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Cation Selectivity of the Resting Membrane of Squid Axon

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Summary. Permeability constant ratios among monovalent cations were studied in the resting membrane of a giant axon of a Pacific squid, *Loligo opalescens*, by observing the relationship between the membrane potential and the ion concentration.

The average permeability ratios are: Tl, 1.8; K, 1.0; Rb, 0.72; Cs, 0.16; Na, <0.08; Li, <0.08. These permeability ratios suggest that neither valinomycin nor nonactin are adequate models for the sites producing the resting permeability in the axonal membrane.

Cyclic polyether *bis*(*t*-butyl cyclohexyl) 18-crown-6 does not increase the permeability ratio P_{Cs}/P_K except when applied at concentrations (5×10^{-5} M) at which the surfactant properties of this molecule may become significant.

The resting membrane of the squid giant axon is predominantly permeable to K^+ ions. Although the permeability to Na^+ ions is smaller than that to K^+ ions, it is not negligible. The permeability constant ratio P_{Na}/P_K as determined by various investigators, ranges between 0.04 and 0.10 (Hodgkin & Katz, 1949; Baker, Hodgkin & Shaw, 1962; Baker, Hodgkin & Meves, 1964). The membrane of the squid giant axon is also permeable to other alkali cations, and the permeability sequence has been shown to be $K^+ > Rb^+ > Cs^+ > Na^+ > Li^+$ (Baker *et al.*, 1962). The actual values of the permeability constant ratios were not determined; however, quantitative estimates of these ratios could be valuable in suggesting the characteristics of permeation in light of recent findings on thin lipid bilayers (Eisenman, Szabo, Ciani, McLaughlin & Krasne, 1972). In addition to the alkali metal cations, some other univalent ions are likely to be permeable. In particular, Tl^+ ion is of interest since its ionic radius approximates that of the alkali cations; and Hille (1972) has recently shown it to be the most permeant ion for the K^+ channel of frog node; however, neither its position in the permeability sequence nor its permeability ratio in the squid giant axon are known. The major purpose of the present work is to determine the permeability ratios between K^+, Rb^+, Cs^+, Na^+, Li^+ and Tl^+ in the resting

membrane of the giant axon of a Pacific squid, *Loligo opalescens*, and to compare these ratios with those of other cell membranes as well as with those of artificial bilayer membranes containing molecular ion carriers.

A further objective of this work was to examine how these permeability ratios might be altered by application to the membrane of a cyclic polyether that is known to act as a carrier molecule in artificial thin lipid bilayers. The permeability sequence and mechanism of ion permeation *via* such neutral molecular carriers has been studied in various artificial membranes (Eisenman, Ciani & Szabo, 1968; Finkelstein & Cass, 1968; Eisenman, Szabo, McLaughlin & Ciani, 1972). Eisenman *et al.* (1972) have shown that cyclic polyether, *bis* (*t*-butyl cyclohexyl) 18-crown-6, (cyclic polyether XXXII) has the affinity sequence $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$, and that in a bilayer membrane the Cs^+ permeability can become almost 100 times the K^+ permeability. We tested this particular carrier since the normal Cs^+ permeability of the squid axon membrane is very small and, therefore, a small additional increase in the Cs^+ permeability caused by the polyether might be detected as an increase in the permeability ratio P_{Cs}/P_K. Stillman, Gilbert, and Robbins (1970) applied monactin to the squid axon membrane but were unable to notice an increase in the K permeability. The lack of effect could be explained as follows. The K permeability is high in the normal untreated membrane so that any additional increase produced by monaction treatment may not be noticeable. Because of the low Cs permeability of the normal squid axon, cyclic polyether XXXII may be more suited for observing an effect of neutral molecular carriers.

Materials and Methods

The giant axons of a Pacific squid, *Loligo opalescens*, were used. Squid were netted from the afterdeck of the R/V Alpha Helix in Isthmus Harbor off Catalina Island, California. The diameter of the axons ranged from 250 to 350 μm.

An isolated axon approximately 5 cm in length was placed in a Lucite chamber. About 3 cm of the central section of the axon was covered with saline. A glass pipette, about 50 to 80 μm in diameter and filled with 0.5 M KCl, was introduced longitudinally into the axon until the tip of the pipette reached the center of the portion of the axon in the saline. A large chlorided silver plate immersed in the saline was connected to ground. Continuous flow of the saline at a rate of about 5 ml/min was maintained by introducing saline through an inlet at one end of the chamber. Excess saline was removed by suction from the other end. For experiments with the alkali metal cations, membrane potential changes were recorded between the internal electrode and a glass microelectrode filled with 3 M KCl (1 to 2 MΩ) placed in the external saline close to the outlet of the chamber. To measure Tl permeability, a microelectrode filled with 1 M KNO_3 was used since TlCl is relatively insoluble. The potential of each electrode was fed to a model M4 electrometer (W.P. Instruments) and their potential difference was recorded by either a Hewlett Packard chart recorder or a Tektronix oscilloscope in conjunction with a Grass camera.

The composition of the normal saline was NaCl, 470 mM; KCl, 10 mM; $CaCl_2$, 10 mM; $MgCl_2$, 50 mM and the pH of the saline was adjusted to 7.8 with 10 mM Tris-HCl buffer. NaCl, KCl, CsCl, RbCl, LiCl and NH_4Cl solutions were obtained by replacing 470 mM NaCl and 10 mM KCl in the normal saline with 480 mM of chloride salt of the respective species. Because of the low solubility of TlCl, the permeability of Tl^+ was compared with K^+ in NO_3 solutions. The composition of the $NaNO_3$ solution was $NaNO_3$, 550 mM; $Ca(NO_3)_2$, 10 mM and the pH was adjusted to 7.8 with 10 mM Tris-HNO_3 buffer. To obtain KNO_3 or $TlNO_3$ solutions of a desired concentration, an appropriate amount of $NaNO_3$ in the $NaNO_3$ solution was replaced with equimolar KNO_3 or $TlNO_3$.

Cyclic polyether XXXII was obtained from Dr. G. Eisenman. The compound was first dissolved in methanol at a concentration of 5×10^{-3} M. This was added to the solution so that the final polyether concentration became 1 to 5×10^{-5} M. The solution, therefore, contained methanol at a concentration of 0.2 to 1.0 cc/100 cc. This concentration of methanol itself neither altered the excitability nor the permeability ratio between Cs and K.

The amplitude of the resting potential of the axon in the normal saline was obtained as a difference between the potential when the tip of the 0.5 M KCl-filled electrode was in the external saline and that found after the insertion of the electrode. It ranged between -48 and -55 mV. This may include an error due to the liquid junction potential between 0.5 M KCl and the external saline or the axoplasm. To measure the permeability constant ratios the change in the membrane potential was observed when the external solution was varied. Since the change was completed within a few minutes no significant modification of the internal ionic composition should have occurred and, therefore, the liquid junction potential between the internal electrode and the axoplasm should have been constant. Then only the liquid junction potential between the external saline and the 3 M KCl electrode had to be considered. Corrections were made in the observed potentials by calculating the junction potential between each solution and 3 M KCl from the Henderson-Planck equation (MacInnes, 1961). The correction had a negligible effect on the calculated values of the permeability ratios except for P_{Cs}/P_K. This was due to the low permeability of the membrane to Cs^+ and to a large difference between the mobilities of Cs^+ and Li^+. A pair of silver wire electrodes was placed at the end of the axon to apply stimulus current pulses. The conducted action potential recorded through the internal 0.5 M KCl-filled electrode had an amplitude of 90 to 100 mV in the normal saline. When the amplitude of the action potential was smaller than 90 mV the experiment was terminated.

All the experiments were performed at room temperature (20 to 21 °C).

Results

Permeability Ratio between K^+ and Li^+ or Na^+

The axon was immersed in LiCl saline since Li^+ is the least permeant of the cations tested. After the membrane potential had reached a steady level, the test solution was applied. The test solution was obtained by replacing various amounts of LiCl in the LiCl saline with equimolar KCl, RbCl or CsCl. After switching from the LiCl saline to the test solution, the membrane potential changed with a relatively slow time course and attained a steady-state level in about 2 min. This potential change was

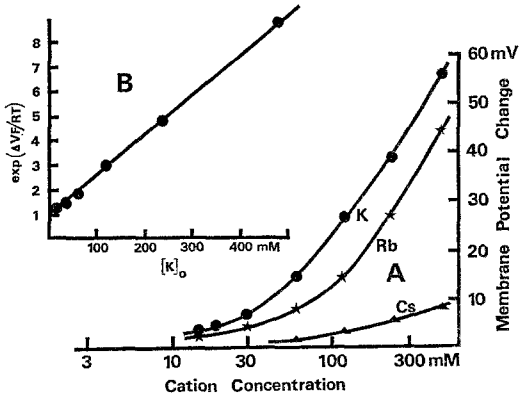

Fig. 1. (*A*) Membrane potential changes produced by various concentrations of different cations. The membrane potential was measured from the potential found in LiCl saline. (*B*) K^+ concentration plotted *vs.* exp ($\Delta VF/RT$). (*See* text for details)

measured as the difference between the potential in the test solution and the potential in the LiCl saline. The axon was frequently returned to the LiCl saline to detect error caused by any drift in the system. Thus, a family of relations between the membrane potential and the concentration of each ion was obtained for each axon as shown in Fig. 1*A*. The permeability ratio P_{Li}/P_K can be obtained from the relationship between the membrane potential and the K^+ concentration using the Goldman, Hodgkin, Katz constant field equation. Since, in the present experiments ($[K^+]_0 + [Li^+]_0$) is always 480 mM, and the internal ionic composition should remain essentially unaltered during application of the test solutions, the difference between the membrane potential found in LiCl saline and that found in different concentrations of K^+ should be:

$$\Delta V = \frac{RT}{F} \ln \frac{f_K[K]_0 + \frac{P_{Li}}{P_K} f_{Li}(480\ \text{mM} - [K]_0) + \frac{P_{Cl}}{P_K} f_{Cl}[Cl]_i}{\frac{P_{Li}}{P_K} f_{Li}(480\ \text{mM}) + \frac{P_{Cl}}{P_K} f_{Cl}[Cl]_i}. \quad (1)$$

f_K, f_{Li}, and f_{Cl} are the activity coefficients of K^+ and Li^+ in the external solution and that of Cl^- in the axoplasm, respectively.

From Eq. (1)

$$\Delta V = \frac{RT}{F} \ln \left\{ 1 + \frac{\frac{P_K}{P_{Li}} \cdot \frac{f_K}{f_{Li}} - 1}{480\ \text{mM} + \frac{P_{Cl}}{P_{Li}} \frac{f_{Cl}}{f_{Li}} [Cl]_i} [K]_0 \right\}. \quad (2)$$

Table 1. Permeability constant ratios

	Cs^+	Rb^+	Tl^+	K^+	Na^+	Li^+	Resting potential (mV)
Axon 1	0.17	0.72		1		<0.08	−55
2	0.18	0.66		1		<0.08	−50
3	0.18	0.71		1		<0.07	−48
4	0.21	0.75		1		<0.09	−48
5			1.70	1	<0.07		−55
6			1.86	1	<0.07		−50
7			1.89	1	<0.09		−55
Average	0.18	0.71	1.82	1	<0.08	<0.08	

Eq. (2) indicates that plotting exp ($\Delta VF/RT$) as a function of $[K]_0$ should result in a straight line which intersects the Y-axis at $Y=1$. Fig. 1*B* is such a plot, obtained from the relation for K in Fig. 1*A*, and shows that this is, in fact, the case. To determine P_{Li}/P_K from the slope of the relation in Fig. 1*B*, the internal Cl concentration and $P_{Cl}f_{Cl}/P_{Li}f_{Li}$ should be known. In the present calculation, 61 mM was taken for the former (Steinbach, 1941) and the latter was assumed to be unity. P_{Li}/P_K thus obtained for the case of Fig. 1*B* was 0.08. In this assumption, P_{Cl} and P_{Li} are assumed to be nearly equal. However, even if P_{Cl} is larger than P_{Li} by a factor of 3, the ratio becomes 0.07. The data obtained by previous workers (Hodgkin & Katz, 1949; Baker *et al.*, 1962; Adelman & Fok, 1964) all indicate that P_{Cl} is larger than P_{Na} and that P_{Na} and P_{Li} are approximately equal. The data obtained here represent, therefore, the upper limit of P_{Li}/P_K. Data for Li obtained for four axons in Cl media and that for Na obtained with three axons in NO_3 media are summarized in Table 1. Almost the same values were found for Na and Li. This was supported by the fact that the replacement of NaCl saline with LiCl saline resulted in either no change in the resting potential or a depolarization of less than 2 mV.

Permeability Ratio between K^+ and Rb^+ or Cs^+

The permeability ratios P_{Rb}/P_K and P_{Cs}/P_K were obtained from a family of relations between the membrane potential and the concentrations of K, Rb and Cs in the same axon (Fig. 1*A*). If the membrane potential, found when the Rb concentration is X, is equal to the membrane potential found when the K concentration is Y, then the following relation is obtained from

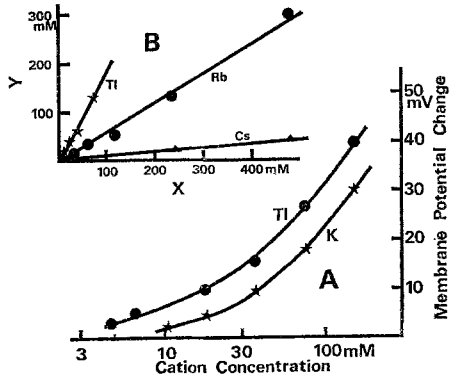

Fig. 2. (*A*) Membrane potential changes produced by various concentrations of K and Tl ions. The potential change was measured from the potential found in $NaNO_3$ saline. (*B*) Cation concentration X is plotted against the K concentration Y which produces the same change in membrane potential

Eq. (1):

$$f_K Y + \frac{P_{Li}}{P_K} \cdot f_{Li}(480\ \text{mM} - Y) = \frac{P_{Rb}}{P_K} f_{Rb} X + \frac{P_{Li}}{P_K} \cdot f_{Li}(480\ \text{mM} - X). \tag{3}$$

Hence,

$$\frac{P_{Rb}}{P_K} = \frac{Y}{X} \frac{f_K}{f_{Rb}} + \frac{P_{Li}}{P_K} \cdot \frac{f_{Li}}{f_{Rb}} \left(1 - \frac{Y}{X}\right)$$

or

$$\frac{Y}{X} = \frac{\dfrac{P_{Rb}}{P_K}\dfrac{f_{Rb}}{f_K} - \dfrac{P_{Li}}{P_K}\dfrac{f_{Li}}{f_K}}{1 - \dfrac{P_{Li}}{P_K}\dfrac{f_{Li}}{f_K}}. \tag{4}$$

A similar equation can also be obtained for Cs^+. The experiment was performed at constant ionic strength and the activity coefficients were obtained from Robinson and Stokes (1959). The K^+ concentration Y which gives the same membrane potential as the potential found at each observed Rb^+ or Cs^+ concentration X was obtained from the relation between $[K]_o$ and the membrane potential. This value of Y was then plotted against the cation concentration X as shown in Fig. 2*B*. Eq. (4) indicates that the plot should be a straight line which intersects the origin if the permeability ratio is independent of the concentration of the test cations; Fig. 2*B* confirms this. To obtain P_{Rb}/P_K or P_{Cs}/P_K the first term of Eq. (3) can immediately be obtained from the slope of the relation and the second term is calculated by using the observed value of P_{Li}/P_K for the same axon. The results are summarized

in Table 1. The second term is negligible for Rb^+ but significant for Cs^+ since the Cs^+ permeability is low and the activity of Cs^+ is less than the activity of Li^+.

Besides the alkali cations, in a few cases NH_4^+ was also applied to the axon. Although no extensive measurements were done, the data indicated that P_{NH_4}/P_K ranged between 0.3 and 0.2. This is approximately the same as that determined by Binstock and Lecar (1969) in another species of squid.

The Permeability to Tl^+

Replacement of 10 mM $NaNO_3$ by equimolar KNO_3 in the normal $NaNO_3$ solution usually resulted in a depolarization of a few millivolts. The action potential was maintained in this solution. Fig. 2*A* shows relations between the membrane potential and the K or Tl concentrations when a varying amount of $NaNO_3$ of the $NaNO_3$ solution was replaced by equimolar KNO_3 or $TlNO_3$. The reference membrane potential was the potential found in pure $NaNO_3$ solution. The permeability ratio P_{Tl}/P_K was obtained in a manner similar to that used for the Rb and Cs ratios. The ionic strength of the external solution was maintained constant. Activity coefficients were obtained for $NaNO_3$ and KNO_3 from Robinson and Stokes (1959). The activity coefficient for Tl was obtained by extrapolation of the activity coefficient *vs.* ionic strength curve obtained from the same source with a parabolic function as the approximation. Table 1 summarizes the data obtained with three axons. The results indicate that the permeability of the membrane to Tl^+ is almost twice that to K^+.

When 4 mM of Na^+ in the $NaNO_3$ saline was replaced with Tl^+, the resting potential was similar to that found when 10 mM of $NaNO_3$ was replaced with KNO_3. The effect of Tl upon the resting potential was reversible and did not affect the potential change produced by the subsequent application of K^+ solutions; however, if the axon was kept in high concentrations of Tl^+ (above 75 mM) for more than 20 min the membrane often underwent irreversible depolarization. Although the action potential could be maintained in a 4-mM solution of Tl^+, its amplitude often showed a gradual decrease during a prolonged immersion.

Because of its chemical similarity to Tl^+ ion, the effect of Ag^+ on thin lipid bilayers has been examined (Eisenman, Szabo, Ciani, McLaughlin & Krasne, 1972); however, Ag is considered very toxic to biological membranes. In a few of the present experiments, 5 mM of the $NaNO_3$ in the $NaNO_3$ external saline was replaced with $AgNO_3$. This immediately resulted

in an irreversible elimination of the resting potential of the axon. This is probably because of the ability of Ag^+ to act as a better oxidizing agent than Tl^+ ion.

Effect of Cyclic Polyether XXXII

The dependence of the membrane potential upon the external K^+ or Cs^+ concentration was observed before and after the application of cyclic polyether XXXII. The K^+ or Cs^+ concentration was altered by diluting with LiCl saline. In concentrations of 10^{-5} M, the polyether after 10 min did not alter the resting potential or the permeability ratio P_{Cs}/P_K. If the effect of the polyether is similar to that found in artificial bilayers, one would expect this concentration to increase the permeability of the membrane to Cs significantly. The polyether did abolish the action potential, indicating that it had gained access to the membrane. In higher concentrations (5×10^{-5} M) and after 30 min the ratio P_{Cs}/P_K still remained unaltered. During prolonged immersion in 5×10^{-5} M polyether, the membrane gradually depolarized (about 5 mV/hr). After 1 hr or more, the permeability ratio P_{Cs}/P_K did increase by a factor of 2 to 4; however, this was always soon followed by a rapid irreversible loss of membrane potential.

Some of these phenomena could be due to surfactant properties of the compound rather than its action as a neutral molecular carrier. This molecule appears to cause disruption of thin lipid bilayers at concentrations comparable with those that caused changes in the axon membrane (G. Szabo, *personal communication*).

Discussion

The permeability ratios obtained above for the resting squid axon membrane are summarized in Table 2 together with the data obtained in other preparations. The similarity between the permeability ratios in the various preparations is striking.

According to Eisenman (1965) the sequence of cation binding to a membrane site can be explained by considering the interaction of the ions with membrane negative charges on the one hand and with water molecules on the other. When the membrane site has a high electric field strength, the differences in the free energy of interaction of the various cations with the site will play the predominant role in determining the selectivity sequence. Conversely, for a weakly negative site, the affinity should be primarily determined by the difference in hydration energies of the ions. The sequence observed for the squid axon $K > Rb > Cs > Na \geqq Li$ is an intermediate sequence between the sequence for a strong site and that for a weak site

Table 2. Permeability ratios of the cell membrane in various tissues

Preparation	Cs	NH_4	Rb	Tl	K	Na	Li	Source
Squid axon (resting)	0.18	(0.2–0.3)	0.71	1.82	1	<0.08	<0.08	Present data
	—	—	—	—	1	0.04	—	Hodgkin & Katz, 1949
	—	—	—	—	1	0.06–0.08	—	Baker, Hodgkin & Shaw, 1962
	—	—	—	—	1	0.05	—	Baker, Hodgkin & Meves, 1964
	—	0.2	—	—	1	—	—	Binstock & Lecar, 1969
Frog skeletal muscle (resting)	0.11	—	0.23	—	1	—	—	Sjodin, 1959
	—	—	—	—	1	0.01	—	Hodgkin & Horowicz, 1959
Barnacle muscle fiber (resting)	0.19	—	0.77	—	1	0.1	0.07	Hagiwara, Toyama & Hayashi, 1971
Lobster muscle (resting)	0.16	—	0.6	—	1	—	—	Gainer (*see* Eisenman, 1963)
Neuroglia cell	0.32	—	0.62	—	1	<0.01	—	Braucho *(personal communication)*
Squid axon (K channel)	—	—	1	—	1	0.03	—	Baker, Hodgkin & Shaw, 1962 (*see* Eisenman, 1963)
	—	0.3	—	—	—	—	—	Binstock & Lecar, 1969
Frog Ranvier node (K channel)	<0.1	0.13	0.9	2.1	1	<0.05	<0.1	Hille, 1972
Valinomycin	0.76	0.039	1.8	0.105	1	3.6×10^{-4}	4.7×10^{-5}	Eisenman, Szabo, Ciani, McLaughlin, Krasne, 1972
Nonactin	0.033	8	0.58	7.4	1	7.1×10^{-3}	$<10^{-3}$	

and corresponds to series IV of Eisenman. If the permeability ratio is mainly determined by the partition of ions between the membrane and the water phases, Eisenman's results suggest that the permeability ratios become identical to the binding ratio and a function of the field strength of the membrane site or the site in the carrier molecule. In other words, $P_K : P_{Rb} : P_{Cs} : P_{Na} : P_{Li}$ should be uniquely determined if the field strength of the site is given. In fact, Eisenman (1965) has shown that if the ratio of any pair, e.g., P_{Rb}/P_K is given, then the other ratios are determined. The permeability ratios obtained with two neutral molecular carriers, valinomycin and the macrotetralide, nonactin, are included in Table 2. The selectivity characteristic of valinomycin (Eisenman, Szabo, Ciani, McLaughlin & Krasne, 1972, and G. Szabo, *personal communication*) resembles the data obtained with biological membranes; the resemblance is less marked for nonactin. One of the important features is that the NH_4^+ permeability ratio, P_{NH_3}/P_K, is significantly smaller than unity both for valinomycin and for the resting axonal membrane, whereas it is significantly larger than unity for nonactin. From this observation, Eisenman, Szabo, McLaughlin and Ciani (1972) suggest that the membrane site or carrier responsible for the resting cation permeability or the K channel of the axon may resemble the structure of valinomycin in having six centrally directed carbonyl oxygens. If the membrane sites are like valinomycin, then the Tl^+ permeability should also be relatively low. However, our data for Tl^+ shows that the permeability of this cation is approximately twice that of K^+ ions. A similar result has been obtained for the K^+ channel of the nodal membrane of a frog nerve fiber (Hille, 1972). Since the Tl^+ permeability ratio suggests that nerve membrane channels resemble the selectivity of nonactin rather than valinomycin, neither of these carriers would seem to be accurate models of the permeability site for K in the axonal membrane.

A second finding of these experiments is that cyclic polyether XXXII does not increase the Cs permeability in concentrations which leave the resting potential unaltered. This is somewhat surprising in light of the results obtained with artificial lipid bilayers. Eisenman, Szabo, McLaughlin and Ciani (1972) measured the conductance of asolectin membrane in 1 mM alkali cation solutions at various concentrations of polyether XXXII. To compare these data to the results obtained with squid axon, the following conditions were assumed to be true: (a) The conductance increases linearly with the ion concentration. (b) The conductance g_{AB} of the membrane which separates solution A from solution B is given by the geometric mean of g_{AA} and g_{BB} which are obtained under symmetrical conditions (Ciani, Szabo & Eisenman, 1969). (c) The axoplasm is assumed to be a solution of

500 mM K. (d) The negative surface potential of the axon is considered much smaller than that of an asolectin membrane. Under these conditions, membrane conductance produced by 2.5×10^{-5} M polyether XXXII in normal seawater (10 mM K) should be about 5 kΩ cm^2 if the axonal membrane is similar to bilayers. This is much larger than the resting membrane resistance (1 kΩ cm^2) of the real axon in the normal seawater. In other words, the effect of polyether may not be detected. When 10 mM K in the seawater is replaced with 10 mM Cs in the presence of 2.5×10^{-5} M polyether, the resistance of this hypothetical membrane should decrease to a value smaller than 400 Ω cm^2. This value is significantly smaller than the resting membrane resistance of the real axon. In the present experiment, no measurement was made to determine the membrane resistance. However, for a decrase in resistance of this magnitude, a significant increase of the Cs permeability should have been observed. The lack of effect of polyether XXXII on the axon membrane, therefore, may provoke reconsideration of the differences between biological membranes and artificial bilayers. There is, however, one possible explanation for the ineffectiveness of polyether in the axonal membrane. Polyether XXXII has a very high affinity for K with which it forms a one-to-one aqueous complex. This complex cannot act as an ionic carrier in the membrane. Because of the high concentration of K inside the axon, the concentration of polyether in the membrane could be reduced by the formation of the complex to a level much lower than that expected from the external concentration and the partition coefficient into the membrane. This reduction would continue until the one-to-one complex and free polyether inside the axon had reached equilibrium with the external polyether concentration. Since this may take an extremely long time, the actual concentration of polyether in the membrane during the time course of our experiments may have been too small to produce an observable effect. This difficulty could be overcome by perfusing internally and externally with symmetrical Cs^+ solutions; however, if this were necessary to observe an effect, the utility of polyether XXXII as a probe of biological membranes would be significantly reduced since most preparations are not easily depleted of their internal K^+.

Addendum

After submission of this paper, Krasne and Eisenman (Membranes—A series of Advances, Vol. 2. G. Eisenman, editor. Marcel Dekker, New York, *In Press*, 1972) have shown that polyether XXXII has selectivity ratios for Tl^+ and NH_4^+ similar to those found in the resting axon membrane. Consequently, they suggest that the site associated with resting permeability in biological membranes should have sixfold coordination like valinomycin; but rather than carbonyl oxygens, the site ought to have ether oxygens as ligand groups similar to the ligands in nonactin.

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