtained when varying fractions of 100 mM KCl are replaced with Tris-Cl (Fig. 3*A*). The major effect of Rb^+ is to reduce the membrane conductance. The membrane conductance at zero current and the limiting slope conductance were calculated and plotted against the K concentration in the external solution in Fig. 6*A*. They were normalized by taking their values at 100 mM K in the absence of Rb as unity. Comparison between Figs. 6*A* and 4*C*, which were obtained in the presence and absence of Rb, respectively, indicates: (1) the decrease in the conductance is greater if the same amount of K is replaced with Rb instead of Tris even though P_{Rb} is much greater than P_{Tris}, and (2) for a given replacement the

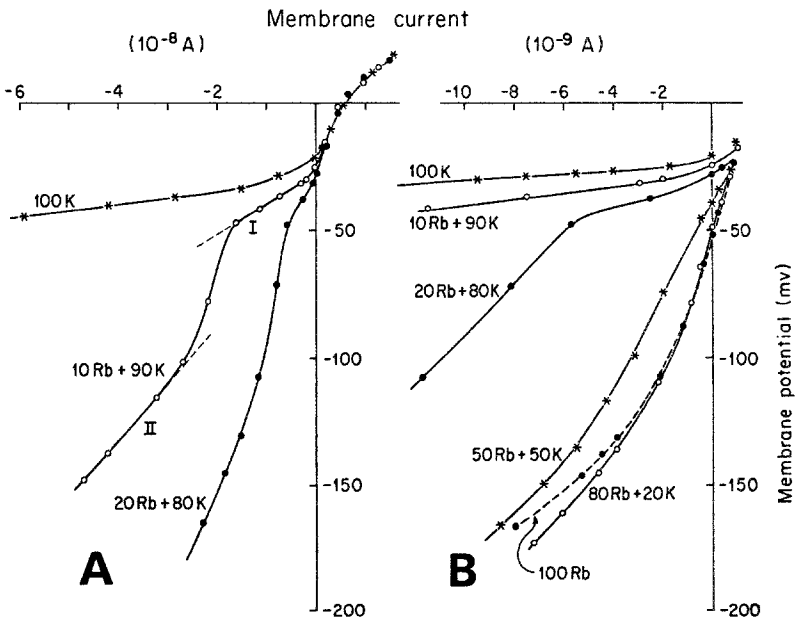

Fig. 5. Steady-state current-voltage relation obtained from the same cell membrane when a varying fraction of 100 mM KCl was replaced with RbCl in Tris saline. *B* is illustrated with an expanded scale of the current axis. Cell diameter, 360 μm

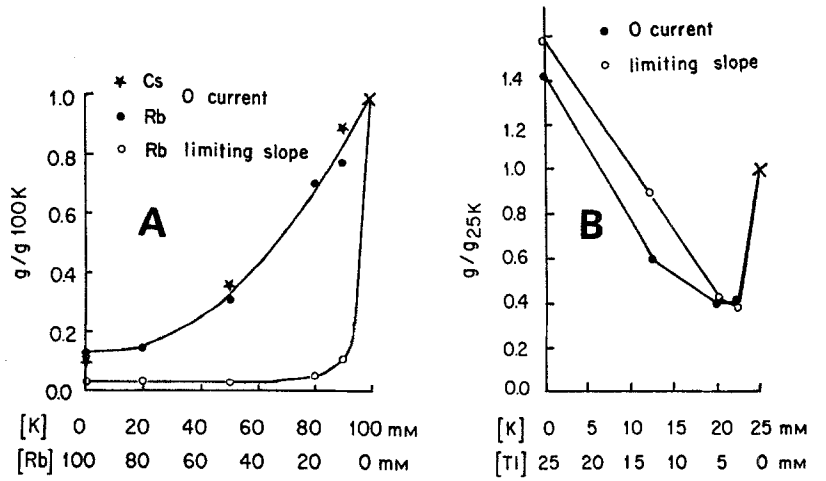

Fig. 6. The relative membrane conductance at zero current and the relative limiting slope conductance when a varying fraction of 100 mM K^+ is replaced with Rb^+ or Cs^+, and of 25 mM K^+ with Tl^+ in Tris media

decrease in the limiting slope conductance is much greater than the decrease in the membrane conductance at zero current. For example, 50% replacement of 100 mM KCl with Tris-Cl reduces both types of conductances to 70% of the original value whereas the same replacement with Rb^+ reduces the conductance at zero current to 30% and the limiting slope conductance to 5% of the original value. In other words, Rb^+ reduces the K conductance and the degree of reduction is greater in the membrane under a large hyperpolarization than when it is at the resting potential. This tends to eliminate the inward-going rectification. This can be demonstrated by showing the ratio between the limiting slope conductance and the conductance at zero current. When an increasing fraction of 100 mM K was replaced with Rb, the ratio became: 10 mM Rb (2.4), 20 mM (1.3), 50 mM (1.3), 80 mM (2.6) and 100 mM (4.5). All these values are substantially smaller than that (18) which is found in K media in the absence of Rb. Besides the decrease in the ratio, the above data suggest an additional property of the ratio as a function of the mole fraction of Rb in the Rb-K medium. The ratio becomes a minimum when the mole fraction of Rb is 0.2 to 0.5. With an increase of the Rb mole fraction above 0.5 there was an increase instead of a decrease. In other words, the curve obtained at 100 mM Rb tended to show a greater inward-going rectification than that obtained at 90 mM Rb + 10 mM K. This corresponds to the fact that the two curves cross each other at about −80 mV. The difference between the two curves, however, was relatively small and, therefore, it may not be safe to conclude that the crossing is actually

significant in the present case. As will be described later, a similar and more evident crossing is found in Cs-K and Tl-K mixtures. Therefore, the above phenomenon has to be considered as one of the general properties of the egg cell membranes exposed to media containing more than one permeant species.

As the fraction of Rb was increased the amplitude of the limiting slope conductance approached that of the zero current conductance. This predicts that the current-voltage relation is approaching linearity. This was, in fact, the case when a large fraction of K^+ was replaced with Rb^+ (*see* curves for 50, 80 and 100 mM Rb in Fig. 5*B*). When the fraction of Rb^+ was small (*see* curves for 10 and 20 mM Rb in Fig. 5*A*), however, two portions of the relation with similar slopes could not be connected with a simple smooth curve. This behavior can be characterized by a transition from one relation to another. For a small negative shift of the membrane potential from the resting potential, the current-voltage relation behaves as if an inward-going rectification similar to that found in K media still exists. For the case for 10 mM Rb + 90 mM K in Fig. 5*A*, the relation would continue along the broken line listed as I if the membrane remained at this state. With further negative shift of the membrane potential, however, the membrane undergoes another stable state whose current-voltage relation is characterized by the line indicated by II. In this state the inward-going rectification is substantially suppressed. The portion of curve between I and II represents the transitional state of the membrane.

Cesium. The current-voltage relation obtained when varying fractions of 100 mM K were replaced with Cs^+ is shown in Fig. 7. It shows that the effect of introducing Cs^+ into K media is essentially similar to that of Rb^+ although the Cs^+ effect is more pronounced in certain aspects. The relative membrane conductance at zero current was calculated and plotted against the K concentration of the Cs-K media in Fig. 6*A*. The result shows that Cs^+ also reduces the K conductance in the resting membrane. In the present experiment the range of the membrane potential was not made large enough to calculate the limiting slope conductance reliably. However, it is likely to be reduced by Cs^+ and hence the inward-going rectification is substantially reduced. The transition type behavior of the current-voltage relation became very marked and striking in Cs-K media. The transition occurs so abruptly that no intermediate state could be demonstrated experimentally. Curves obtained at 100 mM Cs and at 50 mM Cs + 50 mM K cross each other at −150 mV.

Thallium. The four current-voltage relations illustrated in Fig. 8*A* were obtained at 100 mM Tl, 100 mM K, 25 mM Tl and 25 mM K in Tris-NO_3

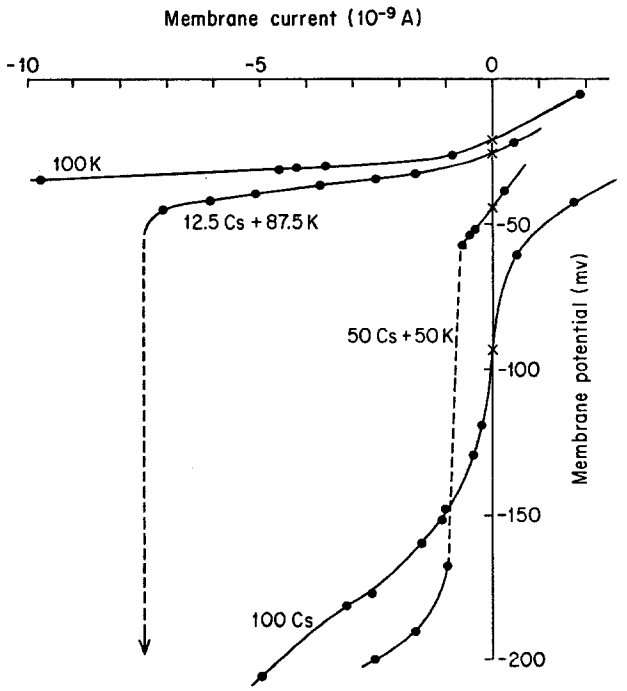

Fig. 7. Steady-state current-voltage relations obtained from the same cell membrane when a varying fraction of 100 mM K^+ was replaced with Cs^+ in Tris media. Cell diameter, 300 μm

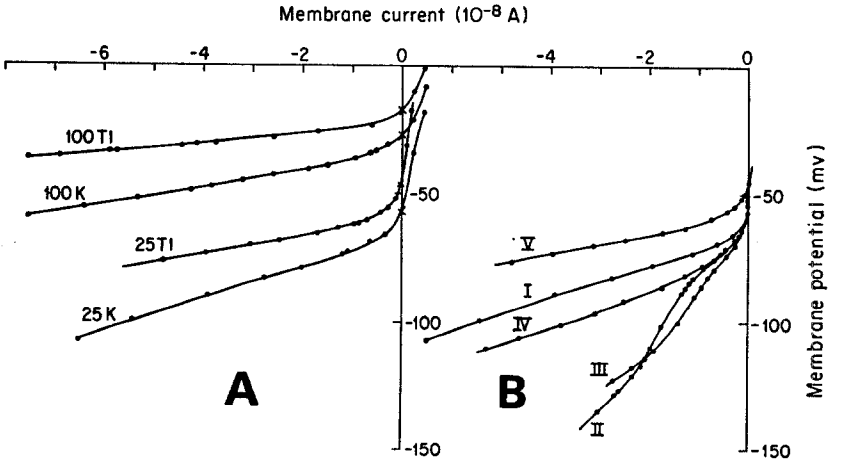

Fig. 8. (*A*) Steady-state current-voltage relations of the same cell membrane obtained at 100 and 25 mM KNO_3 and 100 and 25 mM $TlNO_3$ in Tris media. (*B*) Steady-state current-voltage relations obtained when a varying fraction of 25 mM KNO_3 in Tris media was replaced with $TlNO_3$. I, 25 mM K^+; II, 2.4 mM Tl^+ and 22.5 mM K^+; III, 5 mM Tl^+ and 20 mM K^+; IV, 12.5 mM Tl^+ and 12.5 mM K^+; and V, 25 mM Tl^+. Cell diameter, 300 μm

media, respectively. They show that the effect of the replacement of K^+ with equimolar Tl^+ upon the current-voltage relation is very similar to that found for an increase in the K concentration. The above replacement increased both the membrane conductance at zero current and the limiting slope conductance by a factor of 1.3 to 1.5. If chord conductance g is defined in Tl media by equation $I=g(V-E_o)$, the above finding suggests that g as a function of $(V-E_o)$ has a shape similar to that of g_K as a function of $(V-E_K)$ obtained in K media. As described already, the permeability ratio P_{Tl}/P_K obtained from changes in the resting potential is about 1.5. This indicates that as far as the resting membrane potential is concerned, the equimolar replacement of the total K^+ with Tl^+ is equivalent to an increase of the K concentration by a factor of 1.5. The increase of the K concentration by a factor of 1.5 increases g_K for a given $(V-E_K)$ by a factor of 1.2. The present experimental data suggest that the equimolar replacement of K^+ with Tl^+ increases the chord conductance by a factor of 1.3 to 1.5, which is not very different from that expected from the permeability ratio and the independence principle.

Curves I through V in Fig. 8*B* show current-voltage relations obtained when an increasing fraction (I, 0; II, 1/10; III, 1/5; IV, 1/2; V, 1) of 25 mM K was replaced with Tl^+. The most remarkable result found in the Tl-K media is that both the membrane conductance at zero current and the limiting slope conductance shows an "anomalous mole fraction dependence" in such a way that they become minimum when the fraction of Tl^+, X_{Tl} $(=[Tl^+]_o/([Tl^+]_o+[K^+]_o))$ is $0.1 \sim 0.2$ (*see* Fig. 6*B*). This results in numerous crossings among current-voltage relations obtained at different mole fractions. Transition type behavior in the current-voltage relation was found when X_{Tl} was 0.1 and 0.2. This does not imply direct Tl^+-K^+ interaction, but more likely a change in properties of the 'channel' (e.g., allosteric). It seems analogous to that found in a simple hydrated ion exchanger glass membrane by Eisenman, Sandblom and Walker (1967).

Ammonium. Fig. 9*A* shows current-voltage relations obtained when a varying fraction of 100 mM K in the Tris medium was replaced with NH_4^+. The result does not differ significantly from that obtained when the same replacement was performed with Tris (*see* Fig. 3*A*). In other words, the effect of replacement with NH_4^+ on the current-voltage relation can simply be explained by the decrease in $[K^+]_o$ except for the range of small $[K^+]_o$ $(<10$ mM). This can be shown by plotting the membrane conductance at zero current and the limiting slope conductance against the K concentration

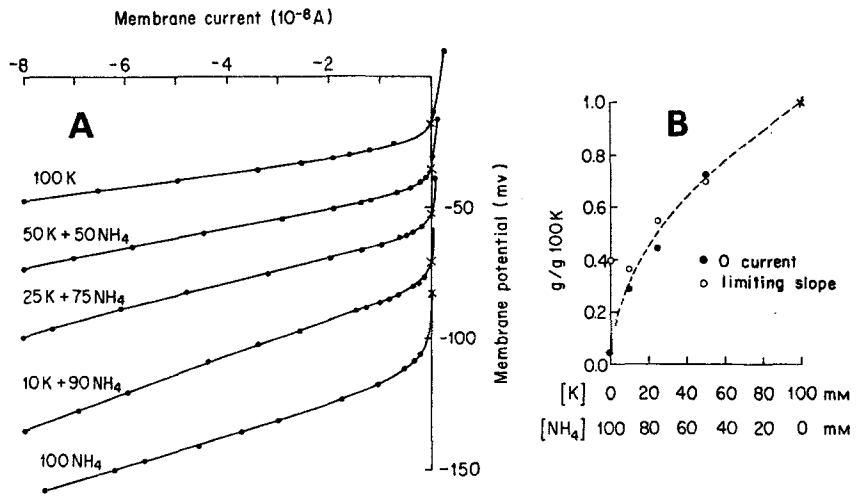

Fig. 9. (*A*) Steady-state current-voltage relations obtained when a varying fraction of 100 mM K^+ in Tris media was replaced with NH_4^+. (*B*) Relative membrane conductance at zero current and relative limiting slope conductance are plotted against the K and NH_4 concentrations. The broken line was drawn from the assumption that the relative conductance is proportional to $\sqrt{[K^+]_o}$. Cell diameter in *A*, 300 μm

of the NH_4-K medium (Fig. 9*B*). The result indicates that NH_4^+ does not interfere with the K current. This does not seem to be due to the low permeability of NH_4^+. The permeability ratio P_{NH_4}/P_K obtained from changes in the resting potential is at least greater than P_{Cs}/P_K and yet Cs^+ reduces the K conductance significantly.

Discussion

The present results show that the K conductance for inward membrane current is expressed by a product of g' and g''; g' being an exclusive function of the electrochemical potential of K ions and g'' an exclusive function of the K concentration. The form of the function g' [Eq. (3)] is identical to the one used by Hodgkin and Huxley (1952) to describe the steady-state potential dependence of the Na inactivation in the squid axon. In both cases a positive shift of the potential inside the cell increases the inactivation. One of the differences, however, is that the resting membrane represents the inactivated state for the inward-going rectification while it represents the absence of inactivation for the Na channel. The other difference is that for the former the important potential is the electrochemical potential of

K ions, whereas it is the membrane potential alone for the latter. This form of dependence is expected if the activation of the K conductance depends on the movement of charged groups within the membrane. One particular example might be the following. When $V = E_K$, positively charged particles locate at a certain site of the K channel thereby inactivating the K conductance. When $\Delta V = V - E_K$ is made negative the particles leave from the sites toward the inner membrane surface and the K conductance is activated. At a large negative ΔV all particles are at the inner surface and the activation of the K conductance reaches a maximum. If P_s and P_i represent the probabilities for the particles to be at the sites and at the inner surface, respectively, and if $P_s + P_i = 1$, P_i can be calculated from Boltzman's principle,

$$P_i = \left(1 + \exp\left(\frac{\alpha z F(\Delta V - \Delta V_h)}{RT}\right)\right)^{-1}. \tag{6}$$

The site and the inner surface are assumed to see a difference in voltage proportional to ΔV and α is the proportionality constant. ΔV_h is ΔV at $P_s = P_i$. R, T, z and F have their usual meaning. The experimental result indicates that $RT/\alpha zF$ is 7 mV. If α is approximately unity, the valence of the charged group is about 3. The present situation can be compared to the suppression of the K channel in the squid axon by quaternary ammonium ions, such as tetraethylammonium (TEA) (Armstrong, 1971). TEA moves from inside into the K channel when the potential inside the axon is made more positive. The molecule occupies the crucial site of the channel, thereby inactivating the channel. The activation of inward-going rectification can then be considered as a reverse of the above phenomenon. The form of function g' is, however, equally compatible with the idea that the K permeation is mediated by negatively charged carrier molecules in the membrane (Horowicz *et al.*, 1968; Adrian, 1970). Therefore, the form of the function alone does not distinguish between carrier and channel mechanisms. Recently, Schneider and Chandler (1973) showed that there is a voltage-dependent charge movement in the membrane of a frog skeletal muscle fiber. The movement of charges is in the same direction as that considered for the inward-going rectification in the membrane of a starfish egg cell. The estimated valence of the charged group is also 2 to 3.

As described already, the zero current membrane conductance and the limiting slope conductance (which corresponds to the K chord conductance at the saturation level) both depend on $[K^+]_o$ approximately with the same function. A similar result has been obtained in frog skeletal muscle fibers

by Almers (1971). The relationship can be approximated by a square root function. It is reasonable to assume that the internal K concentration is unaltered in this case since the egg cell membrane is practically impermeable to Cl. Under such conditions, the dependence upon $[K^+]_o$ of the K conductance can be calculated from the constant field theory. The predicted functions for the conductance at zero current and the square root function are both concave downward. The accuracy of the present measurements does not distinguish between these two cases. Almers (1971) showed that the relation between the zero current membrane conductance and $[K^+]_o$ in frog skeletal muscle fiber significantly deviate from that predicted by the constant field assumption. Although he did not mention it in his paper, Almer's experimental curves suggest that the square root function may also be applicable to a frog muscle fiber. The constant field assumption predicts that the limiting slope conductance depends linearly upon the $[K^+]_o$. The experimental result, therefore, deviates significantly from the above prediction. The present measurements were done only for $[K^+]_o \geqq 10$ mM. Therefore, there is a possibility that the limiting slope conductance may depend linearly upon $[K^+]_o$ when $[K^+]_o$ is very small. Under such a condition, our experimental results represent the portion of a relation when it approaches a saturation level.

The permeability ratios of various monovalent cations with respect to K^+ obtained from the measurement of membrane potential changes are:

$$Tl(1.5) > K(1.0) > Rb(0.3 \text{ to } 0.4) > NH_4(0.03 \text{ to } 0.04) > Na, Cs, Tris.$$

The above sequence is the same as that found for the resting membrane of a squid axon (Binstock & Lecar, 1969; Hagiwara *et al.*, 1972) and for the membrane of Ranvier's node during delayed rectification (Hille, 1973). The actual permeability ratios, however, show significant differences. P_{Rb}/P_K and P_{NH_4}/P_K in the present preparation are substantially smaller than those found for the above nerve membranes, i.e., discrimination of K^+ over Rb^+ and NH_4^+ is much greater in the resting membrane of the egg cell. The difference may reflect differences in the electric field strength at the sites crucial for ion permeation (Eisenman, 1965).

Current-voltage relations obtained when a part of K^+ in the K solution has been replaced with Rb^+, Cs^+ or Tl^+ are complicated but include a number of properties which may suggest the mechanism of cation permeation in the egg cell membrane. Under such polyionic conditions, the term

"K conductance" has to be used operationally since some fraction of the membrane current should be carried by cations other than K^+, at least by Rb^+ and Tl^+. When Rb^+ or Cs^+ is added to the K solution, the K conductance is reduced and the degree of reduction becomes greater with a negative shift of the membrane potential from the resting potential; thereby the inward-going rectification is reduced substantially. The above effect of Rb^+ or Cs^+ upon the K conductance resembles the blocking effect of internal Na^+ or Cs^+ upon the K channel during the outward-going rectification in a squid giant axon, described by Benzanilla and Armstrong (1972). The overall mobility of Rb^+ across the K channel seems to be very small and the mobility of Cs^+ may be completely negligible. Their affinity to the sites of the K channel may be significant, however. Thus, Rb^+ and Cs^+ block the K channel by occupying sites. As the membrane potential is made more negative than the resting potential, more Rb^+ or Cs^+ enters the channel and consequently the proportion of sites occupied by Rb^+ or Cs^+ increases. This mechanism is similar to that proposed for the removal of positively charged particles from the sites which activate the inward-going rectification. For a similar potential gradient across the membrane these particles move towards the inside of the membrane, whereas Rb^+ or Cs^+ move from outside into the channel. In other words, activation and the inactivation of the inward-going rectification occur simultaneously. The transition type behavior of the current-voltage relation often found in Rb-K or Cs-K media suggests that the membrane undergoes metastable states during this process. Both the zero current and limiting slope conductance show an "anomalous mole fraction dependence" in Tl-K media, i.e., the conductance becomes minimum at a certain mole fraction. A similar anomalous dependence of the membrane conductance has been observed for anion permeation in the subsynaptic membrane of a crayfish muscle fiber (Takeuchi & Takeuchi, 1971), in the membrane of a stingray muscle fiber (Hagiwara & Takahashi, 1974) and for cation permeation through a hydrated glass membrane (Eisenman *et al.*, 1967). The last authors have suggested that local structure and, therefore, mobilities and chemical potentials change as a function of the particular ion present. A similar explanation may be applicable to the present case.

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