

# Anomalous Permeabilities of the Egg Cell Membrane of a Starfish in $K^+$ - $Tl^+$ Mixtures

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**ABSTRACT** The electrical properties of "inward" rectifying egg cell membranes of the starfish *Mediastera aequalis* have been studied in the presence of  $K^+$ - $Tl^+$  mixtures. When the ratio of the external concentrations of these ions is changed while their sum is kept constant, both the conductance and the zero-current membrane potential go through a minimum, showing clear discrepancies from theoretical results based on conventional electrodiffusion models (e.g., Goldman's equation). By contrast, when the ratio of the two concentrations is fixed and their sum varied, the potential follows an ideal Nernst slope, consistent with Goldman's equation. The membrane conductance which, according to previous studies on similar membranes, is to be viewed as a function of the displacement of the membrane potential from its resting value  $\Delta V$ , shows marked differences between the cases in which  $K^+$  or  $Tl^+$  are the predominant ions: when  $K^+$  is the predominant permeant ion in solution, the addition of small amounts of  $Tl^+$  inhibits the current, while corresponding blocking effects of  $K^+$  on the current are not observed when  $Tl^+$  is the predominant permeant ion. Also, the time course of the conductance during voltage clamp is different in the two cases, being much faster in  $Tl^+$  than in  $K^+$  solution for comparable values of  $\Delta V$ . Most of the above features are accounted for by a model in which it is assumed that the ionic channels have external binding sites for cations and that their permeability properties depend on the species of the cation bound ( $K^+$  or  $Tl^+$  in the present experiments).

## INTRODUCTION

The egg cell membrane of a starfish shows an inward rectification (Hagiwara and Takahashi, 1974*a*; Miyazaki et al., 1975; Hagiwara et al., 1976), and its properties are very similar to those of the inward, or anomalous, rectification of a frog skeletal muscle fiber (Katz, 1949). Under normal conditions, the membrane current is carried predominantly by  $K^+$  ions during the inward rectification.  $Tl^+$  ions are as permeant as  $K^+$  ions through the membrane when the  $K^+$  in the external solution is replaced with equimolar  $Tl^+$ . However, when a fraction of the  $K^+$  in the solution is replaced by  $Tl^+$ , the membrane conductance does not change monotonically with the molar fraction of  $Tl^+$  [defined as  $c_{Tl}/(c_K + c_{Tl})$ ] but goes through a minimum (Hagiwara and Takahashi, 1974*a*). Eisenman et al. (1967) have previously described similar behavior of ion conductance in a thin, hydrated glass membrane in the presence of  $K^+$ - $Na^+$  mixtures. In addition,

analogous properties of conductance have been found in various biological systems such as in the anion-permeable subsynaptic membrane of a crayfish muscle fiber (Takeuchi and Takeuchi, 1971) and in the nonsynaptic membrane of a stingray muscle fiber (Hagiwara and Takahashi, 1974*b*). Most recently, anomalous molar fraction dependencies of the conductances observed for single gramicidin channels in lipid bilayer membranes have been reported for  $\text{Na}^+\text{-Tl}^+$  (Neher, 1975; Eisenman et al., 1976) and for  $\text{K}^+\text{-Tl}^+$  mixtures (Andersen, 1975). The present paper characterizes the behavior of the anomalous molar fraction-dependent permeability of the egg cell membrane of a starfish *Mediaster aequalis* to  $\text{K}^+$  and  $\text{Tl}^+$  ions, as analyzed by the voltage clamp technique.

#### MATERIALS AND METHODS

Immature egg cells of the starfish *M. aequalis* were used. The collection of eggs and the experimental techniques were similar to those described previously (Hagiwara et al., 1975).

Normal saline used had the following composition:  $\text{KCl}$ , 10 mM;  $\text{NaCl}$ , 470 mM;  $\text{CaCl}_2$ , 10 mM;  $\text{MgCl}_2$ , 50 mM;  $\text{Tris-OH}$ , 10 mM (titrated by  $\text{HCl}$  to pH 7.7). Because of the low solubility of  $\text{TlCl}$ ,  $\text{NO}_3^-$  salts were used in most of the experiments. Some control experiments were performed with acetate salts which yielded identical results. 4 M  $\text{K}$  acetate-filled glass micropipettes (2–5  $\text{M}\Omega$ ) were used instead of  $\text{KCl}$ -filled pipettes. The  $\text{K}^+$  or  $\text{Tl}^+$  solution had the following compositions:  $\text{KNO}_3$  or  $\text{TlNO}_3$ , 150 mM;  $\text{Ca}(\text{NO}_3)_2$ , 10 mM;  $\text{Mg}(\text{NO}_3)_2$ , 50 mM;  $\text{Tris-OH}$ , 406 mM;  $\text{HNO}_3$ , 271 mM (pH 7.7). The composition of  $\text{K}^+\text{-Tl}^+$ -free solution was  $\text{Ca}(\text{NO}_3)_2$ , 10 mM;  $\text{Mg}(\text{NO}_3)_2$ , 50 mM;  $\text{Tris-OH}$ , 586 mM;  $\text{HNO}_3$ , 391 mM (pH, 7.7). Test solutions were made up by mixing these three solutions in appropriate proportions. The experiments were performed at room temperature (21–22°C).

#### RESULTS

The average resting potential of the egg cell in normal saline (10 mM  $\text{K}^+$ ) was  $-73 \pm 3$  mV (SD,  $n = 10$ ). The membrane potential was not altered by replacing either  $\text{Na}^+$  with  $\text{Tris}^+$  or  $\text{Cl}^-$  with  $\text{NO}_3^-$ . When the  $\text{K}^+$  concentration was altered by replacing various fractions of the  $\text{K}^+$  solution with  $\text{Tris}$ , the membrane potential changed with a nearly perfect Nernst slope (Fig. 1 A, filled circles). The cell membrane was also significantly permeable to  $\text{Tl}^+$  ions. When a similar experiment was performed with  $\text{Tl}^+$  instead of  $\text{K}^+$ , the membrane potential changed with concentration also with a nearly perfect Nernst slope (Fig. 1 A, open circles). The two straight lines obtained for  $\text{K}^+$  and  $\text{Tl}^+$  are parallel, and the membrane potential at a given concentration is more positive in  $\text{Tl}^+$  than in  $\text{K}^+$  by  $5.1 \pm 0.4$  mV (SEM,  $n = 6$ ). This result indicates that  $\text{Tl}^+$  is slightly more permeant than  $\text{K}^+$ ,  $P_{\text{Tl}}/P_{\text{K}}$  being 1.2.

If the above permeability ratio were independent of the composition of the external solution, membrane potentials in solutions containing both  $\text{K}^+$  and  $\text{Tl}^+$  could be predicted from the Goldman-Hodgkin-Katz equation. Observed membrane potentials, however, deviated significantly from predicted ones as the solution composition was changed. For example, when one-half of the  $\text{K}^+$  in the  $\text{K}^+$  solution was replaced with  $\text{Tl}^+$  [i.e.  $y = c_{\text{Tl}}/(c_{\text{K}} + c_{\text{Tl}}) = 0.5$ ], while the total concentrations  $c_{\text{K}} + c_{\text{Tl}}$  were kept constant, the predicted membrane potential should have been intermediate between those in pure  $\text{K}^+$  and pure  $\text{Tl}^+$  solutions;

whereas the actual membrane potential observed for a molar fraction of Tl<sup>+</sup>,  $y$ , equal to 0.5 was more negative than those observed in either pure K<sup>+</sup> or Tl<sup>+</sup>, as illustrated by the cross symbols in Fig. 1 A. When the total concentration  $c_K + c_{Tl}$  was altered while keeping  $y$  equal to 0.5, the membrane potential again

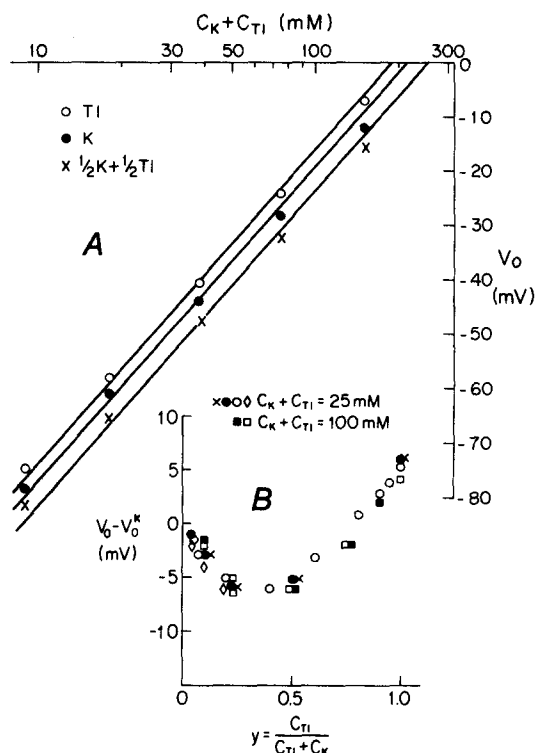


FIGURE 1. A, This figure illustrates the dependence of the zero-current membrane potential (ordinate) on the external K<sup>+</sup>-Tl<sup>+</sup> concentration (abscissa) at a constant molar fraction of Tl<sup>+</sup> (or K<sup>+</sup>). The data points represent observations made on a single egg cell when the permeant cation in the external solution was either K<sup>+</sup> (filled circles) or Tl<sup>+</sup> (open circles), or a 1:1 mixture of K<sup>+</sup> and Tl<sup>+</sup> (crosses). The solid lines are drawn with an ideal Nernst slope of 58 mV per 10-fold increase in the external permeant ion concentration. B, The manner in which the zero-current potential, observed at a total external concentration of K<sup>+</sup> and Tl<sup>+</sup> equal to either 25 mM or 100 mM, varies as a function of the molar fraction of Tl<sup>+</sup>,  $y$  [defined as  $y = c_{Tl}/(c_K + c_{Tl})$ ]. The ordinate gives the difference between the zero-current potential observed at the value of  $y$  indicated by the abscissa and that observed when  $y$  equals zero (i.e. K<sup>+</sup> is the only permeant ion in the external solution). The various symbols represent data obtained from different cells.

changed with a Nernst slope (crosses, Fig. 1 A). This relationship was observed for all values of  $y$  examined. Since the mobility of Tl<sup>+</sup> in the solution is very close to that of K<sup>+</sup>, errors originating from changes in the liquid junction potential between the 4 M K-acetate electrode and the external solution during the replacement of K<sup>+</sup> with equimolar Tl<sup>+</sup> are negligible.

Denoting by  $V_0$  and  $V_0^K$  the experimentally observed membrane potentials in a test solution and in a pure  $K^+$  solution of the same total concentration, respectively, the above result indicates that  $V_0 - V_0^K$  depends only on  $y$  and is independent of the total concentration. The data points in Fig. 1 B, which represent the values of  $V_0 - V_0^K$  as a function of  $y$  show very similar behavior at the total concentrations of 25 mM and 100 mM, characterized in both cases by a minimum between  $y = 0.25$  and  $y = 0.5$ . Identical results were obtained in media in which  $NO_3^-$  had been replaced by acetate. These results strongly suggest that in the presence of  $K^+$  and  $Tl^+$ , membrane permeability depends on the ratio of the concentrations of these ions.

#### Membrane Conductance in $Tl^+$ Media

When  $Tl^+$  is the only permeant ion present in the external solution, the starfish egg membrane shows an anomalous rectification similar to that found in  $K^+$  media. In Fig. 2, the upper set of traces illustrates the time course of the current for membranes clamped at various potentials from the zero-current membrane potential,  $V_0^K$ , in 25 mM  $KNO_3$ . The initial jump of the current (the instantaneous current) is followed by an exponential increase to a steady-state amplitude ( $I_s$ ). The lower traces of Fig. 2 illustrate currents in 25 mM  $TlNO_3$ , showing that the behavior of the membrane current is similar in  $Tl^+$  media. The steady-state  $Tl^+$  conductance  $G_{Tl}$  at a given membrane potential  $V$  is defined as

$$G_{Tl} = I_s / (V - V_0) = I_s / \Delta V. \quad (1)$$

The steady-state  $Tl^+$  conductance can be formally described by the same type of dependence on external permeant-ion concentration and membrane potential as that deduced empirically (Hagiwara and Takahashi, 1974a) and derived theoretically<sup>1</sup> for the case in which only one permeant cation, namely  $K^+$ , was present both outside and inside. This behavior is illustrated in Fig. 3, in which the data points represent the steady-state conductances observed in 10, 25, 50, and 100 mM external concentrations of  $TlNO_3$  upon clamping the membrane to various values of voltage, and the solid curves have been drawn according to the relationship derived by Krasne and co-workers (see footnote 2),

$$G_{Tl} = \frac{B_{Tl} c_{Tl}^{1/2}}{1 + \exp\left(\frac{\Delta V - \Delta V_h^{Tl}}{v}\right)}, \quad (2)$$

where  $c_{Tl}$  is the external concentration of  $Tl^+$ ,  $\Delta V$  is the displacement of the membrane potential from  $V_0$  observed for the particular external  $Tl^+$  concentration, and  $B_{Tl}$ ,  $\Delta V_h^{Tl}$ , and  $v$  are constants for a particular cell. For the data of Fig. 3 these are  $B_{Tl} = 235 \mu\text{mho} \cdot \text{M}^{-1/2}$ ,  $\Delta V_h^{Tl} = 12.2 \text{ mV}$  and  $v = 8.5 \text{ mV}$ .

When the data obtained in  $Tl^+$  solutions were compared with those for the same cell in the  $K^+$  solution, characteristic differences were found in the parameters of Eq. (2):  $B_{Tl}$  is always greater than  $B_K$ , indicating that the saturation value of the steady-state conductance at a given permeant ion concentration is greater for  $Tl^+$ . The value of  $v$  is the same for  $Tl^+$  as for  $K^+$  solutions and is approxi-

<sup>1</sup> Ciani, S., S. Krasne, S. Miyazaki, and S. Hagiwara. Submitted for publication.

<sup>2</sup> Krasne, S., S. Ciani, S. Hagiwara, and S. Miyazaki. Submitted for publication.

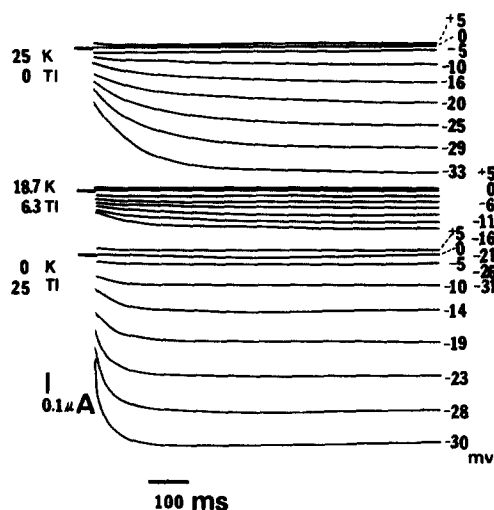


FIGURE 2. Membrane currents recorded during voltage clamp of the same egg cell in three different external solutions. The number to the right of each trace indicates membrane potential during voltage clamp measured from the zero-current membrane potential. The zero-current potential was  $-56$  mV for  $25$  mM  $K^+$  +  $0$  mM  $Tl^+$ ,  $-60$  mV for  $18.7$  mM  $K^+$  +  $6.3$  mM  $Tl^+$ , and  $-53$  mV for  $0$  mM  $K^+$  +  $25$  mM  $Tl^+$ . The diameter of the cell was  $900$   $\mu$ m.

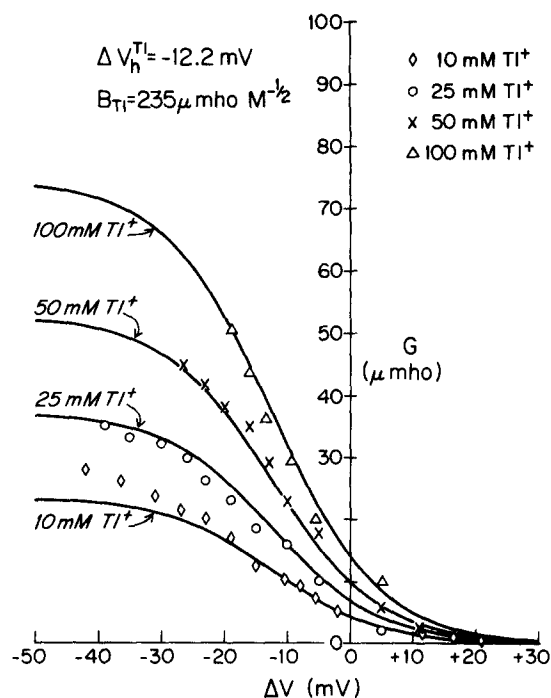


FIGURE 3. Dependence of the steady-state conductance (ordinate) on  $\Delta V$  (abscissa) for four different  $Tl^+$  concentrations in  $K^+$ -free bathing media. The solid curves have been drawn according to Eq. (2) by using the values of the parameters indicated in the figure as well as the value  $v = 8.43$  mV.

mately 8.5 mV. For the anomalous rectification of the  $K^+$  current this value of  $v$ , which is approximately equal to  $RT/3F$ , has been rationalized in terms of a model for gating in which the formation of permeant channels is mediated by orientation of membrane-bound aggregates carrying three charged groups (see footnote 1). The same value of  $v$  in  $Tl^+$  media indicates that a similar interpretation can be applied to the anomalous rectification of the  $Tl^+$  current as well. Fig. 4 shows relations between the steady-state conductance and the membrane potential of the same cell obtained in 25 mM  $K^+$  and 25 mM  $Tl^+$ , respectively. The solid curves were drawn with  $B_{Tl} = 304 \mu\text{mho} \cdot M^{-1/2}$  and  $B_K = 226 \mu\text{mho} \cdot M^{-1/2}$ ;  $v = 8.5 \text{ mV}$ ,  $\Delta V_h^{Tl} = -10 \text{ mV}$ , and  $\Delta V_h^K = -18 \text{ mV}$ .  $\Delta V_h$  is the value of  $\Delta V$  at which the conductance equals one-half of its saturating value.  $\Delta V_h^{Tl}$  was invariably less negative than  $\Delta V_h^K$  in all three other cases examined, the difference being 6 ~ 8 mV. This difference in  $\Delta V_h$  indicates that the degree of

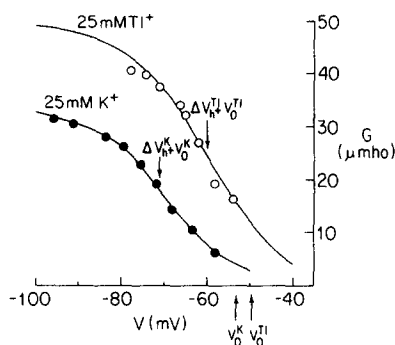


FIGURE 4. Dependence of the steady-state conductance (ordinate) on the membrane potential (abscissa) obtained at 25 mM  $K^+$  and 25 mM  $Tl^+$  in the same egg cell. The solid curves have been drawn according to Eq. (2). For values of the parameters, see text.

activation of the membrane conductance is substantially greater in  $Tl^+$  than in  $K^+$  solution when compared at a given, small  $\Delta V$ .

#### *Membrane Conductance in $K^+ - Tl^+$ Mixtures*

When both  $K^+$  and  $Tl^+$  are present in the external medium, the membrane current (or conductance) at a given value of  $\Delta V$  cannot be predicted simply by summing the currents (or conductances) observed for the same individual concentrations of  $K^+$  or  $Tl^+$  at that value of  $\Delta V$ . In Fig. 2, the upper set of traces illustrates the time course of the current for membranes clamped at various potentials from the zero-current membrane potential  $V_0^K$  in 25 mM  $KNO_3$ . The middle and lower sets of traces were obtained with the same cell after 25% and 100%, respectively, of the 25 mM  $KNO_3$  had been replaced with  $TlNO_3$ . For a given potential shift  $\Delta V$  from  $V_0$ ,  $I_s$  was significantly greater in 100%  $TlNO_3$  but smaller in 25%  $TlNO_3$  than that observed in 25 mM  $KNO_3$ . In other words the membrane conductance shows an anomalous dependence on the molar fraction of the two permeant ions.

Relationships between  $I_s$  and the transmembrane potential  $V$  obtained when various fractions of 25 mM  $K^+$  were replaced with  $Tl^+$  are shown in Fig. 5 A. The following two conclusions can be drawn from this figure: (a) The currents observed at a given transmembrane potential are lower when the bathing

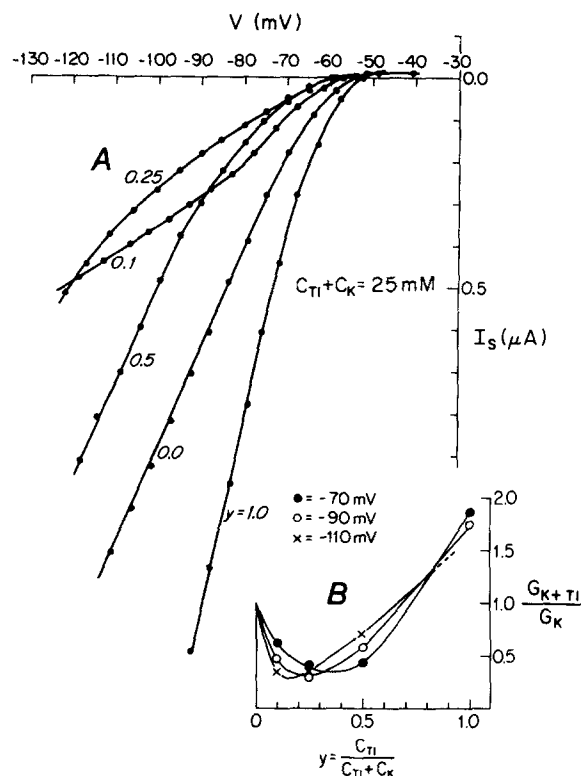


FIGURE 5. A, Steady-state current-voltage relations of the same egg cell membrane obtained at different molar fractions of  $Tl^+$ ,  $y$ . The abscissa is the potential at which the membrane was clamped. The zero-current membrane potentials were -55 mV at  $y = 0.0$ , -57 mV at  $y = 0.1$ , -60 mV at  $y = 0.25$ , -60 mV at  $y = 0.5$ , and -53 mV at  $y = 1.0$ . The cell diameter was 900  $\mu m$ . B, Relationships between the "normalized" conductance and the molar fraction of  $Tl^+$  at different membrane potential  $V$ . The total concentration  $c_{Tl} + c_K$  is 25 mM. The "normalized" conductance is defined as the ratio between the conductance observed at a given  $y$  and a given membrane potential  $V$ , and that observed at the same membrane potential and the same permeant ion concentration when  $K^+$  is the only permeant ion.

medium contains a molar fraction of  $Tl^+$ ,  $y$  equal to 0.1, 0.25, or 0.5, than when the bathing medium contains either pure  $K^+$  or pure  $Tl^+$ ; (b) the current-voltage relationships obtained at different values of  $y$  cross each other, so that  $y$  at which the conductance goes through a minimum varies as a function of the voltage.

The steady-state conductance  $G_{K+Tl}$  defined as  $I_s/\Delta V$  was calculated at each  $y$ . The normalized conductances  $G_{K+Tl}/G_K$  were obtained by dividing  $G_{K+Tl}$  at a

given membrane potential  $V$  by the steady-state conductance observed for 25 mM  $K^+$  (i.e.  $y = 0$ ) at the same  $V$ . When the normalized conductances at different  $V$ 's are plotted as a function of  $y$ , as in Fig. 5B, the most apparent feature is that the conductance goes through a minimum between  $y = 0.2$  and  $y = 0.4$ . The precise molar fraction at which the minimum occurs is membrane potential dependent and shifts toward lower values of  $y$  with an increase in the negative membrane potential. Essentially similar results were obtained for the total concentration  $c_K + c_{Tl} = 100$  mM.

#### *Different Kinetics of the Membrane Current in $K^+$ and $Tl^+$ Media*

The membrane current  $I(\Delta V, t)$  associated with a negative voltage shift  $\Delta V$  from the zero current membrane potential  $V_0$  is expressed by the following equation in  $K^+$  media (Hagiwara et al. 1976).

$$I(\Delta V, t) = I_s(\Delta V) - [I_s(\Delta V) - I_0(\Delta V)]e^{-t/\tau(\Delta V)}. \quad (3)$$

$I_0$  is the instantaneous current, which depends on  $\Delta V$  as well as  $c_K$ , and  $\tau$  is the time constant, which also depends on  $\Delta V$  but has little or no dependence on  $c_K$ . In Fig. 6, the mean values of the logarithm of  $\tau$  observed in  $K^+$  (open circles,  $n = 5$ ) and  $Tl^+$  (filled circles,  $n = 3$ ) solutions are plotted as a function of  $\Delta V$ . Clearly, the kinetics of the conductance increase is much faster in  $Tl^+$  than in  $K^+$  solutions.

In addition, the dependencies of the time constants on  $\Delta V$  measured for a single egg cell at different  $K^+ - Tl^+$  portions are illustrated by the broken curves in Fig. 6. For this cell, the relaxation of the current could be approximately described by Eq. (3) at any given  $y$ . However, the data are not sufficiently accurate or extensive to determine precisely whether the time course of the current reflects a single time constant or multiple time constants. In any case, it is clear that the value deduced for  $\tau$  generally decreases as  $y$  is increased (the apparent small increase between  $y = 0$  and  $y = 0.1$  is not statistically significant), and in contrast to the steady-state conductance and zero-current potential behaviors, there is no reversal in the trend of  $\tau$  vs.  $y$  between  $y = 0.2$  and  $y = 0.5$ .

#### *Blocking Effect of $Tl^+$ upon $K^+$ Current*

The behavior of the zero-current potential data for the very small molar fraction of  $Tl^+$ ,  $y$ , in Fig. 1 B resembles that expected if the membrane were impermeable to  $Tl^+$ ; that is, if one plots the potential against  $\log [K^+]_0$  for the range of  $y$  smaller than 0.2, a Nernst slope is obtained. This suggests that when the major permeant ion in the solution is  $K^+$ , the membrane channels may be in a state in which they are permeable only to  $K^+$  and almost impermeable to  $Tl^+$ , whereas when the major permeant ion is  $Tl^+$ , the membrane channels may be in another state in which the permeability to  $Tl^+$  is significant.

Fig. 7 A shows steady-state membrane currents obtained in 25 mM  $K^+$  solutions containing 0, 0.5, 1.0, and 2.0 mM  $TlNO_3$ , respectively. Addition of  $Tl^+$  at these low concentrations resulted in no observable changes in the zero-current potential. If  $Tl^+$  were simply impermeant, no differences would be expected among current-voltage relations obtained in these solutions, since the  $K^+$  con-



centration is constant. The fact that the amplitude of the current at a given membrane potential decreases as the concentration of Tl<sup>+</sup> increases (see Fig. 7 A) suggests that Tl<sup>+</sup> is not only impermeant but also has a "blocking" effect on the channel. This effect increases as the concentration of Tl<sup>+</sup> increases from 0.5 mM to 2 mM. At a given Tl<sup>+</sup> concentration, the blocking effect increases as the membrane potential becomes more negative. A similar potential-dependent blocking effect on the K<sup>+</sup> current has also been found in the starfish egg membrane in the presence of Cs<sup>+</sup> (Hagiwara et al., 1976). The degree of

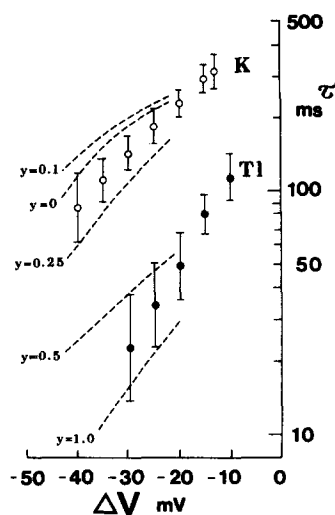


FIGURE 6. The dependence of the time constant,  $\tau$ , for the membrane current on the displacement of the voltage,  $\Delta V$ , from the zero-current potential and on the K<sup>+</sup>-Tl<sup>+</sup> molar fraction in the external solution. The ordinate denotes the logarithm of  $\tau$ , the abscissa denotes the values of  $\Delta V$ , and all data were obtained in external solutions containing K<sup>+</sup> and Tl<sup>+</sup>,  $c_{\text{Tl}} + c_{\text{K}}$  being 25 mM. The data points represent mean values of  $\tau$  and the bars represent standard deviations for the cases in which the only permeant ion in the external solution is K<sup>+</sup> (open circles,  $n = 5$ ) or Tl<sup>+</sup> (filled circles,  $n = 3$ ). The broken curves represent the log  $\tau$  vs.  $\Delta V$  behaviors of a single egg cell when the external solution contained the indicated molar fractions of Tl<sup>+</sup>,  $y$ .

suppression, defined as the ratio, at a given transmembrane voltage, between the steady-state currents in the presence to that in the absence of the blocking ion  $I^+$ , was found to be described by

$$\frac{I'_s}{I_s} = \frac{1}{1 + A[I^+] \exp(-\mu FV/RT)}, \quad (4)$$

where  $\mu$  is an empirically deducible parameter for the membrane-potential dependence of blocking and  $A$  is the constant for a particular cell when the external K<sup>+</sup> ion concentration is constant. In order to examine the applicability of this equation to the blocking effect of Tl<sup>+</sup> on the K<sup>+</sup> current,  $I'_s/I_s$  was calculated

from the data in Fig. 7 A and  $\ln(I_s/I'_s - 1)$  was plotted against  $V$  (Fig. 7 B). Eq. (4) predicts a linear relationship for which  $\mu$  can be determined from the slope and  $A$  can be determined from the intercept at zero voltage. The solid lines in Fig. 7 B were drawn according to Eq. (4) with  $A = 12 \text{ M}^{-1}$  and  $\mu = 1.0$ , the values of  $\mu$  obtained in two other cases being 0.9 and 0.8. These values obtained for  $\mu$  are substantially smaller than those found for the blocking effect of  $\text{Cs}^+$  (Hagiwara et al., 1976,  $\mu = 1.4 - 1.5$ ).

In contrast to the effect of  $\text{Tl}^+$  on the  $\text{K}^+$  current, the addition of small amounts of  $\text{K}^+$  (1 and 2 mM) to a 25 mM  $\text{Tl}^+$  solution results in no significant changes in the membrane current, as shown by Fig. 7 A. Thus, the blocking

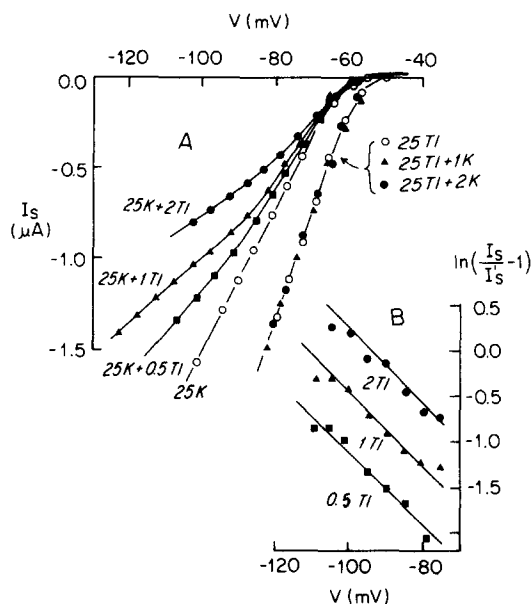


FIGURE 7. A, Steady-state current voltage relations of the same egg cell membrane obtained either in 25 mM  $\text{K}^+$  solutions containing 0, 0.5, 1.0, and 2.0 mM  $\text{Tl}^+$  or in 25 mM  $\text{Tl}^+$  solutions containing 0, 1.0, and 2.0 mM  $\text{K}^+$ . B, See text.

effect of  $\text{K}^+$  on the  $\text{Tl}^+$  current is negligible when the major permeant ion is  $\text{Tl}^+$ . The experimental data in Fig. 7 B are fit reasonably well by eq. (4); however, when the  $\text{Tl}^+$  concentration was increased above 2 mM, the observed current became greater than that predicted by this equation, assuming the values for  $A$  and  $\mu$  given above. This increase corresponds to the fact that increasing the  $\text{Tl}^+$  concentration also increases the  $\text{Tl}^+$  molar fraction,  $\gamma$ , which, above a certain level, appears (cf. Figs. 1 B and 5 B) to increase the membrane permeability to  $\text{Tl}^+$ .

#### DISCUSSION

The following features of the experimental results suggest that membrane channels have different properties when either  $\text{K}^+$  or  $\text{Tl}^+$  is the only permeant ion present in the external solution. (a) The value of  $\Delta V_h$ , corresponding to the

voltage displacement from  $V_0$  at which the steady-state conductance reaches half its saturation value, is constant for a given cell and independent of the ion concentration when the external permeant ion is either  $K^+$  or  $Tl^+$  alone. However, the value of  $\Delta V_h$  differs between  $K^+$  and  $Tl^+$  solutions.  $\Delta V_h^{Tl}$  is always more positive than  $\Delta V_h^K$  by 6 ~ 8 mV when measured on the same cell; that is, the fraction of conductance activated at a given small  $\Delta V$  is much greater in  $Tl^+$  than in  $K^+$  solution. (b) The development of the inward current during the voltage clamp in the  $K^+$  solution follows first-order kinetics. The time constant of the process depends on  $\Delta V$  but has little or no dependence on the  $K^+$  concentration (Hagiwara et al., 1976). The time course of the  $Tl^+$  current also follows first-order kinetics and the time constant depends on  $\Delta V$ . However, the time constant for the  $Tl^+$  current is substantially smaller than that for the  $K^+$  current when compared at a given  $\Delta V$ ; that is, the membrane channels activate much faster in  $Tl^+$  than in  $K^+$  solution. (c) When the major permeant ion in the external solution is  $K^+$ ,  $Tl^+$  not only acts as an impermeant ion, but also has a blocking effect on the current carried by  $K^+$ . These results cannot be explained by simply assuming different selectivities of the same channel for  $K^+$  and  $Tl^+$ .

Ciani and co-workers (see footnote 1) proposed a model which describes anomalous rectification of the  $K^+$  current as being due to a voltage-dependent orientation of charged molecules in the membrane followed by a voltage-independent binding of external  $K^+$  ions to the "gating site" of the oriented molecules. For the sake of brevity, the latter step will be referred to as "ion stabilization." Assuming that the gating site of each channel consists of three negative charges and that three  $K^+$  ions are bound to the gating site of a stable, permeable channel, an expression similar to Eq. (2) can be deduced theoretically. Since Eq. (2) also describes the membrane current in  $Tl^+$  media and since  $v$  is also approximately 8.5 mV (which is one-third of  $RT/F$ ), the same model, with three negative charges on the gating site, is also applicable to the  $Tl^+$  current.

Krasne and co-workers (see footnote 2) have extended the model to the case in which the membrane has both  $K^+$ -stabilized and  $Tl^+$ -stabilized channels, and for simplicity, considered the limiting case in which a single channel binds either three  $K^+$  or three  $Tl^+$  ions.  $Tl^+$ -stabilized channels are assumed to have permeability properties different from those of  $K^+$ -stabilized channels. From a model based on these assumptions, most of the results on the anomalous molar fraction dependences could be accounted for. The larger conductances and greater membrane depolarization observed in  $Tl^+$  compared to  $K^+$  solution are due to the combination of a higher permeability of single  $Tl^+$ -stabilized channels to  $Tl^+$  than of single  $K^+$ -stabilized channels to  $K^+$  and of a larger equilibrium constant for complexation between  $Tl^+$  and the gating site than between  $K^+$  and the gating site. The latter factor was found to be slightly more important and corresponds to the fact that  $\Delta V_h^{Tl}$  is less negative than  $\Delta V_h^K$ . The minimum observed in the membrane conductance and zero-current potential when the molar fraction of  $Tl^+$ ,  $y$ , is varied from 0 to 1 is due to two factors. First, the  $K^+$ -stabilized channel is much more permeable to  $K^+$  than to  $Tl^+$ , whereas the  $Tl^+$ -stabilized channel is slightly more permeable to  $Tl^+$  than to  $K^+$ . Thus, the permeability of a single,  $K^+$ -stabilized channel decreases as the molar fraction of  $Tl^+$  increases, while that of a single  $Tl^+$ -stabilized channel increases with the

molar fraction of  $Tl^+$ . Second, the change in the "state" of the channel occurs much more steeply than the change in molar fraction since the relative proportion of  $K^+$ - and  $Tl^+$ -stabilized channels depends upon the third power of the  $K^+ : Tl^+$  concentration ratio (the concentrations of  $K^+$ - and  $Tl^+$ -stabilized channels being about equal, according to this model, when  $y$  is approximately 0.45). Thus, at small values of  $y$ , virtually all the channels are in the  $K^+$ -stabilized state. In this state, the channel is virtually impermeable to  $Tl^+$ , so that the conductance decreases and the zero-current potentials become more negative as  $K^+$  is replaced by  $Tl^+$ . On the other hand, at large values of  $y$ , virtually all of the channels are in the  $Tl^+$ -stabilized state, in which they are even more permeable to  $Tl^+$  than to  $K^+$ . Finally, the slight shift in the conductance minimum to smaller values of  $y$  with increasing membrane potential is due to the voltage dependence of the blocking effect of  $Tl^+$  on the  $K^+$ -stabilized channels, no such blocking effect having been observed for  $K^+$  in  $Tl^+$ -stabilized channels. The details of the above model will be described in a separate paper (see footnote 2). Although the above model can explain most of the results on the anomalous molar fraction dependence, other approaches (such as to assume that the permeating ion interacts with the channel, altering the permeability properties for the next entering ion) could probably also be used to explain these experimental results. However, thus far no attempt has been made to formulate the predictions expected from other such models.

As noted earlier, anomalous molar fraction dependencies of conductance have been studied in two artificial membrane systems: the glass electrode (Eisenman et al., 1967), and the single gramicidin channel in bilayer membranes (Andersen, 1975; Neher, 1975; Eisenman et al., 1976). Whereas the conductance phenomena observed for these two systems are similar to those observed for the starfish egg cell membrane, important differences appear in the zero-current potential behaviors and the permeability ratios predicted from these behaviors. Thus, in the gramicidin-bilayer system, in the presence of  $Tl^+$  and  $K^+$ , the permeability ratios deduced from zero-current potential measurements vary as a function of the total concentration of  $Tl^+$  and  $K^+$ , whereas in the starfish egg cell membrane the permeability ratios at a given molar fraction of  $Tl^+$  and  $K^+$  are independent of ion concentration. In the case of the glass electrode, the zero-current potential changes monotonically with a change in the  $Na^+ - K^+$  molar fraction (Eisenman et al., 1967), yielding permeability ratios which are constant and independent of the  $Na^+$  and  $K^+$  molar fractions. In the starfish egg cell membrane, on the other hand, the zero-current potential goes through a minimum, increasing the  $Tl^+$ , or decreasing the  $K^+$ , molar fraction. Unfortunately, certain types of observations among the various systems are difficult to compare directly because in the artificial systems measurements can be made as a function of a variation in the solution at either side of the membrane, whereas in the starfish egg cell only the external solution can be readily altered. Also, in the case of gramicidin, conductance minima upon varying  $Na^+ - Tl^+$  or  $K^+ - Tl^+$  molar fractions have been demonstrated for the single channel but, thus far, not for the macroscopic system, while in the starfish cell (and glass electrode), the minimum has been demonstrated only for the macroscopic system, no attempt at measuring single channels having yet been made.

Finally, some precedent for the notion that a membrane conductance pathway may have different properties in Tl<sup>+</sup> solutions than in K<sup>+</sup> solutions comes from the observations reported by Landowne (1975) on radioactive Tl<sup>+</sup> and K<sup>+</sup> flux measurements across the giant axon membrane of the squid. This investigator found that the ouabain-insensitive ratio of influx to efflux was different for K<sup>+</sup> and Tl<sup>+</sup>, the flux ratio for Tl<sup>+</sup> being consistent with a passive, noninteracting flux and that for K<sup>+</sup> being consistent with "single file" passage through the membrane. The possible explanations put forward by the investigator were that there are entirely different permeation pathways for Tl<sup>+</sup> and K<sup>+</sup> ions or, alternatively, that Tl<sup>+</sup> and K<sup>+</sup> ions interact with the same permeation pathway in different ways.

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