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TRANSIENT PERMEABILITY OF A MODEL LIPID MEMBRANE TO $^{22}Na^+$

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SUMMARY

1. A permeability cell is described which allows tracer diffusion studies to be carried out on model lipid membranes for an extended time.

2. The permeability of a model lipid membrane to $^{22}Na^+$ drops to an undetectable level in 10–12, 6–7, and 4 h for membranes formed in distilled water, 0.1 M NaCl, and 0.5 mM $MgCl_2$, respectively.

3. In 0.1 M NaCl and distilled water the permeability constant is dependent on the amount of *n*-tetradecane, added to the lipid solution from which the membranes are formed.

4. The permeability constant is also dependent on Δc, the $^{22}Na^+$ concentration difference across the membrane. Values, as $\Delta c \rightarrow 0$, of $3.71 \cdot 10^{-8}$, $0.27 \cdot 10^{-8}$, and $0.25 \cdot 10^{-8}$ cm/sec were obtained for the $^{22}Na^+$ permeability constant for membranes formed in 0.1 M NaCl, distilled water, and 0.5 mM $MgCl_2$, respectively.

5. Solvent drag, the ionic composition of the aqueous medium, and the hydrocarbon region of the membrane are considered in accounting for the results.

INTRODUCTION

The thermodynamic aspects of solute and solvent flow across a permeability barrier, such as the model lipid membrane, have been developed[1] and emphasize as energy sources the chemical potential and osmotic pressure gradients. A more recent kinetic derivation of the thermodynamic equations of flow considers the energy-barrier model of membranes[2]. It takes into account the interface potential energies which, depending on the nature of the solute, may either hinder or aid the movement of ions across a water–lipid interface. In addition negative entropy changes in moving ions across the interface have been measured[3]. Their relative values for LiCl, NaCl, KCl, RbCl, and CsCl agree with the observed order of the flux rates across the interface for the five cations, $Li^+ < Na^+ < K^+ < Rb^+ < Cs^+$. Solvent drag, another restraint to ion flow across model lipid membranes, has been considered but not demonstrated[4].

The low conductivity of model lipid membranes suggests that their permeability to ions is low. Permeability data, based on conductivity and/or Nernst potential measurements, may be in error[5] due to the effect of applied electric currents on a membrane, composed probably of regions of different dielectric constant. To obviate this effect it is necessary to use an ion diffusion technique to obtain permeability data

that are more easily interpretable. In this communication Na^+ permeability data, based on tracer diffusion studies, are presented which indicate that solvent drag, counterions, and the hydrocarbon layer are all involved in determining the rate at which the ions diffuse through the membrane.

EXPERIMENTAL PROCEDURE

Cell with perfusion chamber

The cell adopted for the Na^+ permeability experiments is shown schematically in Fig. 1 in an oblique view from the top. It consists of two lucite chambers, each of approx. 3 ml volume, separated by a teflon septum with a 2- or 2.8-mm diameter aperture for membrane location machined in it. The proximal chamber is bordered on the free side by a curved glass window through which the behaviour of membranes applied to the aperture can be microscopically observed. The perfusion chamber consists of two parts. The larger part nearest the teflon septum carries the perfusion inlet which enters from the bottom so that the perfusate streams upwards and past the membrane in the aperture. The perfusate flow is then streamed away from the membrane by means of the curved, grooved bottom surface. It streams towards the smaller part of the perfusion chamber which communicates through a 2-mm diameter hole with the larger part. The smaller part of the perfusion chamber is cylindrical in shape, 6 mm in diameter, and contains the drain inlet. The meniscus in the cylindrical small part maintains the drain inlet always below the liquid surface and prevents air from being entrained in the flow through the drain tube. The drain tube is approx. 8 cm long and makes an angle of about 58° with respect to the vertical and has a U-shaped capillary outlet. Drop formation is by capillary flow through the opening and surface flow along the outside surface of the tube. This effectively separates the release of the drop from the aqueous contents of the perfusion chamber and prevents any hydrostatic pressure backlash from rupturing the membrane.

The perfusate is delivered to the perfusion chamber through a stainless steel micrometer valve from a 4-l polyethylene reservoir bottle by way of a gravity-fed drip set, normally used for intravenous infusions. The flow rate of the perfusate is

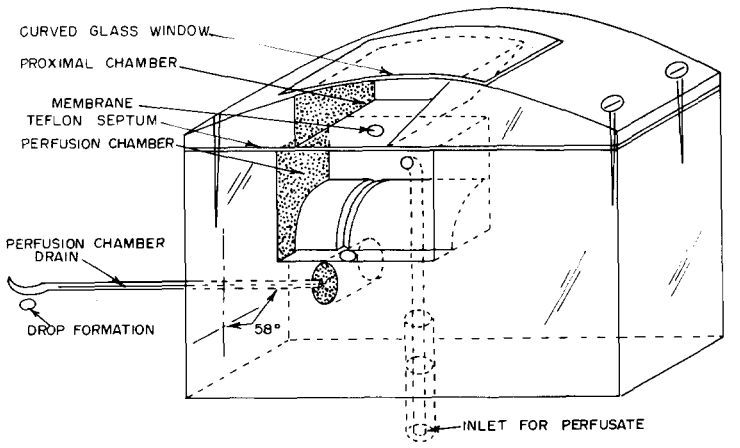

Fig. 1. Oblique view from the top of the permeability cell.

then controlled by the height of the reservoir bottle over the perfusion chamber, the setting of the micrometer valve, the difference in elevation of the water level in the perfusion chamber and the outlet of the drain tube and the size of the capillary opening of the drain tube outlet. The micrometer valve and the capillary opening are adjusted to give a perfusion rate of 15–20 ml/30 min which, in 30 min and at a 100 % clearance efficiency, is sufficient to clear a volume 5–7 times that of the perfusate normally in the perfusion chamber. Chamber clearance studies with slugs of radioactive Na^+, two orders of magnitude greater than those normally encountered in the permeability experiments, indicate that at least 98 % of the radioactive slug is cleared in 30 min.

A short length of fine stainless steel wire was inserted into the inlet portion of the drain tube to improve the entry of the perfusate. Under these conditions the flow rate of the perfusate was very uniform for the duration of the permeability experiments. During an experiment some loss of water occurred in the proximal chamber through evaporation. Because the level of the perfusate in the perfusion chamber was kept constant by the perfusion technique, a small hydrostatic pressure gradient therefore developed across the membrane. This process was followed by microscopically observing the changing light reflex of the membrane. By periodically adding μl volumes of distilled water to the proximal chamber the light reflex and hydrostatic equilibrium were simultaneously restored. The addition of the small amounts of distilled water maintained the solute concentration in the proximal chamber at its initial value (see below).

Membrane formation

The phospholipid extract used in these experiments has been described previously[6]. The only new feature is that the extract was stored in chloroform–methanol (2:1, v/v) at −50°. To the phospholipid extract varying amounts of *n*-tetradecane were added to make up the membrane solution from which the membranes were made. The amount of *n*-tetradecane added varied between 14 and 23.6 % (v/v) and was governed by the criterion that the membrane thinned fully in 60 min (ref. 6).

Prior to membrane application the flow of the perfusate was started and allowed to fill both the proximal and perfusion chambers. Initially, therefore, the solute composition of the aqueous medium in the proximal chamber was identical to that of the perfusate in the perfusion chamber but was subsequently increased by the addition of $^{22}Na^+$ (as $^{22}NaCl$, see below). Flow adjustments were then made to achieve the desired flow rate of 15–20 ml/30 min. The perfusates selected were 0.1 M NaCl, 0.5 mM $MgCl_2$, and distilled water.

Application of the membrane solution to the aperture in the teflon septum was made with a fine sable hair brush. The membranes were then allowed to thin fully to the "secondary black" stage before two or more 30-min control samples of perfusate were collected in separate, previously unused, 20-ml liquid scintillation bottles for subsequent background counting rate determinations. The experiments were run at 37° in a controlled-temperature room.

$^{22}Na^+$ permeability experiments

Following the collection of the control samples $^{22}NaCl$ solution (specific activity approx. 16 μC/mg) was added to the proximal chamber to an activity level of approx.

$1 \cdot 10^6$ counts/min per cm^3 as determined by liquid scintillation counting (resulting in the addition of approx. 0.25 mM $^{22}NaCl$). The contents of the proximal chamber were stirred briefly with a small stainless steel rod. Continuous collection of perfusate was then made in new 20-ml liquid scintillation bottles with a fresh bottle placed under the drain outlet every 30 min.

All the $^{22}Na^+$ activity diffusing through the membrane was thus collected for the duration of the experiments. 10–15 h were considered to be necessary for reasons that will become apparent later.

The samples were then reduced to less than half volume by drying. The two samples obtained per h for each hour were pooled and evaporated to dryness at 90°. The two or more control samples were also dried as separate samples. 2 ml NE220 liquid scintillator solution (Nuclear Enterprises, San Carlos, Calif.) were added to each sample and the latter then counted for $^{22}Na^+$ activity (mostly 0.54 MeV positron) to a statistical accuracy of $\leqslant 1\%$.

Three 50-μl samples were withdrawn from the proximal chamber at the conclusion of an experiment and counted for $^{22}Na^+$ activity both by NaI(Tl) crystal spectrometry (1.28 MeV photopeak) and by the liquid scintillation counting technique described above. The permeability cell was then exhaustively decontaminated prior to re-use.

RESULTS

The net $^{22}Na^+$ activity per hourly sample was determined by subtracting the mean counting rate of the control samples from the gross counting rate of the hourly

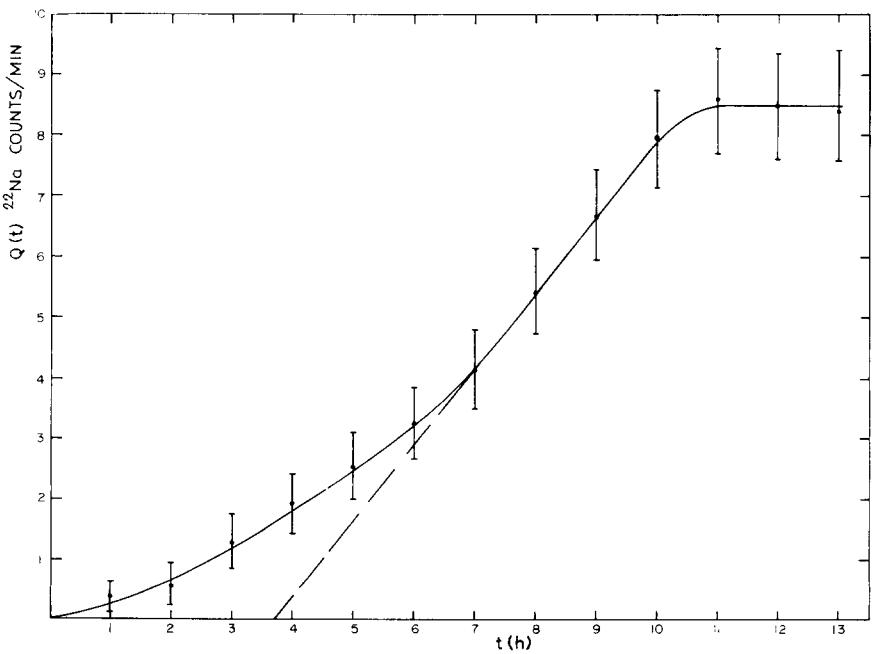

Fig. 2. $^{22}Na^+$ permeability of membrane with area 0.06 cm^2 and distilled water as the aqueous medium and perfusate. Permeability constant $k = 0.45 \cdot 10^{-8}$ cm/sec, calculated from the straight line portion.

samples. The time integral of the $^{22}Na^+$ counts, $Q(t)$, was then calculated by summation of the net counts per hourly sample over the total number of samples included at time t. In Fig. 2 a typical curve of $Q(t)$ *vs.* t is shown for a model lipid membrane of area 0.06 cm^2 and distilled water as the perfusate and initial aqueous medium. The vertical bars associated with points give the standard deviation.

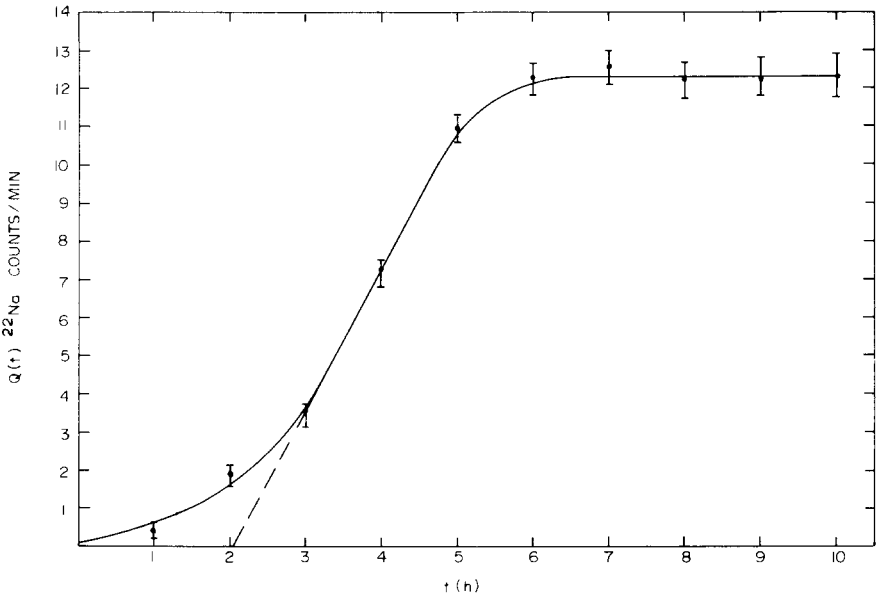

Fig. 3. $^{22}Na^+$ permeability of membrane with area 0.03 cm^2 and 0.1 M NaCl solution as the aqueous medium and perfusate. Permeability constant $k = 2.6 \cdot 10^{-8}$ cm/sec, calculated from the straight line portion.

TABLE I

RELATIONSHIP BETWEEN THE $^{22}Na^+$ PERMEABILITY CONSTANT AND THE % *n*-TETRADECANE IN THE MEMBRANE SOLUTION

Aqueous medium	*Number of experiments*	$^{22}Na^+$ *permeability duration (h)*	*Equation of line through set of points of k vs. % n-tetradecane* by least squares fit*	*Standard error of estimate* (δy)
0.1 M NaCl	5	6–7	$y = -0.39\,x + 8.85$	± 0.19
Distilled water	12	10–12	$y = -0.02\,x + 0.60$	± 0.21
0.5 mM $MgCl_2$	7	4	$y = -0.004x + 0.15$	± 0.04

* k is the permeability constant for $^{22}Na^+$ in $1 \cdot 10^{-8}$ cm/sec, k and % *n*-tetradecane represented by y and x, respectively.

From the curve it is seen that the amount of $^{22}Na^+$ diffusing through reaches a steady state value in 7 h and continues at this rate to 10 h. In a few experiments the perfusate samples were collected every 15 min and counted for $^{22}Na^+$ activity to the same statistical accuracy as the hourly samples of the other experiments. In the curve of $Q(t)$ *vs.* t four points for every hour were then obtained. After 10 h the amount diffusing through decreases and becomes undetectable after 11 h. $^{22}Na^+$ permeability

appears to have been shut off. In all of the twelve experiments in distilled water the shutting off occurred in 10–12 h (Table I). The samples of the experiment, from which Fig. 2 was derived, were also counted for $^{22}Na^+$ by NaI(Tl) crystal spectrometry (1.28 MeV photopeak) and a similar curve was obtained.

From the steady state portion of the curve in Fig. 2 the amount of $^{22}Na^+$ permeating in a unidirectional manner through the membrane per unit time, J_i, can be calculated in counts/min per sec. The permeability constant k (cm/sec) may be calculated from the equation:

$$J_i = k \cdot A \cdot \Delta c$$

where A is the area of membrane in cm^2, and $\Delta c = c_p - c_{pf} \simeq c_p$, since c_p is the concentration of $^{22}Na^+$ in the proximal chamber (approx. $1 \cdot 10^6$ counts/min per cm^3) and c_{pf} is the concentration of $^{22}Na^+$ in the perfusion chamber ($\leqslant$0.1 counts/min per cm^3).

In Fig. 3 a typical curve of $Q(t)$ *vs.* t is shown for a model lipid membrane of area 0.03 cm^2 and 0.1 M NaCl as the perfusate and initial aqueous medium. The vertical bars again give the standard deviation for each point. In this instance the

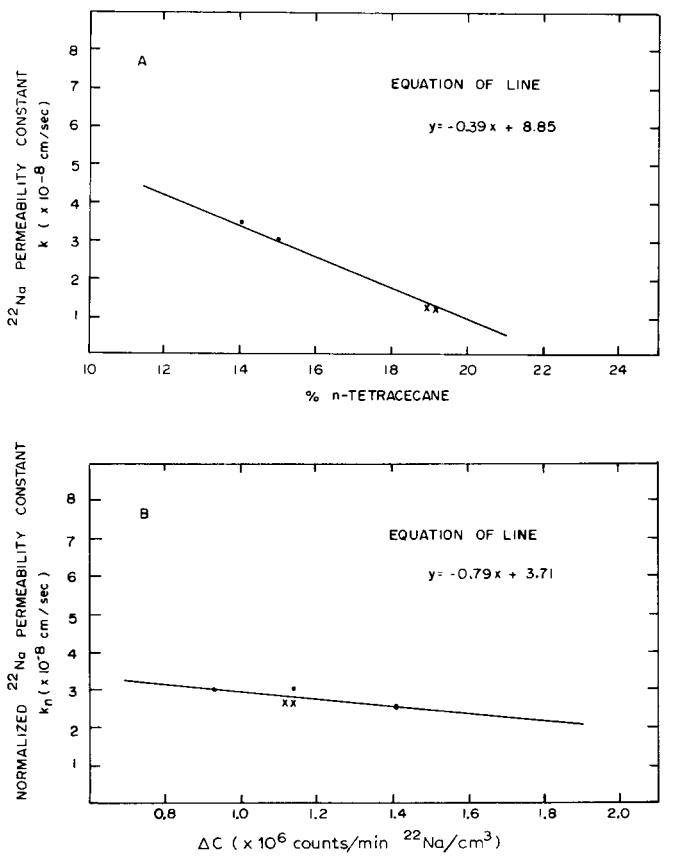

Fig. 4. A. Permeability constant k *vs.* % n-tetradecane in the membrane solution. B. Permeability constant k_n, normalized to 15% n-tetradecane, *vs.* Δc, the $^{22}Na^+$ concentration difference across the membrane. Aqueous medium: 0.1 M NaCl. Membrane area: 0.03 cm^2 (●) and 0.06 cm^2 (×), respectively.

steady state flux of $^{22}Na^+$ through the membrane begins at 3 h and declines after 5 h. Between the 6th and 7th h the $^{22}Na^+$ permeability appears to have been shut off. In all of the five experiments in 0.1 M NaCl the shutting off occurred in 6–7 h (Table I) and similar curves were again obtained by counting the samples of all the experiments

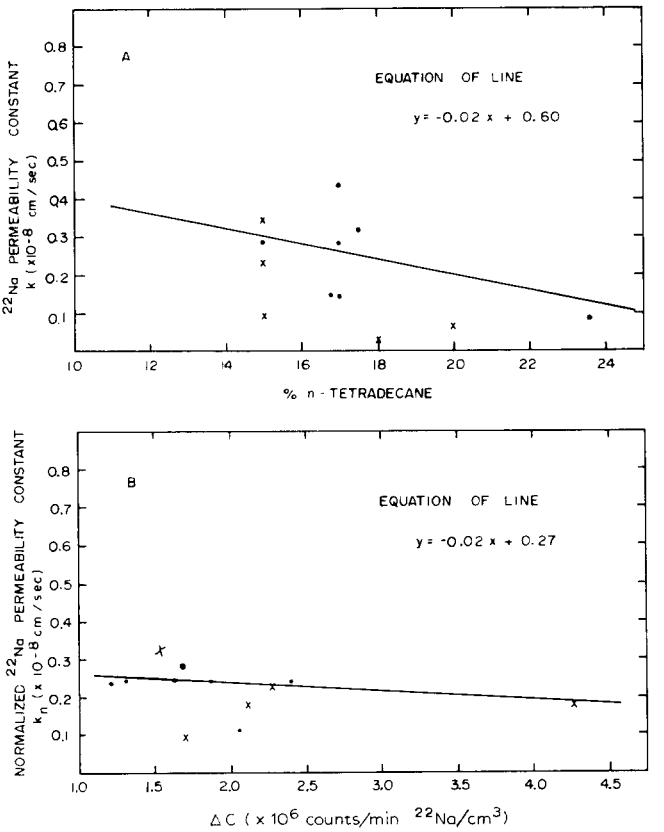

Fig. 5. A. Permeability constant k *vs.* % n-tetradecane in the membrane solution. B. Permeability constant k_n, normalized to 15 % n-tetradecane, *vs.* Δc, the $^{22}Na^+$ concentration difference across the membrane. Aqueous medium: distilled water. Membrane area: 0.03 cm^2 (●) and 0.06 cm^2 (×), respectively.

by NaI(Tl) crystal spectrometry (1.28 MeV photopeak). In seven similar experiments run in 0.5 mM $MgCl_2$ the shutting off time was reduced to 4 h.

The permeability constants for the series of experiments in 0.1 M NaCl and distilled water correlate with the percent n-tetradecane in the membrane solution as shown in Figs. 4A and 5A, respectively. The equations of the lines through the two sets of points of k *vs.* percent n-tetradecane by least squares fitting are given in the figures and in Table I. The last column of Table I gives the standard error of estimate. The correlation of k with the percent n-tetradecane in the membrane solution for the series in 0.5 mM $MgCl_2$ was not as strong but has been included in the table.

In the 0.1 M NaCl and distilled water series, the values of k were normalized to 15 % n-tetradecane in the membrane solution by using the respective line equations given in Table I. The normalized permeability constants k_n were then plotted against

Δc, and Figs. 4B and 5B show the results. The equations of the lines through the points, obtained by least squares analysis, are given in the figures and in Table II (third column). The standard errors of estimate are given in the fourth column of the table.

TABLE II

RELATIONSHIP BETWEEN THE $^{22}Na^+$ PERMEABILITY CONSTANT k_n AND Δc, THE $^{22}Na^+$ CONCENTRATION DIFFERENCE ACROSS THE MEMBRANE

Aqueous medium	*Number of experiments*	*Equation of line through set of points of k_n vs. Δc by least squares fit***	*Standard error of estimate (δy)*	*$k_n^* \times 10^8$ as $\Delta c \to 0$ (cm/sec)*
0.1 M NaCl	5	$y = -0.79\ x + 3.71$	± 0.09	3.71
Distilled water	12	$y = -0.02\ x + 0.27$	± 0.10	0.27
0.5 mM $MgCl_2$	7	$y = -0.09\ x + 0.25$***	± 0.01	0.25
	7	$y = -0.005x + 0.05$	± 0.06	0.05

* k_n = value of k normalized to 15% n-tetradecane in the membrane solution.
** k_n and Δc represented by y and x, respectively.
*** Values of k not normalized to 15% n-tetradecane in the membrane solution in this instance.

For the series in 0.5 mM $MgCl_2$ the correlation of k_n with Δc was not as good as the correlation of the permeability constants k with Δc. The standard error of estimate of the former is 6 times that of the latter (Table II, fourth and third row, respectively). Therefore for this series k is plotted against Δc, Fig. 6. In all three series, however, the fitted lines have a negative slope and suggest that solvent drag has a significant effect on $^{22}Na^+$ diffusion through the lipid membrane.

The permeability constants for the series of experiments in 0.1 M NaCl and distilled water, normalized to 15% n-tetradecane in the membrane solution, were found not to vary with the area of the membrane, a necessary and sufficient condition to rule out peripheral leakage. The result is consistent with earlier observations[4]. In the 0.5 mM $MgCl_2$ series the 0.03-cm^2 membrane was used only once and the permeability constant obtained for it compares with the values obtained for the 0.06-cm^2 membranes.

In the equations of the fitted lines of Figs. 4B, 5B, and 6 the calculated values of the y intercepts are given and they are the permeability constants as $\Delta c \to 0$ (Table II, fifth column). The values of the $^{22}Na^+$ permeability constants, thus defined, for the lipid membranes in 0.1 M NaCl, distilled water, and 0.5 mM $MgCl_2$ are $3.71 \cdot 10^{-8}$, $0.27 \cdot 10^{-8}$, and $0.25 \cdot 10^{-8}$ cm/sec, respectively.

In Figs. 2 and 3 the time integral $Q(t)$ of the $^{22}Na^+$ flux through the membrane is shown to approach a linear or steady state portion. Extrapolation of this linear portion to the time axis provides a measure of the diffusion time lag t_l (ref. 7). In Table III, second column, the mean values of t_l are given, along with the standard deviation, for the three series of experiments. The diffusion time lag for the $^{22}Na^+$ is longest in 0.1 M NaCl and shortest in 0.5 mM $MgCl_2$. Qualitatively it may be accounted for on the basis of the relative energies of adsorption of the ions in the polar layer of the membrane. Since Mg^{2+} is more strongly bound to the polar layer than Na^+ (ref. 8), the density of the latter in the layer will be relatively less in 0.5 mM $MgCl_2$

TABLE III

DIFFUSION TIME LAG, FLUX, CONCENTRATION, CONDUCTIVITY, AND TRANSFERENCE NUMBER OF Na^+ IN MODEL LIPID MEMBRANE IN VARIOUS MEDIA

t_l, diffusion time lag; $J_i = k_{\Delta c \to 0} \cdot A \cdot \Delta c$, unidirectional Na^+ flux; A, membrane area (0.06 cm^2); Δc, Na^+ concentration difference across the membrane (0.25 mM); $m_i = 6 J_i t_l$, amount of Na^+ in the membrane; $[Na^+]$, molar concentration of Na^+ if m_i is distributed uniformly in a 100-Å thick membrane; $[Na^+]_p$, molar concentration of Na^+ if m_i is distributed in the two approx. 2-Å thick negative regions of the polar layers only; $K_i = J_i F^2/RT$, the Na^+ contribution to membrane conductivity; K, electrical conductivity of membrane; Na^+ transference number $t_i = J_i F^2/RTK$; F, R, and T have their usual meaning; temperature 37°.

Aqueous medium	t_l (*min* ± σ)	$k_{\Delta c \to 0} \times 10^8$ (*cm/sec*)	$J_i \times 10^{16}$ (*moles/sec*)	$m_i \times 10^{12}$ (*moles*)	$[Na^+]$ (*M*)	$[Na^+]_p$ (*M*)	$K_i \times 10^9$ (Ω^{-1})	$K \times 10^9$ (Ω^{-1})	$K - K_i/K_i$	t_i
0.1 M NaCl	160 ± 40	3.71	5.5	31	0.5	12.7	1.9	33	16.3	0.06
Distilled water	104 ± 53	0.27	0.4	1.5	0.025	0.60	0.14	1	6.1	0.14
0.5 mM $MgCl_2$	16 ± 13	0.25	0.4	0.21	0.004	0.10	0.14	7.5	53	0.02

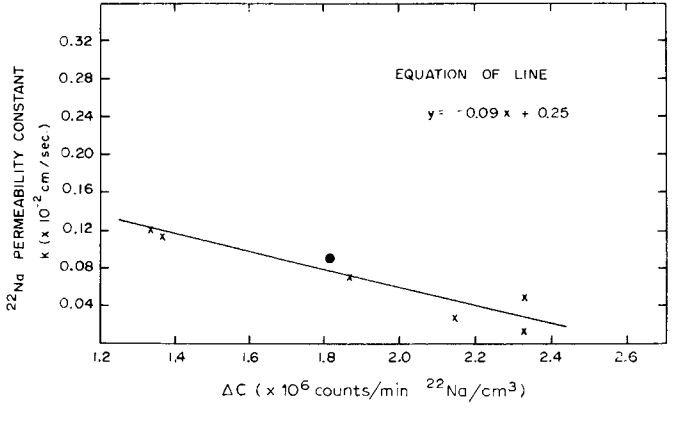

Fig. 6. Permeability constant k *vs.* Δc, the $^{22}Na^+$ concentration difference across the membrane. Aqueous medium: 0.5 mM $MgCl_2$. Membrane area: 0.03 cm^2 (●) and 0.06 cm^2 (×), respectively.

than in 0.1 M NaCl (ref. 9). For a constant Na^+ partition coefficient, therefore, t_l in 0.5 mM $MgCl_2$ will be less than its value in 0.1 M NaCl.

The values of t_l are useful in calculating m_i, the amount of Na^+ in the membrane[10]. The values of m_i, calculated from $m_i = 6 J_i t_l$, are given in Table III, column five. The values of J_i, used in the calculation were obtained from: $J_i = k_{\Delta c \to 0} \cdot A \cdot \Delta c$, and are given in Table III, column four for values of A and Δc that are specified in the same table. In column six of the same table m_i is expressed as a molar concentration on the assumption that it is uniformly distributed throughout the volume of the membrane of thickness 100 Å and area 0.06 cm^2. It is more reasonable to assume, however, that most of the Na^+ within the membrane is located in the two hydrophilic polar layers[9]. Column seven of Table III gives the local concentration if the amount of Na^+ given by m_i is uniformly distributed within two approx. 2-Å thick negative regions of the polar layers. The highest value of 12.7 molar, obtained for the series in 0.1 M NaCl, may not be in excess of the water solubility of Na^+ (ref. 9). As suggested earlier the concentration of Na^+ in the polar layers of the membrane is much less in 0.5 mM $MgCl_2$ than in 0.1 M NaCl.

The Na^+ contribution to the membrane conductivity K_i is of interest[10] and has been calculated from:

$$K_i = J_i F^2 / RT$$

where F, R, T have their usual meaning. The values of K_i are given in Table III, column eight for a temperature of 37°. The stable values for the electrical conductivity (K) of the model membrane at 37° in 0.1 M NaCl, distilled water, and 0.5 mM $MgCl_2$ are given in column nine of the same table. In column ten the ratio $(K - K_i)/K_i$ is given for the three aqueous media. It is seen that the electrical conductivity in each case is considerably larger than the Na^+ contribution to membrane conductivity in the absence of an applied electric field.

The last column of Table III gives the Na^+ transference numbers t_i calculated from:

$$t_i = J_i F^2 / RTK$$

They suggest that the major charge carrier through the membrane is not Na^+ but some other charged species. The relative values of t_i in 0.1 M NaCl, distilled water, and 0.5 mM $MgCl_2$ are consistent with the view that divalent cations are more effective than monovalent cations in bringing about close packing of the membrane[11,12] and that such a transition in its structure increases its resistance to the diffusion of Na^+.

DISCUSSION

The dependence of the permeability constant k on the amount of n-tetradecane in the membrane solution suggests that the amount of n-tetradecane in the membrane is variable. This dependence is also consistent with the view that the apolar solvent acts as a filler molecule in the hydrocarbon layer of the membrane. It has been suggested that these molecules are oriented perpendicularly to the plane of the membrane[13]. They might therefore be expected to increase the resistance of the membrane to the diffusing $^{22}Na^+$ if the hydrocarbon layer is sufficiently close packed.

The lack of a dependence of k on the amount of n-tetradecane with 0.5 mM $MgCl_2$ as the perfusate may perhaps be attributable to the substantially lower value of k. Also the Mg^{2+} increases the close packing of the head groups of the phospholipid molecules, decreases the free energy of configuration, and increases the Van der Waals forces between fatty acids of adjacent phospholipids[14]. The interaction of the $^{22}Na^+$ with n-tetradecane may then become relatively unimportant.

A limitation on Δc exists by virtue of the radiation sensitivity of the phospholipid membrane (in preparation). Concentrations equivalent to a $^{22}Na^+$ activity greater than $1 \cdot 10^8$ disint./min per cm^3 in the proximal chamber ruptured the membranes within a few hours and precluded the 10–15-h experiments desired. Suitable experiments with non-radioactive NaCl solutions established that the early rupture of the membrane was not due to an excessive osmotic pressure gradient. Δc was therefore held at about $1 \cdot 10^6$ counts/min per cm^3 or lower.

At these concentrations the permeability constants are not primarily dependent on any effect due to irradiation damage. Radiation effects such as desaturation and scissions of fatty acid chains in the membrane[15] would lead to a loosening of the membrane structure. The ion permeability constants might then be expected to increase with Δc and this was not observed. In all three series of experiments the permeability constants, normalized to 15% n-tetradecane in the membrane solution or not, are inversely proportional to Δc. Now the osmotic water permeability constant for the lipid membrane in the presence of a 0.25 mM NaCl concentration gradient in 0.1 M NaCl was found to be $7.3 \cdot 10^{-4}$ cm/sec, a result consistent with published values[16]. The value exceeds the highest $^{22}Na^+$ permeability constant obtained in these experiments by more than four orders of magnitude. The much higher water permeability of the membrane must influence the ionic movements by their interaction with water. The dependence of the permeability constants on Δc may be attributed, therefore, to water drag on the $^{22}Na^+$ and indicates common pathways for the countercurrent diffusion of ions and water in the model membrane.

The permeability constant of $3.71 \cdot 10^{-8}$ cm/sec for $^{22}Na^+$ in 0.1 M NaCl as $\Delta c \to 0$ is larger than the upper limit of $1 \cdot 10^{-9}$ cm/sec suggested earlier[1]. The two values are not inconsistent, however, when it is considered that the slope of the steady state portion of the graph in Fig. 3 is approx. 7 times that of a straight line approximation through the points corresponding to the first 2 h, the length of the earlier experiments[4]. Furthermore, the value of $3.71 \cdot 10^{-8}$ cm/sec is a maximum value, obtained by extrapolating to $\Delta c \to 0$, a condition not obtained in the earlier study. In addition the membrane used here is probably different in composition.

Comparisons with permeability data obtained for biological membranes can only be of an approximate nature since different solute compositions are invariably used. The parameters describing solute–membrane, membrane–solvent, and solvent–solute interactions are not sufficiently well established to permit normalization of ion fluxes to the same experimental conditions. Nevertheless it is interesting that the permeability constant of $3.71 \cdot 10^{-8}$ cm/sec compares in order of magnitude with the passive Na^+ permeability ($6 \cdot 10^{-8}$ cm/sec) of amphibian skins[17]. For $\Delta c = 0.23$ M NaCl the permeability constant of $3.71 \cdot 10^{-8}$ cm/sec corresponds to a unidirectional Na^+ flux of $8.5 \cdot 10^{-12}$ moles/cm^2 per sec, a value that is within an order of magnitude of the average resting Na^+ influx in perfused squid giant axons for a 0.23 M NaCl concentration difference across the membrane[18].

As shown in Figs. 2 and 3 the time integral $Q(t)$ of the $^{22}Na^+$ flux through the membrane reaches a constant value at a time that depends on the ionic composition of the medium, Table I, third column. It means that, within the sensitivity of the tracer technique used, the membrane becomes impermeable to $^{22}Na^+$. A number of properties of the membrane may be considered in a preliminary general account of the drop off in Na^+ permeability.

The durability of the unmodified lipid membranes (up to 21 days) is long compared to the duration of the permeability experiments (10–15 h). This stability of the membrane suggests that oxidation of its unsaturated fatty acids is minimal and that the hydrocarbon region exists as a tight system[19]. Cross linking between neighbouring molecular chains, resulting from secondary chemical reactions initiated by the radiation from the $^{22}Na^+$ tracer[15], may play a part in producing membrane stability, although the limitation on Δc, described earlier, is evidence that it is not a predominant factor. It has also been shown that radiation increases the electrical conductivity of model lipid membranes[20], a feature that appears to indicate an increase in membrane permeability if it is assumed that the current through the membrane is ionic.

More important perhaps, than the foregoing, are factors which lead to a closely packed structure, or that increase the energy required for ion permeation. Counterions, sorbed to the polar layers of the membrane, probably play an important part with respect to these aspects of the transition[9]. The dependence of the duration of membrane permeability on the ionic composition of the aqueous phase is evidence in support of it.

The transition of the membrane to a closed configuration is promoted by decreasing the amount of water contained in it. Membrane hydration may be reduced through manipulation of the ionic composition and strength of the aqueous phase[11,21]. Divalent cations, through their stronger interaction with the polar layer of the membrane, are more effective than monovalent cations in reducing the water content of model membranes. A transition time of 4 and 6–7 h for membranes in 0.5 mM $MgCl_2$ and 0.1 M NaCl, respectively, is therefore qualitatively correct. For the permeability experiments with membranes in distilled water the ionic strength in the proximal and perfusion chambers was approx. 0.25 and approx. 1 mM NaCl, respectively. A longer time for the appearance of the transition to the impermeable state might be expected and was obtained (10–12 h).

Periodic microscopic observations of the membranes throughout the experiments did not suggest any apparent change in the fluid nature of their structure after the transition to the impermeable state. No pH change of the perfusate passing through the perfusion chamber during additional, but similar, permeability experiments was observed. The degree of ionization of the polar groups would appear not to have been changed significantly. The transition of the membrane therefore, is not due to the movement of the Na^+ involving a H^+-linked exchange.

The reduction in membrane hydration must lead to a decrease in its dielectric constant, particularly of the hydrocarbon region. The additional energy required by a $^{22}Na^+$, permitting it to diffuse into the hydrocarbon region from the aqueous medium, may easily exceed the mean thermal and electrochemical potential energies available to it under the present experimental conditions[21]. The effective permeability barrier to the $^{22}Na^+$ is then the hydrocarbon region of the membrane, a conclusion

consistent with the description of the manner in which the $^{22}Na^{+}$ permeability constant is believed to depend on the amount of *n*-tetradecane in the membrane solution.

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