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ION-SELECTIVE PROPERTIES OF SOME LIPOID SYSTEMS AND THEIR RELATION TO BIOLOGICAL MEMBRANES

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SUMMARY

Bi-ionic potentials and single ionic potentials have been determined across membranes containing mixtures of sodium and potassium stearate interposed between aqueous NaCl/KCl solutions. Ionic transport numbers evaluated from these data compare well with values obtained by a direct current method.

The results indicate ion-exchange properties and preferential sorption of KCl by the membrane.

The discrimination of the lipoids towards Na^+ and K^+ is found to be caused by the hydrophobic part of the stearate aggregates. Some biological implications related to lipoprotein structures are briefly discussed.

INTRODUCTION

The selectivity towards Na^+ and K^+ is an important feature of many living systems. Various explanations have been proposed for the selective accumulation of K^+ within biological cells. The most general phenomenological description was recently given by KATCHALSKY[1]. It states that a combined action of metabolic processes, matter transport and structural effects are involved in natural membranes. The role of ion-selective carrier molecules may be very important, yet no complete model system has been developed thus far to test the theory.

The present investigation deals only with structural effects related to lipoidic systems and to lipoidic *plus* gelatine systems as determined by a different uptake of ionic species.

Some years ago, ILANI[2] observed that apolar and lipoid substances supported on a suitable grid could act as selectors not only between Na^+ and Cl^-, but also between Na^+ and K^+. This was deduced by means of electric potential measurements.

However, no discussion could be given about the molecular causes of this effect, which might be due to an increased ionic mobility ratio.

In the following we attempt to carry ILANI's investigation somewhat further. We shall discuss the Na^+/K^+ selectivity using more defined systems within a wider range of Na^+/K^+ ratios.

We first make a theoretical approach to the problem of electric ion transport, using the formalism of nonequilibrium thermodynamics.

THEORY

Transport processes in bi-ionic membrane systems have been discussed in a formal theory by STAVERMAN[3,4].

Writing the relevant transport equations in a systematic scheme:

$$J_{\mathrm{NaCl}} = w_{\mathrm{NaCl}}\Delta\mu_{\mathrm{NaCl}} + w_{\mathrm{Na^+K^+}}\Delta\mu_{\mathrm{KCl}} + t_{\mathrm{Na^+}}\frac{I}{F} \qquad (1a)$$

$$J_{\mathrm{KCl}} = w_{\mathrm{Na^+K^+}}\Delta\mu_{\mathrm{NaCl}} + w_{\mathrm{KCl}}\Delta\mu_{\mathrm{KCl}} + t_{\mathrm{K^+}}\frac{I}{F} \qquad (1b)$$

$$I = \frac{t_{\mathrm{Na^+}}k}{F}\Delta\mu_{\mathrm{NaCl}} + \frac{t_{\mathrm{K^+}}k}{F}\Delta\mu_{\mathrm{KCl}} + kE \qquad (1c)$$

where J is the salt flux in moles·cm^{-2}·sec^{-1}; F is the faraday; $I = (J_{\mathrm{Na^+}} + J_{\mathrm{K^+}} - J_{\mathrm{Cl^-}})$ is the electric current density in A·cm^{-2}; w is the salt permeability or coupled permeability defined as $w = D/RTd$, D is the related Fickean diffusion coefficient, R is the gas constant, T the absolute temperature and d the membrane thickness; t_{i} is the transport number of the ion i; $\Delta\mu$ is the difference in chemical potential across the membrane; E is the membrane potential; k is the specific membrane conductivity.

We can easily derive expressions for the single ionic potential and bi-ionic potential. This is done most conveniently by using relation 1c. If $\Delta\mu_{\mathrm{NaCl}}$ and I values vanish, we have the following expression for the membrane potential between the two solution phases "o" and "i".

$FE = -t_{\mathrm{K^+}}\Delta\mu_{\mathrm{KCl}}$ or more precisely

$$E = -\frac{2RT}{F}\int_{\mathrm{''i''}}^{\mathrm{''o''}} t_{\mathrm{K^+}}\,\mathrm{d}\ln a_{\mathrm{KCl}}$$

where

$$a_{\mathrm{KCl}} = f_{\pm}\, c_{\mathrm{KCl}}$$

$f_{\pm}$ is the mean activity coefficient, and c is the concentration of KCl. For isobaric systems[5], *i.e.*, systems with steady osmotic volume flow (as dealt with in the present case)

$$E = -\frac{2RT}{F}\int_{\mathrm{''i''}}^{\mathrm{''o''}} (t_{\mathrm{K^+}} - 0.018\, t_w \bar{c}_{\mathrm{KCl}})\,\mathrm{d}\ln a_{\mathrm{KCl}} \qquad (2)$$

where t_{w} is the transport number of water and $\bar{c}_{\mathrm{KCl}}$ is the average of the two outer KCl concentrations. If only a few values of E are available, Eqn. 2 reduces to

$$E = \frac{2RT}{F}(t_{\mathrm{K^+}} - 0.018\, t_{\mathrm{w}}\bar{c}_{\mathrm{KCl}})\ln\frac{a_{\mathrm{KCl}}^{\mathrm{''i''}}}{a_{\mathrm{KCl}}^{\mathrm{''o''}}} \qquad (2a)$$

To a first approximation, $\bar{c}_{\mathrm{KCl}}$ is chosen as the mean value[6] between $c_{\mathrm{KCl}}^{\mathrm{''o''}}$ and $c_{\mathrm{KCl}}^{\mathrm{''i''}}$

$$\bar{c}_{\mathrm{KCl}} = \sqrt{c_{\mathrm{KCl}}^{\mathrm{''i''}}\, c_{\mathrm{KCl}}^{\mathrm{''o''}}} \qquad (3)$$

For bi-ionic systems, if we assume $I = 0$ in Eqn. 1c, we get for the case of vanishing osmotic gradient ($\Delta\mu_{\mathrm{Cl}^-} = 0$)

$$E = \frac{RT}{F}(t_{\mathrm{Na}^+} - t_{\mathrm{K}^+}) \ln \frac{c''^{\mathrm{i}''}_{\mathrm{NaCl}}}{c''^{\mathrm{o}''}_{\mathrm{NaCl}}} = \frac{RT}{F}(t_{\mathrm{Na}^+} - t_{\mathrm{K}^+}) \ln \frac{c''^{\mathrm{o}''}_{\mathrm{KCl}}}{c''^{\mathrm{i}''}_{\mathrm{KCl}}} \tag{4}$$

since here the ionic concentration gradients are equal and have opposite sign.

$$\Delta\mu_{\mathrm{Na}^+} = -\Delta\mu_{\mathrm{K}^+} \tag{5}$$

EXPERIMENTAL AND RESULTS

Materials and membranes

Alkali halide solutions were made from stock, prepared by dissolving NaCl or KCl (p.a., Merck) in distilled water (specific resistance $5 \cdot 10^5$ Ω). Membranes were prepared by dissolving sodium and potassium stearate mixtures to have a concentration of 15 g per 100 ml of each form and corresponding to a total amount of 0.90 equiv per kg (wet) theoretical exchange capacity (*i.e.*, complete dissociation of the carboxyl groups) in the appropriate electrolyte solutions. When gelatin was used as a support, this was added to the boiling lipoid solution until complete mixing had been achieved. The gelatin–stearate mixtures were casted (40°) into a perspex grid, with holes of 1 mm diameter and a thickness of 2 mm and then allowed to cool. The gelatin had its isoelectric point at a pH of 5.0.

Single ionic and bi-ionic potential measurements

A cylindrical cell was used to contain the dilute KCl solution. This was placed in a somewhat larger vessel containing the concentrated electrolyte solution. Only the dilute solution was stirred magnetically since stirring the concentrated one as well did not affect the potentials. When the sheet-supported membrane array was used, both the external compartments were stirred. Ag/AgCl electrodes were prepared by anodizing pure silver wire (0.5 mm diameter) in 0.75 M HCl (p.a.) for 10 min at 2 mA·cm^{-2} and conditioning afterwards in the suitable NaCl/KCl mixtures. Potentials were measured with a PYE potentiometer at ± 0.2 mV accuracy. The reading became stable after several hours. Meanwhile the electrolyte solutions were replaced twice. When the membrane was supported by cellulose sheets, it was contained in a glass ring of 3 mm thickness. All measurements were made at 22 ± 1°. The results are given in Tables I and II.

TABLE I

SINGLE IONIC POTENTIAL VALUES FOR GELATIN-SUPPORTED MEMBRANES (x) AND CELLULOSE SHEET-SUPPORTED MEMBRANE (xx)

	c''i''/c''o'' ratio	E_x *(mV)*	E_{xx} *(mV)*
KCl	0.02/0.04	26.9	24.0
KCl	0.06/0.12	17.3	16.0

TABLE II

BI-IONIC POTENTIAL VALUES FOR GELATIN-SUPPORTED MEMBRANES

E_1 at total salt concentration of 0.02 M, E_2 at total salt concentration of 0.20 M.

$c^{''i''}_{Na^+}/c^{''i''}_{K^+}$ ratio	$c^{''o''}_{Na^+}/c^{''o''}_{K^+}$ ratio	E_1 (*mV*)	E_2 (*mV*)
9/1	1/9	7.00	6.35
8/2	2/8	6.04	5.90
7/3	3/7	3.31	—
6/4	4/6	1.35	—

Direct current transport measurements

The total current passing through the membrane was derived from measurements of the potential drop across a standard resistance. The Ag/AgCl electrodes had been prepared by electroplating silver gauze as anode in a 0.75 M HCl solution at 15 mA·cm^{-2} for 30 min. Both the anode and cathode compartments were stirred. Afterwards the cathodic solution was analyzed by means of an Evans Electroselenium LTD flame photometer. The total halide concentration was 0.02 M.

DISCUSSION

Applying Eqn. 2a to the single ionic potential results and substituting a t_w value (W. DORST AND H. L. BOOY, unpublished results) of 50, we find from E_x at $\bar{c}_{KCl} = 0.028$ M (Eqn. 3): $t_{K^+} = 0.71$, $t_{Cl^-} = 0.29$.

For the sheet-supported membrane, the water transport number is 100 equiv/Faraday, close to the limiting value[7]. From Eqn. 2a, we obtain for this case: $t_{K^+} = 0.70$, $t_{Cl^-} = 0.30$. At 0.084 M KCl we find $t_{K^+} = 0.53$, $t_{Cl^-} = 0.47$ so the selectivity almost disappears for the single salt system at higher concentrations.

From the E_1 values in Table II we deduce the values in Table III, and finally, from direct current results the values in Table IV.

TABLE III

TRANSPORT NUMBERS (EMF)

t_{K^+}	t_{Na^+}
0.41	0.29
0.44	0.27
0.43	0.28
0.41	0 29

TABLE IV

TRANSPORT NUMBERS (DIRECT CURRENT)

c_{Na^+}/c_{K^+}	t_{K^+}	t_{Na^+}
7/3	0.30	0.40
1	0.45	0.25
3/7	0.60	0.10

Thus concentrated stearate solutions can behave as quite strong ion exchangers. Similar effects have been observed for cellulose acetate membranes[6]. There is only little variation of the bi-ionic potentials with salt content, indicating "saturating kinetics" of the mixed stearate system[8].

A preferential sorption may explain the high values of t_{K^+}/t_{Na^+}; when $c_{Na^+} \ll c_{K^+}$ as found by the direct current method. A similar retention effect for nonelectrolytes has been observed some years ago[9].

It must be concluded that the co-ion mobility in the stearate membranes strongly depends on the external salt concentration (Table I). This usually occurs in rather dense membranes, where short range forces between co-ions and the membrane matrix exist. In addition, it can be deduced from Table III that the Na^+/K^+ specificity of the stearates (Table IV) is a sorption effect, not a mobility effect.

In this connection, it is interesting to note that the single ionic potentials were found always positive at the dilute side when KCl was replaced by NaCl.

Obviously, structural changes in sodium stearate–potassium stearate systems are of great importance in determining their selectivity[10] towards ions. From Table III we derive for the average mobility ratio of K^+ and Na^+ value of 1.5. Applying this value to the data of Table IV we find for an external Na^+/K^+ ratio of 3/7 an internal ratio of about 1/3 from the direct current data.

We may then state that while the single ionic potential results point to ion-exchange properties of the stearate, the bi-ionic potential results indicate a "normal" (*i.e.*, comparable with the free solution) ratio of cation mobilities and that combination of bi-ionic potential, single ionic potential and direct current transport numbers point to a specific sorption of KCl in the studied mixed stearate gels.

The theoretical and experimental procedure described, differs essentially from the usual experimental approach to bi-ionic potential measurements which consists in varying one salt concentration gradient, whereas the other gradient is kept constant. This would involve averaging transport numbers over various salt concentrations at which potential measurements are made, thus adding an additional parameter in determining ion transport numbers and consequently specific ion sorption in the membrane. Moreover the evaluation of transport data by direct current method is usually done in a similar manner. From Table III it becomes apparent that ion-mobility ratio can be extrapolated quite accurately from bi-ionic potential measurements and their combination with direct current data gives at once the ratio of specific ion sorption, thus avoiding laborious analytical techniques.

By avoiding osmotic gradients the values of anion transport number can be easily combined with the results of bi-ionic potential and direct current data.

In living cells, Na^+/K^+ ratios of 1/20 or smaller are quite common. This is caused by active transport, which induces selective accumulation and osmotic tension in the membrane macromolecules. The latter may in turn pump the ions through the membrane and affect the lipoid structures. Making the lipoid part of an anisotropic membrane will be the subject of our future research.

REFERENCES

1 A. KATCHALSKY, *Neurosciences*, Rockefeller University Press, New York, 1967, p. 300.
2 A. ILANI, *J. Gen. Physiol.*, 46 (1963) 839.
3 A. J. STAVERMAN, *Trans. Faraday Soc.*, 48 (1952) 176.
4 F. BERGSMA AND A. J. STAVERMAN, *Discussions Faraday Soc.*, 21 (1958) 61.
5 J. W. LORIMER, E. I. BOTERENBROOD AND J. J. HERMANS, *Discussions Faraday Soc.*, 21 (1956) 141.
6 W. DORST AND C. BOTRÉ, *Farmaco Ed. Sci.*, 23 (1968) 399.
7 K. S. SPIEGLER, *Trans. Faraday Soc.*, 54 (1958) 1408.
8 G. M. SHEAN AND K. SOLLNER, *Ann. N.Y. Acad. Sci.*, 137 (1966) 759.
9 J. L. TALEN, Ph. D. Thesis, Leiden, 1963.
10 V. LUZZATI, F. REISS-HUSSON, E. RIVAS, T. GULIK-KRZYWICKI, *Ann. N.Y. Acad. Sci.*, 137 (1966) 408.