ELECTROCHEMICAL PROPERTIES OF HYDRATED CATION-SELECTIVE GLASS MEMBRANE

A MODEL OF K^+ AND Na^+ TRANSPORT

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ABSTRACT Electrochemical properties of cation-selective glass microelectrodes made from $NAS_{27\text{-}04}$ were studied. There was a marked fall in electrical resistance of the microelectrodes stored in 3 M KCl solution (aging). The resistance was in the range of 2×10^7 to 10^9 Ω, which were much lower than those estimated from the electrical resistivity of dry glass for the equivalent dimensions of microelectrode working tips. This fall in resistance was accompanied by an increase in microelectrode selectivity for K^+. The low resistance and increased K^+ selectivity are desirable features that make the microelectrode more suitable for application to biologic studies. The changes in microelectrode resistance and selectivity were interpreted to be due to hydration of the entire thickness of the glass membrane, resulting in a change in the field strength of anionic sites and formation of ionic channels in the glass membrane. Thus, the fall in resistance is explained by decrease in energy barrier, which is equivalent to the activation energy of interaction between the cations and anionic sites in the glass membrane. Some of the microelectrodes showed a transient depolarization that resembled the action potential of a biological membrane. This transient depolarization was associated with the changes in microelectrode resistance and selectivity. The transient depolarizations suggest the temporary development of wide channels in the membrane permitting free movement of hydrated cations according to the bulk electrochemical gradient.

INTRODUCTION

Understanding of cation-selective glass membrane microelectrodes is important in the following two aspects. First, these microelectrodes can be applied to measure directly K and Na ion activities in living cells. Second, the membrane made from the cation-selective glass may be considered to be a physicochemical model of biological membranes. In the glass membrane framework, silicon and aluminum atoms hold oxygen atoms which are thought to give the negatively charged sites. In the biological membrane, carbon and phosphorous atoms hold oxygen atoms which are also thought to give the negative charges. Further, glass membranes made from $NAS_{27\text{-}04}$ glass are selectively more permeable to K ions than to Na ions and can show transient depolarization of their membrane potentials like the action potential of biological membranes. Therefore, investigation of the electrochemical properties of the glass membrane may provide some insight into the mechanism of monovalent cation transport through the biological membrane and its electrochemical complexity.

Over the last 15 years, the relative selectivity of the glasses with different composition to the alkali metal cations has been extensively investigated. Eisenmen et al. (1957; 1962) observed systematic relationships between the glass compositions and their relative selectivities to the alkali metal cations. The specificity of the glass (Na_2O—Al_2O_3—SiO_2 system) electrodes to the monovalent cations was studied and discussed at the atomic level (Eisenman, 1962). Further, the origin of these glass electrode potentials was analyzed by Eisenman (1965) from both theoretical and experimental points of view, leading to the conclusion that the origin of the glass electrode potential is a result of an ion-exchange process and that the observed total potential is the sum of diffusion and phase-boundary potentials. As the result of Eisenman's work, it was possible for the cation-selective glass electrodes to be applied to biological and chemical research for the measurement of the monovalent cation activities. One of the most important applications is the development of cation-selective glass microelectrodes for the measurement of K and Na ion activities in the living cells. Hinke (1959) made cation-selective glass microelectrodes which had tip diameters of 20–30 μm, and were insulated by ordinary glass micropipettes so that they had exposed tip lengths of 70–90 μm. On the basis of Hinke's design, Lev and Buzhinsky (1961) constructed the K^+-selective glass microelectrodes with tip diameter of 0.6–1.5 μm and exposed tip length of 3–5 μm. Recently Lee and Armstrong (1972) made cation-selective glass microelectrodes from $NAS_{27\text{-}04}$ glass by modifications of the techniques of Hinke (1959) and Lev and Buzhinsky (1961). These microelectrodes showed increased stability of the potentials and had improved physical dimensions (Lee, 1972; Lee and Armstrong, 1973).

During practical application of the cation-selective microelectrodes, problems appeared that made meaningful experiments difficult. These problems presumably originated from the complicated electrochemical properties of the microelectrodes, including complexity of the glass structure, hydration effects on the glass, and high resistance of the microelectrodes. It has been reported that hydration effects on the glass altered the glass structure as well as ion mobilities in the glass (Eisenman, 1965; 1967). By tracer experiments, Eisenman (1967; 1968) estimated that in $NAS_{27\text{-}04}$ glass a hydrated surface layer was thicker than 10,000 Å. He also reported that the ion-exchange affinities in this hydrated layer were markedly different from those of the dry glass in the interior of the glass membrane, resulting in a dramatic reduction in electrode resistance and a change in specificity (1969). Lev (1969) reported that the resistance of a large number of cation-selective glass microelectrodes made from $NAS_{27.5-4.8}$ glass was almost the same as that of the conventional open-tip micropipette electrodes with tip diameter less than 0.4 μm. The mean resistance value of the microelectrodes was $1.68\ (\pm\ 0.94) \times 10^9\ \Omega$ (36 microelectrodes). To account for this low resistance, he concluded that in a considerable number of the cation-selective glass microelectrodes there are narrow pores in the sealed part of the capillary tips. Such microelectrodes are said to be "incompletely sealed".

In the present study it was observed that the resistance of cation-selective microelectrodes made from NAS_{27-04} glass was quite variable. The range of the microelectrode resistance was from 2×10^7 to $10^{10}\ \Omega$. The resistance was dependent on the time stored in 3 M KCl solution, the thickness of the glass membrane at the microelectrode tip, and the exposed area of thin glass membrane of the microelectrodes to a test solution. Also, the selectivity coefficient (relative specificity) of the microelectrodes changed with the time stored in 3 M KCl solution together with the change in their resistance. It is difficult to explain these phenomena in terms of narrow pores at the tips of "incompletely sealed" microelectrodes.

The present study is concerned with the relationships between hydration effect, electrical resistance, and selectivity coefficient of the cation-selective glass membrane microelectrodes. It offers some help in understanding the electrochemical properties of the microelectrodes, as well as facilitating their application. Very recently it was observed that the thin glass membrane of the microelectrodes made from NAS_{27-04} was transiently depolarized, like the action potentials of the excitable cell membrane. The possible mechanisms of Na and K ion transport in this glass membrane are discussed in terms of ionic channels and energy barriers in the glass membrane, in connection with the transient depolarization.

METHODS

Construction of Cation-Selective Glass Microelectrodes

K^+-selective glass (NAS_{27-04}, Corning Glass Works, Medfield, Mass.) was used for construction of cation-selective glass microelectrodes with sealed tips. The composition of the glass is described according to the notation of Eisenman et al. (1957). NAS_{27-04} glass is composed of 27 mol % Na_2O, 4 mol % Al_2O_3, and 69 mol % SiO_2. This glass was obtained as capillary tubes with an inner diameter of 0.020 ± 0.003 in and an outer diameter of 0.040 ± 0.004 in. Micropipettes were drawn from this glass tube using a vertical micropipette puller (David Kopf Instruments, Tujunga, Calif.; Model 700B). The drawn micropipettes had tip diameters less than 1 μm. The tip of the micropipette was sealed immediately after drawing in the following way. The micropipette was fixed by a holder of a micromanipulator over a microscope table so that the tip portion of the micropipette could be seen in the field of the microscope under 200 times magnification. Then a platinum wire loop was positioned near the tip of the micropipette. The platinum wire loop was then heated by electrical current through the wire, which could be controlled by a power stat (Type 116, Superior Electric Co., Bristol, Conn.) and a transformer. After the tips were sealed, the micropipettes were filled with methanol by evacuation. The methanol in the micropipettes was replaced with distilled water and then the distilled water was again replaced with 3 M KCl solution by diffusion. The sealed micropipettes filled with 3 M KCl solution were kept in 3 M KCl solution throughout experiments. When insulation of these micropipette electrodes was necessary, outer insulating micropipettes were prepared from Kimax glass tubes (Kimble Products, Toledo, Ohio) with an inner diameter of 1.6 mm and an outer diameter of 1.8 mm. The outer insulating micropipettes had tip diameters of about 1 μm and the total length of the micropipettes was about 15 mm. The tips of outer insulating micropipettes were filled with paraffin by immersion of the tips into molten paraffin for a moment. The total tip length filled with paraffin was about 50 μm. Then an insulating micropipette was fixed by a micromanipulator. Using another micromanipulator, a cation-selective micropipette electrode was inserted into the outer insulating micropipette until the tip of the microelectrode was very close to the paraffin portion at the tip of the outer micropipette. Then the paraffin in the tip was slowly melted by the heating platinum loop. When the paraffin was properly melted, the outer insulating micropipette was moved by a fine microdrive of the micromanipulator until the tip of the inner microelectrode was seen outside the tip of outer insulating micropipette under 200 times magnification. The heating current was then decreased slowly and the paraffin was cooled. The opposite end of the outer micropipette was attached to the shaft of the inner microelectrode by 2 or 3 drops of molten paraffin. In this way, the exposed cation-sensitive tip length could be adjusted to the required length. After insulation, the microelectrodes were stored in 3 M KCl solution. The detailed procedure of this construction has appeared (Lee, 1972; Lee and Armstrong, 1973).

Measurement of Microelectrode Potentials and Resistances

For the measurement of cation-selective glass microelectrode potentials, the microelectrode was connected to an agar bridge leading from a calomel half cell. The agar bridges were formed by filling polyethylene 190 tubing with a gel of 3% wt/vol agar in saturated KCl solution. An agar bridge was used as a reference electrode by connecting one end to another calomel half cell and immersing the other end in the test solution. Both calomel half cells were, in turn, connected to an amplifier (Keithley Instruments, Inc., Cleveland, Ohio; Model 610 C) with an input impedance greater than 10^{14} Ω. In order to read the correct values of microelectrode potentials, the output of the amplifier was connected to a digital voltmeter. Also the output was connected to a recorder and an oscillocsope. Fig. 1 shows the schematic diagram for measurement of the microelectrode potentials. To eliminate noise, all the instruments except the voltmeter and the oscilloscope were housed within a Faraday cage.

For measuring the resistances of cation-selective glass microelectrodes, a resistance box assembled in this laboratory was used. The resistance box contained the resistors (Hi-Meg Resistor, Victoreen Instrument Co., Cleveland, Ohio) of 10, 100, 1,000, 10,000, 100,000 MΩ and an open circuit which were connected to one another in parallel. As shown in Fig. 1, the resistance box was connected in parallel to a microelectrode. The resistors in the box could be switched individually into the circuit for measurement of a microelectrode resistance. As shown in Fig. 1, the cation-selective glass microelectrodes may be regarded as having their own voltage source (E_s), a resistive (R_e) and a capacitive element. The microelectrode potential (E_s) can be measured when the resistance box is switched to infinite resistance. For determination of a microelectrode resistance, another potential (E_r) was measured by switching the resistance box to one (R_b) of the other resistors. Usually the resistor was selected so that E_r could be close to a half of E_s. Thus the resistance (R_e) of a microelectrode was calculated according to the following equation:

$$R_e = R_b(E_s - E_r)/E_r. \qquad (1)$$

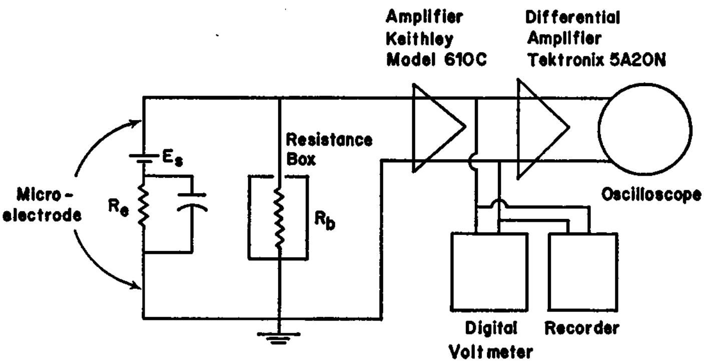


FIGURE 1 Circuit for measurement of potential and resistance of cation-selective glass microelectrodes.

RESULTS AND DISCUSSION

Description of the Microelectrode Behavior

The system shown in Fig. 2 represents a concentration cell consisting of two calomel half cells and a cation-selective glass membrane. This glass membrane made from $NAS_{27\text{-}04}$ is permeable to K and Na ions with different selectivity. The compartments I and II represent a test solution containing electrolytes and the 3 M KCl solution within the cation-selective glass microelectrode. E_{J1} and E_{J2} represent the junction potentials at each saturated KCl agar bridges which are very small and can be neglected. In an ideal state, the potentials of E_1 and E_2 do not contribute to the potential difference across the glass membrane since they are equal and of opposite sign. In the system (Fig. 2), the potential difference (E_M) across the glass membrane can be represented according to the following general equation based on the ion-exchange theory (Eisenman et al., 1957).

$$E_M = E_o\,(\text{const}) + RT/F \ln\,[a_i^{1/n} + (k_{ij}a_j)^{1/n}]^n, \qquad (2)$$

where E_o may include the junction potentials together with the potential at phase boundary of the inside of the glass microelectrodes if the solution inside the microelectrode is constant in its composition (3 M KCl in this study). n is a constant specific for a given glass electrode and pair of cations. a_i and a_j are ionic activities of i and j ion, respectively. k_{ij} is the selectivity coefficient of a microelectrode which is identical to the permeability ratio of i and j ion (P_j/P_i). In the case of K^+-Na^+ mixture, n is equal to 1. Then Eq. 2 gives a simple equation:

$$E_M = E_o + RT/F \ln\,(a_K + k_{KNa}a_{Na}). \qquad (3)$$

This equation is identical to the type given by Nicolsky (1937). Eq. 3 can be re-

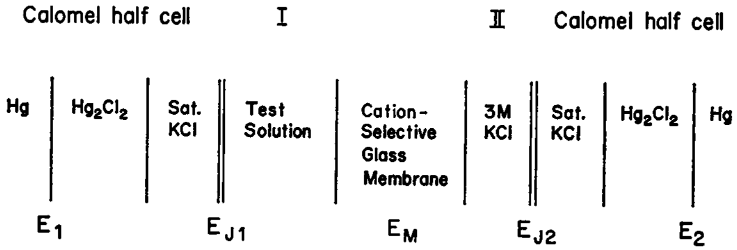


FIGURE 2 The electrochemical system used for measurement of cation-selective glass microelectrode potentials. See text for further description.

written in the following form:

$$E_M = E_o + S \log (a_K + k_{KNa}a_{Na}), \tag{4}$$

where S is an empirical slope of the calibration curve of a microelectrode. When the solution outside the microelectrode contains only KCl, Eq. 4 can be represented in the following form:

$$E_M = E_o + S \log a_K\,, \tag{5}$$

where S can be determined by $\Delta E_M/\Delta \log a_K$. E_o can be obtained by solution of two equations at two different values of $\log a_K$. The selectivity coefficient (k_{KNa}) in Eq. 4 can be determined from the difference of two potentials measured by a microelectrode in a KCl solution and a NaCl solution at the same ionic activity ($a_K = a_{Na}$). Thus the selectivity coefficient (k_{KNa}) for K^+ and Na^+ can be calculated according to the following equation:

$$k_{KNa} = e^{(E_{Na}-E_K)F/RT}, \tag{6}$$

where E_{Na} and E_K are the microelectrode potentials in the two solutions containing Na^+ and K^+ with the same activity ($a_{Na} = a_K$), respectively. When the slope (S) is not ideal ($RT/F = 25$ mV at 25°C; $2.303\ RT/F = 59.1$ mV), the following form should be used:

$$k_{KNa} = e^{(E_{Na}-E_K)/S}. \tag{7}$$

After cation-selective glass microelectrodes have been drawn to the configuration desired, they should be calibrated to determine if they satisfy the theoretical principle described by Eq. 2. This is because the glass properties might be changed during the microelectrode construction. Therefore the microelectrodes made in this study were calibrated in 20 different solutions—NaCl and KCl solutions of 5, 10, 50, and 100 mM, and 12 mixture solutions of NaCl and KCl. In Fig. 3 the values of the measured and theoretical potentials in the calibration solutions for a typical microelec-

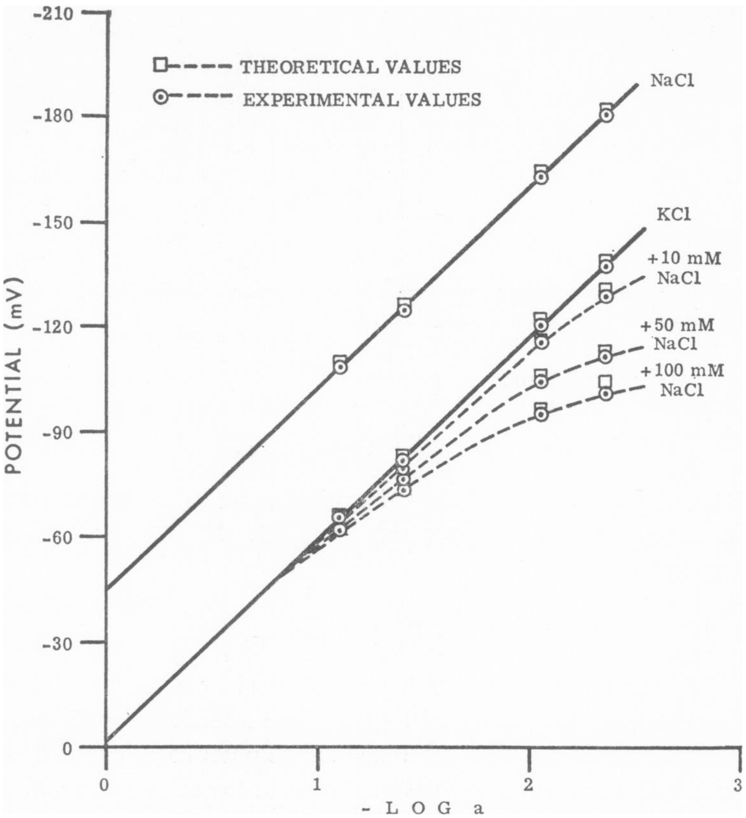


Figure 3 Responses of a typical cation-selective glass microelectrode to NaCl, KCl solutions, and mixtures of NaCl and KCl. Dotted curves indicate the responses for 12 mixture solutions (combination of 5, 10, 50, and 100 mM KCl with 10, 50, and 100 mM NaCl).

trode were plotted against the corresponding $-\log a$. The theoretical potentials were calculated from Eq. 4 using the corresponding ionic activities (Robinson and Stokes, 1965). The measured potentials shown in Fig. 3 are in good agreement with the calculated potentials for each solution. These and similar results indicate that the cation-selective glass microelectrodes used in this study satisfactorily follow the predictions of the ion exchange theory.

To observe the effects of pH on the cation-selective glass microelectrodes, microelectrode potentials were measured in NaCl and KCl solutions at pH 3.0, 4.0, 5.6, 7.2, and 8.0. Fig. 4 shows a relationship between the potentials of a typical microelectrode and the ionic activities of Na and K at pH 3.0 and 5.6. This and similar results indicated that the plots of microelectrode potentials against ionic activities of Na and K were parallel straight lines above pH 5 (Lee and Armstrong, 1972). Therefore, in this range of pH (5.0–8.0) and salt solutions (5–100 mM NaCl and KCl solutions), the microelectrodes have a single selectivity coefficient (k_{KNa}). The

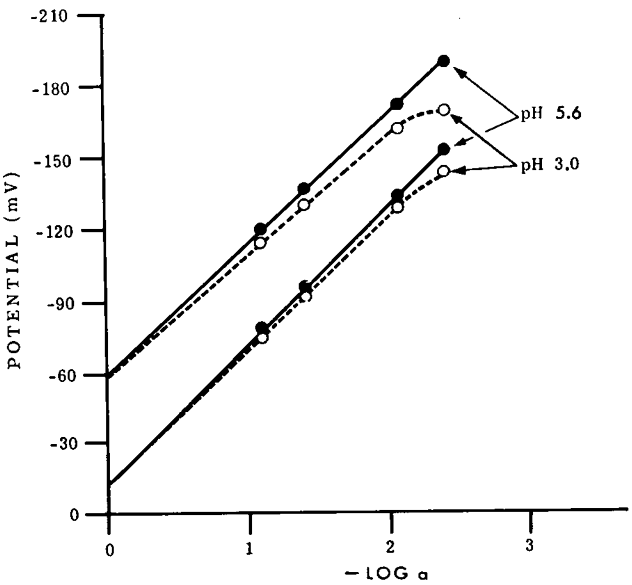


FIGURE 4 Effect of pH change on potentials of a cation-selective glass microelectrode. Upper two curves are potentials in NaCl solutions and lower two curves are potentials in KCl solutions. See text for discussion.

microelectrodes can then be applied for the measurement of ionic activities of Na and K within these ranges of pH and salt solution. As shown in Fig. 4, below pH 4 the relationship between microelectrode potentials and ionic activities was not linear over the concentration range tested. The deviation from linearity increased with decreasing NaCl or KCl concentration and was greater in NaCl solution than KCl solution. In this case, it is impossible to determine a single selectivity coefficient over the concentrations of NaCl and KCl, so that the microelectrodes cannot be used to measure the ionic activities under these conditions.

Resistance of Cation-Selective Glass Microelectrodes and Hydration Effects

One of the most important electrical properties of cation-selective glass microelectrodes is their resistance. The microelectrode resistance is related to other important electrochemical properties: accurate measurement of electrode potentials, stability of electrode potentials, response time of electrodes, conductivity of ions in the glass of electrodes, and hydration effects on the glass and its structural changes. In this section, the relationships are analyzed empirically for $NAS_{27\text{-}04}$ glass microelectrodes and compared with theoretical predictions. Since the bulk resistivity of dry $NAS_{27\text{-}04}$ glass is 2×10^9 Ω-cm (Eisenman, 1965), the microelectrode is expected on the basis of its small dimensions to have a resistance of about 4×10^{11} Ω for the glass thickness of 0.2 μm and the area of 10 μm^2. This high electrical resistance imposes considerable obstacles to the practical application of the microelectrodes.

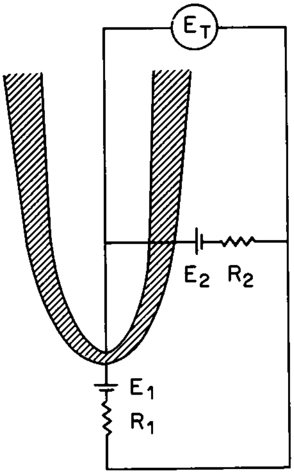


FIGURE 5 Schematic diagram of the tip portion of a cation-selective glass microelectrode with sealed tip and its equivalent electrical circuit. E_1 and R_1 represent the potential and resistance at the tip, respectively. E_2 and R_2 represent those near the tip.

Fig. 5 shows a schematic diagram and simplified equivalent circuit of a microelectrode. In the equivalent circuit, the total potential (E_T) and resistance (R_T) of the microelectrode can be represented by Thevenin's theorem in the following equations:

$$E_T = E_2 - \frac{E_2 - E_1}{R_2 + R_1} \times R_2 = \frac{E_1 + E_2(R_1/R_2)}{1 + R_1/R_2} \tag{8}$$

$$R_T = \frac{R_1 \cdot R_2}{R_1 + R_2}. \tag{9}$$

As seen from Eq. 8, the total potential (E_T) is a function of R_1 and R_2 as well as E_1 and E_2. If E_1 is equal to E_2, E_T does not change with increase in the immersed tip length whatever the values of R_1 and R_2 are. If E_1 is not equal to E_2, E_T depends on R_1 and R_2. In this case, if R_1 is similar to R_2, E_T is greatly affected by increase in the immersed tip length. In other words, if the surface area of the microelectrode in contact with a test solution is increased, the total microelectrode potential changes. If E_2 is greater than E_1, E_T increases from E_1. If E_2 is less than E_1, E_T decreases from E_1. From Eq. 8, it is clear that the change of total electrode potential from E_1 is dependent on R_2. If R_2 is much greater than R_1, the change of E_T is negligible. In order to confirm this theoretical description, uninsulated microelectrodes with sealed tips were tested. Before starting experiments, the microelectrodes were stored in 3 M KCl solution for about 30 h to fill them with 3 M KCl solution. The microelectrode potentials were then measured in 1 and 10 mM KCl solutions. 12 microelectrodes were tested and similar results were obtained for all the electrodes. Fig. 6 shows the typical recordings of microelectrode potentials. As shown in Fig.

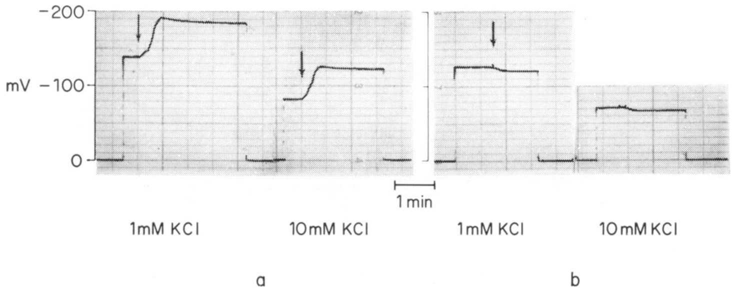


FIGURE 6 Changes of microelectrode potentials with increases (↓) in tip length exposed to the solution. See text for discussion.

6 *a*, when the tip of a microelectrode was dipped in the test solution, a level of potential was obtained. The dipped tip length was then increased, and the recorded potential increased to another level. Fig. 6 *b* shows the potential changes with increase of the dipped tip length of another microelectrode. In this case, the microelectrode potential decreased with increase of the dipped tip length. These experimental results are consistent with the theoretical description mentioned above. In the case of Fig. 6 *a*, E_2 is greater than E_1 . In the case of Fig. 6 *b*, E_1 must be greater than E_2 . This indicates that the potential differences across the glass membrane at different points along its length are not identical. Also, the microelectrode potential changes show that R_1 is relatively similar to R_2 in these microelectrodes. The difference between R_1 and R_2 is expected to be due to the difference in the glass membrane thickness at the two points. If R_2 is much greater than R_1 , the potential changes would be negligible with increases in the dipped tip length. Where microelectrode potentials depend on the dipped tip length, meaningful experiments become difficult. The microelectrodes should therefore be insulated with a material with the resistivity much higher than the glass except for a small portion of the tip. Thus, the surface area of the microelectrode tip contacting a test solution can be kept constant. In the microelectrodes whose potentials depend on the dipped tip length, tip resistances were usually greater than 10^9 Ω.

A more interesting phenomenon was that this potential change of the microelectrodes with increasing dipped length was not observed after the electrodes were stored in 3 M KCl solution more than a few days. Fig. 7 shows typical recordings of such microelectrode potentials. After the microelectrode potential reached a certain level by immersion into a test solution, the potential did not change with increase (↓) of the dipped tip length. This result indicates that in the microelectrode R_2 is much greater than R_1 . The microelectrodes with constant potential have much lower electrical resistances than those with potentials dependent on the dipped tip length. The microelectrodes with constant potential had resistances of 2×10^7–10^9 Ω (42 microelectrodes), depending on the time stored in 3 M KCl solution and the thickness of the glass membrane. Fgi. 8 shows the potential recordings

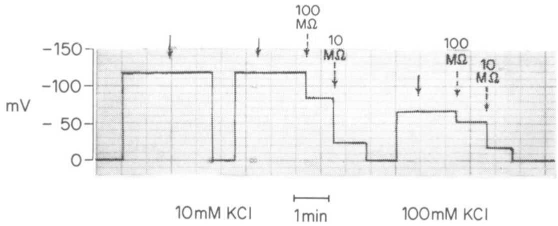


FIGURE 7 Recordings showing no potential changes with increases in the dipped tip length of an aged microelectrode. The solid arrow indicates increases in the dipped tip length. The dashed arrow indicates that the resistance box is switched to the resistance value of 100 or 10 MΩ. See text for discussion.

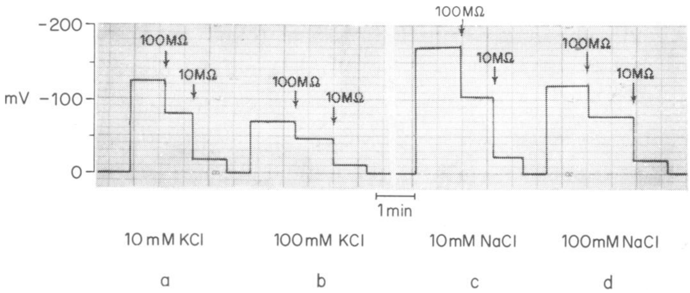


FIGURE 8 Recordings of microelectrode potentials for measurement of resistances of a microelectrode in 10 and 100 mM KCl and 10 and 100 mM NaCl solutions. Each tracing (*a*, *b*, *c*, and *d*) shows the potential changes of the microelectrode where the resistance box is switched to the indicated resistance values.

of a microelectrode for the determination of its resistance. The recordings of Fig. 8 *a*, *b*, *c*, and *d* are the potentials in KCl and NaCl solutions of 10 and 100 mM, respectively. In each tracing of *a*, *b*, *c*, and *d*, the first potential deflections (E_s) were obtained when the resistance box (Fig. 1) was switched to infinite resistance. After a stable potential was reached, another stable potential (E_r) was obtained by switching the resistance box to 10^2 MΩ (R_b). In this way, another potential was obtained when the resistance box was switched to 10^1 MΩ. From the values of E_s, E_r, and R_b, the microelectrode resistance was calculated by Eq. 1. Fig. 9 shows a typical relationship between the resistances of a microelectrode and the time stored in 3 M KCl solution. The microelectrode resistance decreased exponentially with time and finally reached a relatively constant level. The resistances of the microelectrodes are much lower than the values estimated from the resistivity of dry glass for an equivalent dimension of microelectrode working tips. It is difficult to interpret these results in terms of incomplete sealing of microelectrodes, in which the low resistance of microelectrodes was interpreted by Lev (1969) to be due to narrow pores at the sealed part of the capillary tip. If the low tip resistance is due to narrow pores at the

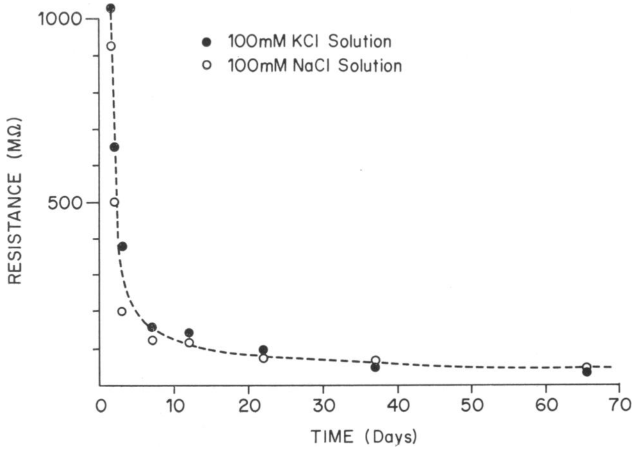


FIGURE 9 Relationship between resistance of a microelectrode and time stored in 3 M KCl solution. The dashed line was drawn to fit by eye.

sealed part of the capillary tip of "incompletely sealed" microelectrodes, the resistance should not decrease with time stored in an aqueous solution.

Our observations for the low resistance of the microelectrodes are understandable from Eisenman's (1967) calculated value of the resistivity of hydrated glass, which he has shown to be as much as 1,000 times lower than the resistivity of the dry glass composition.

In order to analyze more fully the microelectrode properties, electrical resistance of microelectrodes with different exposed tip lengths was measured in KCl and NaCl solutions of various concentrations. For this purpose, the microelectrodes were insulated with a high resistance glass micropipette by the method previously described. Fig. 10 shows the relationship between microelectrode resistance and exposed tip length in 100 mM KCl or 100 mM NaCl solution for 16 microelectrodes. The microelectrode resistance decreased with increasing length of exposed tip and was consistently higher in NaCl solutions than in comparable KCl solutions. However, uninsulated microelectrodes had relatively low resistances and almost same resistances in KCl and NaCl solutions of identical concentration. Table I shows the electrical resistances of five microelectrodes, measured in 5, 10, 50, 100 mM KCl and NaCl solutions. These results (Table I) are similar to those shown in Fig. 10. From these studies it could be concluded that the electrical resistance of microelectrodes decreased with aging in 3 M KCl solution and reached the value at least 100 times less than that calculated from the electrical resistivity of dry glass for the equivalent dimensions of microelectrode working tips. In addition the microelectrode resistances decreased with increasing concentration of KCl and NaCl solutions. The slopes (S, millivolts per unit log a_K or log a_{Na}) of the microelectrodes are close to the calculated value of 59.1 mV (2.303 RT/F) at 25°C. The selectivity coefficient (k_{KNa}) ranged

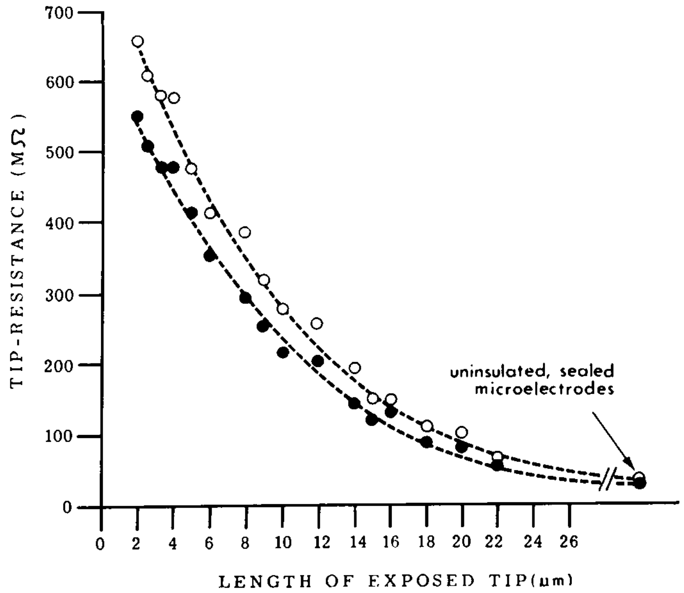


FIGURE 10 Relationship between microelectrode resistance and exposed tip length in 100 mM NaCl (○) and KCl (●) solutions. These microelectrodes were insulated after they were stored in 3 M KCl solution for about 1 wk. The lines were drawn by eye.

TABLE I

CATION-SELECTIVE GLASS MICROELECTRODE RESISTANCES, SLOPES, AND SELECTIVITY COEFFICIENTS AT VARIOUS EXPOSED TIP LENGTHS IN VARIOUS KCl AND NaCl SOLUTIONS

		Cation-selective glass microelectrode resistances					
		Tip length					Un-insulated micro-electrode (SD)
	Conc.	2 μm	3 μm	8 μm	14 μm	20 μm	
	mM	*MΩ*	*MΩ*	*MΩ*	*MΩ*	*MΩ*	*MΩ*
KCl solutions	5	670	641	429	195	105	30 ± 1
	10	600	586	344	176	92	29 ± 1
	50	562	545	316	165	89	28 ± 1
	100	550	514	296	144	87	28 ± 1
NaCl solutions	5	773	688	492	250	126	34 ± 1
	10	709	636	431	237	112	32 ± 1
	50	678	619	406	223	107	30 ± 1
	100	660	580	385	196	104	30 ± 1
S(mV)		58.6	57.9	57.6	59.9	59.8	59.4
k_{KNa}		0.278	0.377	0.336	0.287	0.193	0.113

usually from 0.1 to 0.5, governed primarily by cation-exchange properties of the glass membrane. The selectivity coefficient is one of the most important parameters in the application of cation-selective glass microelectrodes. To observe aging effects on the selectivity coefficients, 12 microelectrodes were studied by storing in 3 M KCl solution. All the results were similar and a typical relationship is shown in Fig. 11. The selectivity coefficient decreased with the stored time in the same manner as the microelectrode resistance decreased with the stored time. In other words, as the resistance decreased and reached a certain level, the selectivity of the microelectrode for K^+ increased and reached a relatively stable level, usually about 0.1 (10 times more selective to K^+ than Na^+). This selectivity change of the microelectrodes is consistent with the results obtained by Eisenman (1965) from the large glass electrodes.

The above properties of the glass microelectrodes can be interpreted as indicating that they are governed primarily by hydration of the entire thickness of the thin glass membrane within a certain length from the tip. This hydration appears to lower the microelectrode resistance by structural changes of the glass (Bates, 1965). In the present study, the hydration of thin glass membrane can be confirmed by the microelectrode resistance. In the diagram of a microelectrode shown in Fig. 12, it is presumed that hydration of entire thickness of the glass membrane starts from the thinnest end of the tip and extends to a thicker part of the membrane with aging in 3 M KCl solution. After a certain time, the middle portion of the relatively thick glass membrane near the tip is still not hydrated. In the simplified equivalent circuit of a microelectrode (Fig. 12), R_1 and R_2 represent the resistance of hydrated glass membrane and R_3 represents the resistance of nonhydrated glass membrane. It is assumed that the differ-

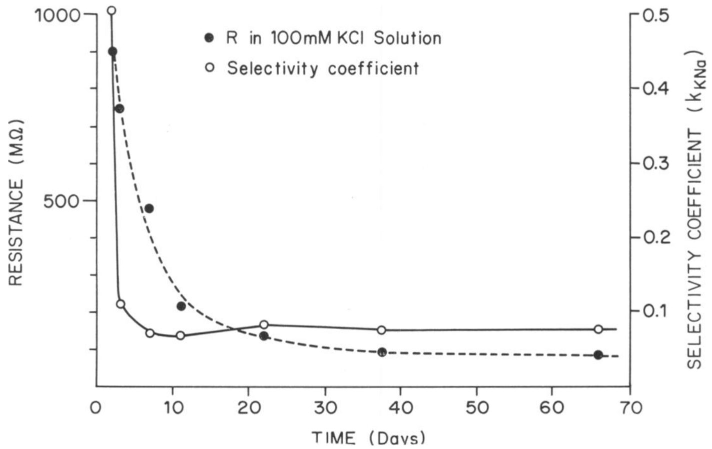


FIGURE 11 Relationship between the selectivity coefficient, the resistance and time stored in 3 M KCl solution for a microelectrode. See text for description. The lines were drawn by eye.

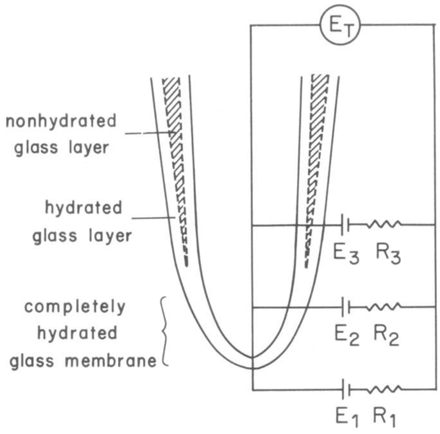


FIGURE 12 Schematic representation of hydrated tip portion of a cation-selective glass microelectrode and its equivalent electrical circuit. See text for discussion.

ence between R_1 and R_2 is due to the difference of glass membrane thicknesses at the two points, and that they are similar in magnitude ($R_1 \approx R_2$). Therefore, as the exposed tip length in the hydrated zone increases, the total resistance of completely hydrated zone of glass membrane will decrease as follows:

$$R_t = (R_1 \cdot R_2)/(R_1 + R_2) = \tfrac{1}{2} R_1 .$$

If the resistance of the nonhydrated glass membrane (R_3) is much greater than R_t, (e.g. 100 times), the effect of R_3 on the total resistance of the microelectrode (R_t) is negligible:

$$R_T = (R_t \cdot R_3)/R_t + R_3) = (R_t \cdot 100\ R_t)/(R_t + 100\ R_t) = 0.99\ R_t .$$

It is therefore likely that increased exposure of the nonhydrated zone would have a negligible effect on the total microelectrode resistance. Under the postulate that hydration in the glass membrane lowers its resistance, the above theoretical description is consistent with the experimental results of the microelectrode resistance.

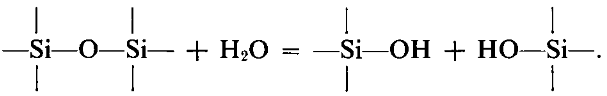
The next question is what is the possible mechanism for lowering the glass resistance due to hydration. This can be considered in terms of ionic conductance mechanisms in the glass in which the interaction of glass molecules with conducting ons and water molecules may occur. In silica glass the silicons known as network formers, are located at the center of tetrahedral coordinates of oxygen atoms. When sodium oxides are added in this silica glass, nonbridging oxygens and sodiums in the interstices of the glass are produced. That is to say, silicon-oxygen bonds are broken by ionic bindings of sodium ions to the oxygen sites. In this sodium silicate glass, the sodium ions, known to act as a network modifier, and the negative oxygen sites are responsible for electrical conduction. When Al_2O_3 is introduced into the glass, the silicons at the center of tetrahedral coordinates are replaced with the aluminums. The ion-exchange property, as well as conductivity of the Na_2O-Al_2O_3-SiO_2 glass, may be changed by altering the field strength of the negative charge sites. In this glass, electrical conduction may be due to the current being carried by sodium ions moving from one site to another site. Hence, the electrical conduction is basically due to interactions of monovalent cations with anionic sites in the glass. So the electrical conduction can be considered as an activation process of their interactions. In this process, monovalent cations jump from one site to another site only when their vibrational energy has reached a certain threshold, equivalent to an energy barrier, so as to break the weak bond of the cations and the sites. Therefore, the conductivity of monovalent cations in the glass can be regarded as the rate of reaction of the cations and the sites, and may be expressed by the following Boltzmann relation:

$$L = A \cdot e^{-E_a/kT}, \tag{10}$$

where A is a constant, E_a is activation energy, and k and T are the Boltzmann constant and the absolute temperature, respectively. According to Eq. 10, the resistance of the cations can be represented by the following equation:

$$R = R_o e^{E_a/kT}, \tag{11}$$

where R_o, the pre-exponential factor is expected to be related to the structural properties of the glass. Therefore, one would expect that the change of the glass resistance to the cations at a certain state is mainly governed by the activation energy (E_a). This activation energy is equivalent to the energy barrier which the cations should attain to jump from one site to another site. From Eq. 11, the decrease in the glass resistance to the cations by the hydration should be the result of a decrease in the energy barrier. It is thus suggested that ionic channels through the thin glass membrane are produced by hydration, and these lower the glass membrane resistance to the cations. It is known that when a glass is exposed to aqueous solutions, the oxygen-silica bonds are broken by a silicate hydrolysis and result in two non-bridging oxygens:



$$\text{—Si—O—Si—} + H_2O = \text{—Si—OH} + \text{HO—Si—}.$$

Accordingly, the formation of a silica film with pores may arise (Holland, 1964). Further it is well known that in the Na_2O—Al_2O_3—SiO_2—H_2O system (synthetic zeolytes) molecular sieve channels are formed. In the Na-modenite of the zeolytes, the diameter of channels was about 4 Å (Eitel, 1966; Sherry, 1969). These channels in the glass membrane may contribute to the decrease in the energy barrier, so that the cations can easily jump from one site to another site. Channel diameter may be related to the degree of hydration, causing a change in the selectivity coefficient. One would also expect that the hydration effects in the glass membrane may alter the field strength of the anionic sites by permitting reaction between the anionic sites and water molecules. A reduction in field strength of the sites could result in a change in the molecular configuration of the glass, altering the shape of the channel and decreasing the electrostatic force between the cations and the sites. This might be an additional factor to decrease the energy barrier, together with the formation of channels. This explanation for the selectivity change (Fig. 11) of the hydrated glass to Na^+ and K^+ is consistent with the Eisenman theory (1962), in which the selectivity depends on the anionic field strength.

At this stage, the higher resistance of relatively small exposed tips in NaCl solutions, compared with the corresponding KCl solutions, should be considered. As long as the glass membrane has a selectivity characteristic due to an ion-exchange process, Na and K ions must lose their bound water molecules (hydration shells) to react with the anionic sites at the phase boundary and move from one site to another site. This may be true in the presence of channels, if they are not large enough to pass the ions with hydration shells. Hence the hydration energy of the ions is important in connection with the energy barrier at the interface. The hydration energy of Na ion (97 kcal/mole) is greater than that of K ion (77 kcal/mole). Therefore the activation energy of Na^+ is greater than that of K^+ for reaction with the anionic sites. According to Eq. 11, the microelectrode resistance should be greater in NaCl solutions than that in corresponding KCl solutions. However, the resistances of microelectrodes in comparable KCl and NaCl solutions become gradually closer to each other with increasing the exposed tip length, and finally it is difficult to differentiate them in uninsulated microelectrodes with infinite exposed tip length. This phenomenon can be explained by the following simple equation:

$$R = \rho \times (L/A), \tag{12}$$

where R, L, and A are the resistance, thickness and area of the glass membrane of microelectrodes, and ρ is the resistivity of the glass. When the exposed area (A) increases, R_{KCl} (resistance in KCl solution) and R_{NaCl} (resistance in NaCl solution) decrease and go toward a same value. Thus the difference between R_{KCl} and R_{NaCl} will be decreased with increase in the exposed tip length.

Transient Depolarization of Glass Membrane Potentials

The thin glass membrane made from $NAS_{27\text{-}04}$ by construction of cation-selective glass microelectrodes showed transient depolarizations that were similar in some respects to the action potentials of biological membranes. These transient depolarizations were observed after storing the microelectrodes in 3 M KCl solution for a few days. Typical transient depolarizations of a microelectrode filled with 3 M KCl solution are shown in Fig. 13. When the microelectrode was immersed in 1 mM NaCl solution (Fig. 13 *a*), a potential of about -215 mV was obtained, which depolarized

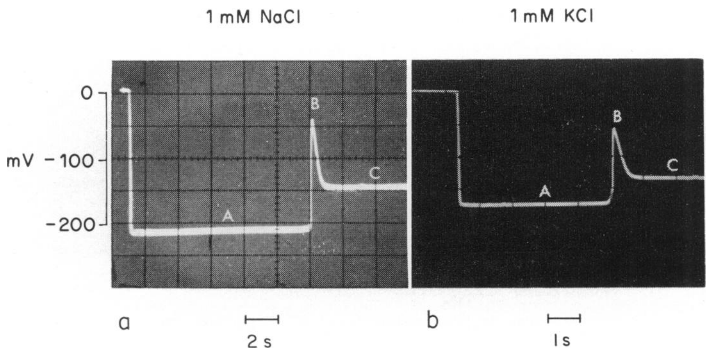


FIGURE 13 Transient depolarizations of cation-selective glass membrane potentials.

slowly. After several seconds, the membrane was transiently depolarized and then returned to a constant potential of about -145 mV, smaller than the original potential. The transient depolarization effect could be repeated by taking the microelectrode out from the test solution into air for about 1 min and reimmersing it in the solution. Fig. 13*b* shows the tracing of a transient depolarization in 1 mM KCl solution, which was obtained using the same microelectrode as that used in Fig. 13*a*. There are three potential levels in the recording, the first slowly depolarized potential (*A*) right after immersing the microelectrode, a spike potential (*B*) due to the sudden depolarization, and the final stable potential (*C*) after repolarization. The potentials at each level in NaCl solution are different from those in the corresponding KCl solution. Therefore, it is possible to determine the selectivity coefficients (k_{KNa}) at each level from the biionic potentials of $E_{\mathrm{Na}} - E_{\mathrm{K}}$ by Eq. 7. At potential level *A*, the glass membrane is selectively more permeable to K^+ than Na^+, with a k_{KNa} of 0.121 ($n = 5$, where n is the number of biionic potentials). At the peak potential, level *B*, there is no longer selectivity of the membrane between K^+ and Na^+. In other words, the glass membrane is almost equally permeable to K^+ and Na^+, although the slightly lower potential in NaCl solution compared to the corresponding KCl solution indicates a slightly greater selectivity for Na^+. This interpretation is consistent with the result that the amplitudes of depolarization are proportional to the original electrochemical potentials before depolarization. The relation of the depolarization amplitudes to the original potentials are clearly seen in Fig. 13 *a*, *b*, and 14*a*. At the potential level *C*, the repolarized potentials are lower than those of the corresponding original potentials. The selectivity coefficient (k_{KNa}) of 0.416 ($n = 5$) indicates that the glass membrane is more selectively permeable to K^+ than Na^+, although the magnitude of the selectivity for K^+ is less than that ($k_{\mathrm{KNa}} = 0.121$) before depolarization. As seen in Figs. 13 and 14, the duration (about 600 ms) of the transient depolarization was almost identical in NaCl and KCl solutions. The rise time of the depolarizations was about 20 ms, so the duration of depolarizations is mainly dependent on the time of repolarization. Also shown in Fig. 14*b* is an ap-

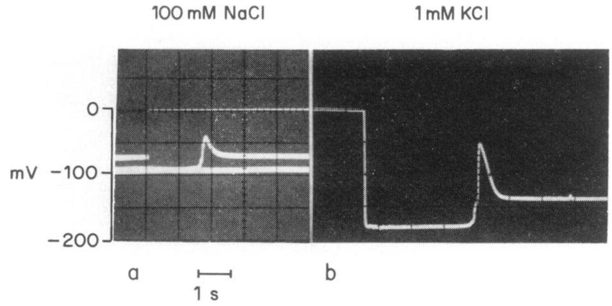


FIGURE 14 (*a*) Transient depolarization of cation-selective glass membrane potential in 100 mM NaCl solution. The amplitude of the depolarization is about 50 mV. (*b*) Transient depolarization in 1 mM KCl solution. A threshold depolarization appears before firing.

parent threshold depolarization before the full and sudden depolarization step. These threshold depolarizations were observed in both NaCl and KCl solutions.

This transient depolarization of the glass membrane is interesting in relation to the action potentials of excitable cell membranes. Eisenman et al. (1967) have made the suggestion that a fixed associated membrane can exhibit a metastability of its electrical resistance characteristics, and they demonstrated such a metastability for Na^+—Cs^+ mixtures in completely hydrated glass membranes. Also they speculated that the metastability properties intrinsic to the biological excitation process may be general features of simple ion-exchange membranes. The observation of these transient depolarizations in the glass membrane is, therefore, not entirely unexpected. One can again involve the energy barrier as an explanation for this resistance change. As pointed out, this energy barrier may include the activation energies for interaction of the anionic sites and the counter-ions in the phase boundary as well as within the glass. Only when this activation energy exceeds the energy barrier can the counter-ions be transported across the membrane. The energy barrier distributed in the glass can be altered by the change of the field strength of the anionic sites due to the degree of hydration. It is assumed that at a certain state the energy barrier is suddenly changed by the electrochemical potential across the glass membrane. At the state (potential level A) with a certain degree of hydration, the conformation of the hydrated glass is unstable and so the energy barrier in the glass is sensitive to the electrochemical potential across the membrane. In this state, the glass membrane potential decreased slowly with time. It was observed that the duration of this unstable period depended on the time that the microelectrode remained in air. As the time in air increased, the unstable period also increased. Fig. 15 shows that the unstable period was about 70 s after keeping the microelectrode in air about 5 min. This result suggests that the degree of hydration may be altered by keeping the microelectrode in air and may lead to some change in the electrical field strength of the anionic sites. At a certain point, the energy barrier decreases drastically and changes to a mini-

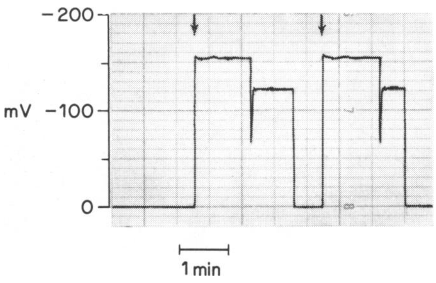


FIGURE 15 Transient depolarization of cation-selective glass membrane potential in 1 mM KCl solution. These tracings show the time required before transient depolarization. This microelectrode remained in air for about 5 min before immersing (↓) it into the test solution.

mum value. This point may be equivalent to the state of the threshold potential. The minimum value of the energy barrier can be regarded as equivalent to the peak potential. The process of decrease in the energy barrier from a certain state to a minimum state may be interpreted as the opening of channels in the glass membrane so that the ions with hydration shells can move freely. At the state of the stable membrane potential (*C* level), the counter-ions can be transported by the equilibrium ion-exchange processes. The hydrated cations should be dehydrated at the phase boundary. In this process, there are considerable energy barriers since the channels are relatively narrow. At the depolarized state of the minimum energy barrier, the diameter of the channel is presumably large enough to allow movement of the hydrated cations. In this state, specific interactions between the counter-ions and the negative charge sites in the channels are not required. Then the cation transport and peak potentials depend on the electrochemical potentials across the membrane before depolarization.

Now we would like to consider an atomic model of the above description for the transient depolarization of the glass membrane potentials. As pointed out already, ionic channels are formed in the system of $Na_2O—Al_2O_3—SiO_2—H_2O$ glass. The structural configuration of the glass membrane with channels is shown in Fig. 16 as a schematic two-dimensional representation. In the $Na_2O—Al_2O_3—SiO_2$ glass, each oxygen atom is coordinated to two silicon atoms or two atoms of silicon and aluminum. In the hydrated glass, some Si—O bonds are broken and the oxygen atoms may bind to one silicon atom. The structural coordination of the monovalent cations is not available from the experimental results. However, Eisenman (1962) gave a hypothetical model in which the cations are moved from their "lattice breaking" positions in the alkali silicate into an "interstitial" position by introducing aluminum oxides. The monovalent cations are not shown in Fig. 16. In the silica glass, each molecule has a certain vibrational energy. Thus the oxygen atoms vibrate with certain frequencies that are not necessarily identical. The motion of oxygen atoms that is dependent on temperature and their frequency response behavior is associated with

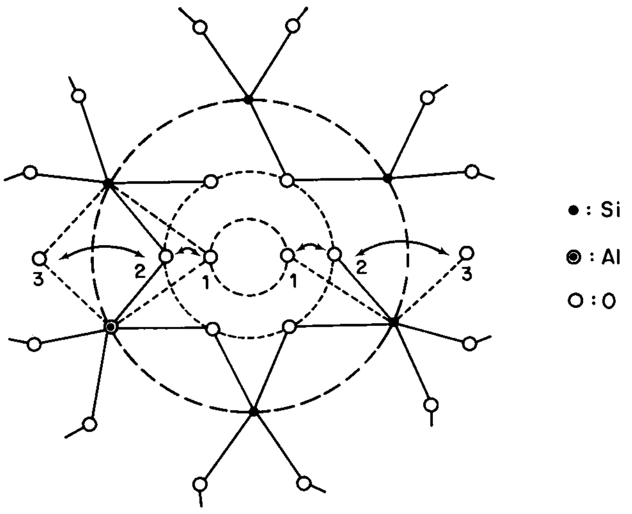


FIGURE 16 Schematic two-dimensional scheme for illustrating the possible motion of oxygen atoms in a hydrated cation-selective glass membrane with a channel. Dotted circles represent the channel diameters at each position (1, 2, or 3) of oxygen atoms. See text for discussion.

the distribution of activation energies (Eitel, 1965). Then it would be expected that the motion of oxygen atoms perpendicular to the bond, Si—O is affected by the electrical field strength of the negative charge sites which can be changed by the degree of hydration. Fig. 16 shows the possible positions of the motion of oxygen atoms which are designated as 1, 2, 3. At a certain degree of hydration, it is assumed that the oxygen atoms are at the stable energy state of position 2 (Fig. 16). This state may be equivalent to the stable potential (Fig. 13, potential level *C*) of the glass membrane. Although the diameter of channels in this state is fairly large in comparison with those of dry glass, they are not large enough to transport the hydrated K^+ and Na^+. So the dehydrated or partially dehydrated cations can move through the channels. As long as the membrane is in a solution, the stable state remains. This stable state can be shifted to a fairly stable state by an external factor (for example, temporary drying in air in these experiments). This shift is thought to be due to the changes in the degree of hydration, with changes in the field strength of the anionic sites. In this state it is assumed that the oxygen atoms are at position 1. The unstable potential (Fig. 13, potential level *A*) may be equivalent to this state where the channels have the most narrow diameter. The energy state of the oxygen atoms in this fairly stable state is sensitive to an environmental force, for example, electrochemical potential across the membrane. So the oxygen atoms can be moved to the position 3 (Fig. 16) by an energy dissipation which might correspond to the change of the threshold potential. At the position 3, the channels have the largest diameter, and pass freely hydrated cations. This process of transient development of wide channels is thought to be initiated by the change in the field strength of surface charge sites, which was induced by the alteration in the degree of hydration. The oxygen atoms at the position 3 may move back to the position 2 since they are again in the unstable energy state.

SUMMARY

Cation-selective glass membrane microelectrodes with tip diameters less than 1 μm were made from $NAS_{27\text{-}04}$ glass. The working tip length of the microelectrodes could be adjusted to a chosen length, with a minimum of about 2 μm. The behavior of the microelectrodes follows satisfactorily the ion-exchange theory.

During storage of the microelectrodes in 3 M KCl solution, their electrical resistance decreased markedly and their selectivity for K^+ increased at the same time, providing desirable features for their practical application. The marked fall in resistance was interpreted to be due to the decrease in energy barrier caused by the complete hydration of the entire thickness of the thinnest tip wall, which would extend upward with stored time.

Transient depolarization of the microelectrode membrane potential, which was associated with a change in electrode resistance and selectivity, was thought to be due to hydration effects on the glass structure. For explanation of the phenomenon we postulated two states: (*a*) a fairly stable state where dehydrated or partially dehyhydrated K^+ and Na^+ interact with negative charge sites on the fairly narrow channels and move through them; (*b*) an unstable state where the hydrated cations can move freely through the transient wide channels without interaction. This unstable state of wide channels is developed by the changes in the field strength of the negative change sites which was induced by the alteration in the degree of hydration. Finally the transient is followed by a second stable state with different selectivity characteristics.

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