Biochimica et Biophysica Acta 900 (1987) 183–190
Elsevier

BBA 73585

Potential study of basement membrane

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(Received 19 December 1986)

Key words: Membrane potential; Basement membrane; Anterior lens capsule; Electrolyte diffusion; (Bovine lens)

The membrane potential of a basement membrane (bovine-anterior-lens-capsule) was studied by use of the Gibbs-Donnan systems. The T.M.S. (Teorell, Meyer, Sievers) theory was applied to these systems for the treatment of experimental data. The results show that, in the basement membrane, the density of fixed charges such as ionogenic groups encountered in heparan sulfate proteoglycan is low. The ratios of the mobilities from the chloride anion and the alkaline cation are different to those in water. In this type of membrane, the mobility of Cl^- is higher than the mobilities of K^+, Na^+ and Ca^{2+}. This result leads us to investigate further the diffusion problem of electrolytes through basement membrane.

Introduction

The importance of basement membrane investigations becomes more and more evident. Biologists and chemists have devoted many efforts to discover the nature of basement membrane [1–10]. Their researches have shown that the basement membrane plays a very important role in attachement, spreading, migration, growth, repair and differentiation of the overlying cell population as well as in the physical phenomena of fluid filtration and gas exchange [10]. Basement membranes act as potential barriers to metastasis. They have been considered as important determinants in neoplasia, and as available substrata for tumour cell attachement and differentiation [6,11]. The last works showed that perhaps the basement membrane should be considered playing a central role in organs and tissue biology [12,13]. These functions of basement membranes depend certainly on their structures and components and also on ionogenic groups such as these of heparan sulfate proteoglycan [9,14–17]. These effects led us to study the electric potential behavior of these membranes.

Membrane potentials have been studied by many authors [18–20]. In this paper, the bovine-anterior-lens-capsules have been taken as the basement membranes with which a Gibbs-Donnan system [19,21] is formed. The membrane potentials were measured by using this system. The theory developed by Teorell, Meyer and Sievers (T.M.S. theory) [22–25] was used to analyze the experimental results. There is another theory developed by Kobatake and co-workers [40].

This theory was derived from the T.M.S. one. It has been developed to account for the behavior of a special experimental system, characterized by the fact that diffusion occurs through a polyelectrolyte layer settled between two semi-permeable membranes. Our experiments concern the membrane itself, in particular the membrane potential. Kobatake's theory does not apply to our experi-

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mental system, which may be described by the T.M.S. theory. We obtained the concentration range of ionogenic groups within the membrane from the calculations used in the T.M.S. theory. We have compared the phenomena in the case of several different salts, when they diffuse through the basement membrane. In the preceding paper [9], we described the diffusion of some electrolytes under the same conditions. We find the maximum concentration of salt in the membrane is dependent only of salt–membrane interactions inside the membrane. From a series of diffusion experiments performed with an applied electric polarization, we concluded that a weak voltage influences greatly the diffusion behaviours. We propose an explanation for these phenomena: an electrostatic barrier, which depends probably on the ionogenic groups such as those of heparan sulfate proteoglycan in the basement membrane, builds up progressively during diffusion of electrolyte solutions. If this hypothesis may be verified, we can understand more about the mechanism of fluid filtration or of other roles played by the basement membrane. Another question should be proposed from our research. That is, when the diffusion of electrolyte occurs within the membrane, which of the cations or the anions diffuses faster? Having examined previously the diffusion velocities for different salts with common anion, we are able to predict which among the cations diffuse faster by comparing one with another according to our calculated results. But up to now no answer can be afforded to the question concerning the relative speeds of cations and anions in the solution. In this paper, we attempt to answer this question and to discuss the problem of diffusion further.

Principle of the method

The membrane potential measurements have been performed with bovine-anterior-lens-capsules. We may admit that this membrane behaves as an ion exchange membrane with fixed charges, due to the presence of heparan sulfate proteoglycan ionogenic groups. These properties allow us to take into account the general Gibbs-Donnan system for the potential measurements and to use the T.M.S. theory in the interpretation of our results. Fig. 1 shows the schema of the Gibbs-Donnan system.

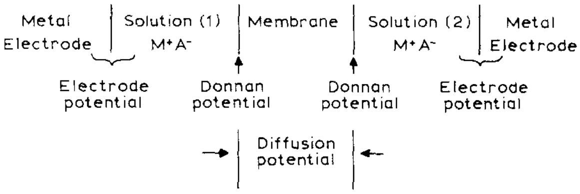

Fig. 1. Scheme of the Gibbs-Donnan system.

Using an Ag-AgCl electrode as metal electrode, we obtain the following system:

$$\text{Ag-AgCl}/\underset{M^+A^-}{\text{solution(1)}}/\text{membrane}/\underset{M^+A^-}{\text{solution(2)}}/\text{Ag-AgCl}$$

Under zero-current condition, the measured potential is the sum of the potential differences at each phase boundary

$$\phi^{M_2} - \phi^{M_1} = (\phi^{M_2} - \phi^{l_2}) + (\phi^{l_2} - \phi^{l_1}) + (\phi^{l_1} - \phi^{M_1}) \qquad (1)$$

where 1, 2 represent the aqueous solution phases. Cl^- being the potential determining ion at the both solution interfaces, Nernst equation gives

$$\phi^{M} - \phi^{l} = E^{\circ}_{Ag/AgCl} - \frac{RT}{F}\ln a_{Cl^-} \qquad (2)$$

Combining Eqns. 1 and 2, we obtain

$$\phi^{l_2} - \phi^{l_1} = (\phi^{M_2} - \phi^{M_1}) - \frac{RT}{F}\ln\frac{(a_{Cl^-})_1}{(a_{Cl^-})_2} \qquad (3)$$

where $\phi^{l_2} - \phi^{l_1}$ is the membrane potential and $\phi^{M_2} - \phi^{M_1}$ is the measured potential.

Another aspect of the T.M.S. theory is that it enables us to calculate the membrane potential under the condition that the membrane composition is known, particularly the concentration of the charged groups. But this concentration is unknown in our system. It can be approximated from the experimental data by deduction using the T.M.S. theory. In the T.M.S. theory the membrane potential is given by the sum of the diffusion potential and the Donnan potential. In the case where the membrane carries some negative charges of concentration $\overline{X}$, the diffusion potential (E_d) can be expressed by Teorell's equation [26]

$$E_d = \frac{\bar{u}_+ - \bar{u}_-}{\bar{u}_+ + \bar{u}_-}\,\frac{RT}{F}\ln\frac{\bar{u}_+(\overline{m}_+)_1 + \bar{u}_-(\overline{m}_-)_1}{\bar{u}_+(\overline{m}_+)_2 + \bar{u}_-(\overline{m}_-)_2} \qquad (4)$$

where $\bar{u}_+$ and $\bar{u}_-$ are the mobilities of cation and anion, respectively, within the membrane; $\bar{m}$ are concentrations which are given by

$$\bar{m}_+ = \frac{\bar{X}}{2} + \left(\frac{\bar{X}^2}{4} + \frac{a^2}{\gamma_\pm^2} \right)^{1/2} \tag{5}$$

$$\bar{m}_- = -\frac{\bar{X}}{2} + \left(\frac{\bar{X}^2}{4} + \frac{a^2}{\gamma_\pm^2} \right)^{1/2} \tag{6}$$

If we consider $\bar{\gamma}_+$ and $\bar{\gamma}_-$, the activity coefficients within the membrane, being equal to unity [22,25–27], and $\bar{U} = (\bar{u}_+ - \bar{u}_-)/(\bar{u}_+ + \bar{u}_-)$, we get

$$E_\mathrm{d} = \bar{U}\frac{RT}{F}\ln\frac{\bar{U}\bar{X}_1 + (\bar{X}_1^2 + 4(a_\pm^2)_1)^{1/2}}{\bar{U}\bar{X}_2 + (\bar{X}_2^2 + 4(a_\pm^2)_2)^{1/2}} \tag{7}$$

The net Donnan potential is given by

$$E_\mathrm{Don} = \frac{RT}{F}\ln\frac{(a_-)_1 \cdot (\bar{a}_-)_2}{(\bar{a}_-)_1 \cdot (a_-)_2} \tag{8}$$

where $(a_-)_1$ and $(a_-)_2$ are the activities of the Cl^- in the solutions 1 and 2, respectively. By substituting Eqn. 8 with Eqn. 6 and adding Eqn. 7 to Eqn. 8, we obtain the total membrane potential

$$\phi^{l_2} - \phi^{l_1} = \frac{RT}{F}\left\{ \ln\frac{(a_-)_2}{(a_-)_1} \cdot \frac{(4(a^2)_1 + \bar{X}^2)^{1/2} + \bar{X}}{(4(a^2)_2 + \bar{X}^2)^{1/2} + \bar{X}} + \bar{U}\ln\frac{(4(a^2)_1 + \bar{X}^2)^{1/2} + \overline{UX}}{(4(a^2)_2 + \bar{X}^2)^{1/2} + \overline{UX}} \right\} \tag{9}$$

where $(a)_1$ and $(a)_2$ are the activities of the electrolytes present at both sides of the membrane.

In order to calculate the experimental membrane potential, Eqn. 3 should be used. In this equation, we must known the activity coefficients at 37 ° C. We can obtain them by using Debye-Hückel's formula:

$$\log f_\mathrm{i} = \frac{-A_\mathrm{i}|Z_1 Z_2| I^{1/2}}{1 + I^{1/2}} \tag{10}$$

where

$$A_\mathrm{i} = N^2 e^3 (2\pi/1000)^{1/2} / (2.303 R^3 / \epsilon_\mathrm{i}^{3/2} T_\mathrm{i}^{3/2})$$

$$= 1.8246 \cdot 10^6 / (\epsilon_\mathrm{i} T_\mathrm{i})^{3/2}$$

ϵ_i = dielectric permittivity

T_i = absolute temperature (K)

f_i = activity coefficient in liquid (not within the membrane)

The following relation is easily deduced from Eqn. 10

$$f_{37^\circ\mathrm{C}} = (f_{25^\circ\mathrm{C}})^{1.0296} \tag{11}$$

Because the activity coefficients at 25 ° C can be found in some constant tables, the activity coefficients at 37 ° C can be derived.

Now we consider Eqn. 9 in which we should calculate $\bar{U}$. In the case when only one electrolyte, under the condition $n_+ = n_-$ (n's are valence numbers), is present in solution, we have $\bar{t}_+ = \bar{u}_+/(\bar{u}_+ + \bar{u}_-)$ and $\bar{t}_- = \bar{u}_-/(\bar{u}_+ + \bar{u}_-)$. Therefore

$$\bar{U} = \bar{t}_+ - \bar{t}_- \tag{12}$$

where $\bar{t}_i$ are transport numbers within membrane. They can't be measured directly without difficulties, but we can calculate them approximately [36] with

$$E/E_\mathrm{max} = 2\bar{t}_+ - 1 \text{ or } E/E_\mathrm{max} = 2\bar{t}_- - 1 \tag{13}$$

We find E/E_max after having drawn the curves $(\phi^{l_2} - \phi^{l_1})$ versus $\log(a_-)_1/(a_-)_2$ (See Figs. 3–6). E/E_max is equal to slopes ratio K/K_max; where K_max is 61.53 mV at 37°C. In the cases when either two electrolytes are present together in a solution or one electrolyte is present with its ions having different valences ($n_+ \neq n_-$), this calculation is only an approximation. Now in Eqn. 9, all terms become known except $\bar{X}$. Since the membrane potential $\phi^{l_2} - \phi^{l_1}$ has been obtained by experiments, $\bar{X}$ can be calculated. This calculation was achieved using the following method

$$\mathrm{d}(\phi^{l_2} - \phi^{l_1}) = \frac{\partial(\phi^{l_2} - \phi^{l_1})}{\partial\bar{X}}\mathrm{d}\bar{X} + \sum_i^2 \frac{\partial(\phi^{l_2} - \phi^{l_1})}{\partial a_\mathrm{i}}\mathrm{d}a_\mathrm{i} \tag{14}$$

In this equation, the terms under the sum are equal to zero, due to the fact that the a_i values are constant. Eqn. 14 becomes

$$\mathrm{d}(\phi^{l_2} - \phi^{l_1}) = \frac{\partial(\phi^{l_2} - \phi^{l_1})}{\partial\bar{X}}\mathrm{d}\bar{X} \tag{15}$$

For every '*i*' point, we get

$$\mathrm{d}(\phi^{l_2}-\phi^{l_1})_i \approx \Delta(\phi^{l_2}-\phi^{l_1})_i = (\phi^{l_2}-\phi^{l_1})_{i\mathrm{cal}} - (\phi^{l_2}-\phi^{l_1})_{i\mathrm{exp}} \tag{16}$$

Subscripts cal and exp represent the calculated value and the experimental one.

$$\begin{aligned}\frac{\partial(\phi^{l_2}-\phi^{l_1})}{\partial\bar{X}} &= \frac{RT}{F}\Bigg(\ln\frac{(a_-)_2}{(a_-)_1} + \frac{(4a_2^2+\bar{X}^2)^{1/2}-(4a_1^2+\bar{X}^2)^{1/2}}{[(4a_1^2+\bar{X}^2)(4a_2^2+\bar{X}^2)]^{1/2}} \\ &\quad + \bar{U}\Bigg[\frac{\bar{U}(4a_1^2+\bar{X}^2)^{1/2}+\bar{X}}{\big((4a_1^2+\bar{X}^2)^{1/2}+\bar{U}\bar{X}\big)(4a_1^2+\bar{X}^2)^{1/2}} \\ &\quad - \frac{\bar{U}(4a_2^2+\bar{X}^2)^{1/2}+\bar{X}}{\big((4a_2^2+\bar{X}^2)^{1/2}+\bar{U}\bar{X}\big)(4a_2^2+\bar{X}^2)^{1/2}}\Bigg]\Bigg)\end{aligned} \tag{17}$$

For every '*i*' point, we get

$$\Delta(\phi^{l_2}-\phi^{l_1})_i = \left(\frac{\partial(\phi^{l_2}-\phi^{l_1})}{\partial\bar{X}}\right)_i \cdot \Delta\bar{X}_i \tag{18}$$

Taking $\Delta\bar{X}_i \approx \Delta\bar{X} = (\Sigma_i^n \Delta\bar{X}_i)/n$ (n is the total point number) and matrices

$$A = \begin{pmatrix} \Delta(\phi^{l_2}-\phi^{l_1})_1 \\ \Delta(\phi^{l_2}-\phi^{l_1})_2 \\ \vdots \\ \Delta(\phi^{l_2}-\phi^{l_1})_n \end{pmatrix} \quad B = \begin{pmatrix} \dfrac{\partial(\phi^{l_2}-\phi^{l_1})}{\partial\bar{X}}_1 \\ \dfrac{\partial(\phi^{l_2}-\phi^{l_1})}{\partial\bar{X}}_2 \\ \vdots \\ \dfrac{\partial(\phi^{l_2}-\phi^{l_1})}{\partial\bar{X}}_n \end{pmatrix} \quad C = (\Delta\bar{X})$$

We get

$$A_{n\times 1} = B_{n\times 1} * C_{1\times 1} \tag{19}$$

$$B^{t}A = B^{t}BC \tag{20}$$

and

$$(B^{t}B)^{-1}(B^{t}A) = C \tag{21}$$

where superscripts t and -1 mean transformation matrice and inverse matrice, respectively. From these operations, $\Delta\bar{X}$ can be obtained. The next cycle of calculation is permitted, until we obtain a value $\bar{X}$ with which the calculated results are close to that of experiments.

Experiments

The potential measurements were carried out at fixed temperature (37 ± 0.5 °C) by means of an air thermostat cabinet. To maintain the concentrations on both sides of membrane at constant values during the experiment, a circulation system for every compartment of the experimental cell with a pump was used (see Fig. 2). Stabilization of the system during the experiment was checked by means of a recorder. We have chosen AgCl-Ag electrodes as metal electrodes and KCl, NaCl, $CaCl_2$ solutions as experimental ones. In every case, as shown in Fig. 2, the cathode (l_1) was immersed in a 0.10 M solution (1), the concentration of which was kept constant during the series of experiments. The anode (l_2) was immersed in solution (2) which may be adjusted between 0.01 M and 1.0 M for a series of experiments. To ascertain that the records of results are correct, every experiment was carried out twice.

Two other experiments have been achieved. In the first one, the KCl and NaCl solutions present on both sides of the membrane, respectively, had the same concentration. In the second, the concentration of KCl was kept constant (0.10 M) and that of NaCl varied during the series of experiments (0.01 M, 0.02 M,···,0.05 M).

Results and Discussion

Figs. 3–6 show the relation of the electrode potential versus $\log((a_-)_1/(a_-)_2)$ and that of the

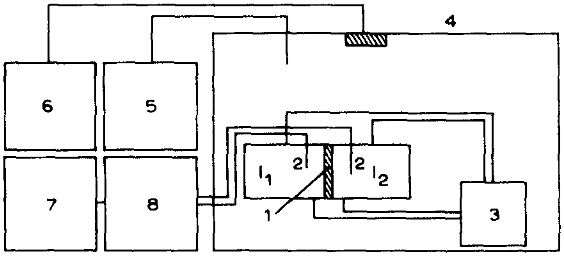

Fig. 2. Scheme of experimental apparatus. 1, Basement membrane; 2, metal electrodes; 3, pump for the circulation of the solutions; 4, air thermostat cabinet; 5, thermometer; 6, thermostat control; 7, recorder; 8, voltmeter.

membrane potential versus $\log((a_-)_1/(a_-)_2)$. The numerical results are classed in Tables I–IV. We can consider that basement membranes act as ion exchanger. This character is due to their internal structure [28]. But the basement membranes are not ideal ion exchangers, because some diffusion proceeds at the same time when we measure the membrane potentials [9,29,30]. As mentioned in the Introduction, we can predict which electrolyte diffuses faster. But we don't known which of the cations or the anions diffuse faster. This question can be answered now by our experimental results. In Tables I–IV and Figs. 3–6, we have noticed that the membrane potential $(\phi^{l_2} - \phi^{l_1})$ is negative when $\log((a_-)_1/a_-)_2)$ is greater than zero, and it is positive in the opposite case. This means that the mobility of anion is more important than that of the cation in the direction of diffusion. Chloride ions diffuse faster than the cations (K^+, Na^+, Ca^{2+}) when a basement membrane is placed in the way. We compare, in Table V, the transport number values of NaCl, KCl and $CaCl_2$ in water with these inside the membrane. The transport number value for Cl^- is greater within membrane than that in water. This result supports our hypothesis in which we supposed that a potential barrier builds up progressively during the diffusion of electrolyte solutions. This barrier, which is a double layer of ions at the immediate neighbouring of the anionic layer in membrane, can hinder further diffusion. If we compare NaCl and KCl, we find that for KCl, $\bar{t}_-$ is nearer to $\bar{t}_+$ than in the case of NaCl and that the difference between the transport number values ($\bar{t}_+$ and $\bar{t}_-$) inside membrane and in water for KCl is less important than for NaCl. The barrier has less influence in the case of KCl diffusion than in the case of NaCl diffusion. The diffusion velocity of KCl can be predicted as more rapid than that of NaCl. This predict-

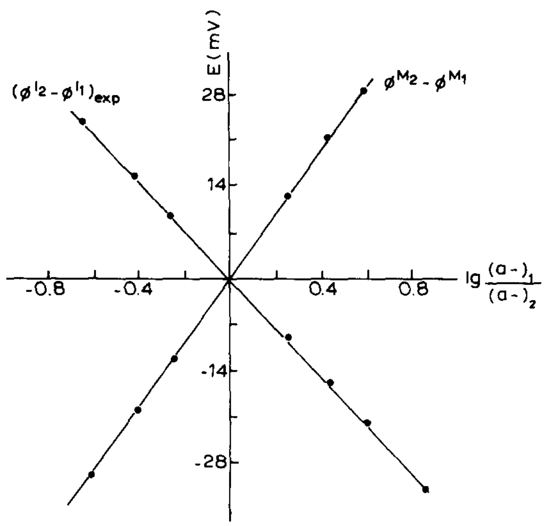

Fig. 4. Relation between the membrane potential and the $CaCl_2$ concentration.

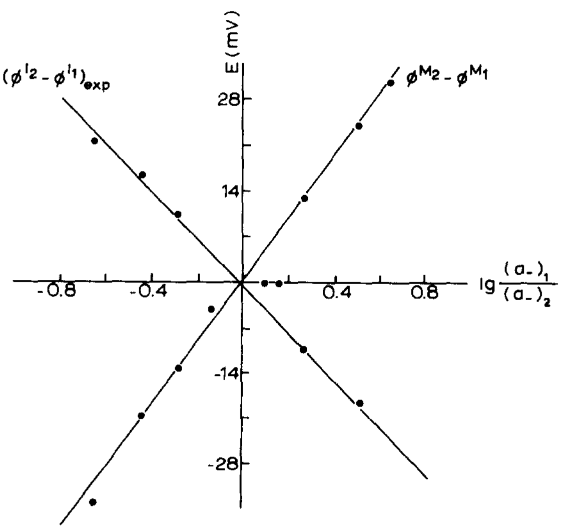

Fig. 3. Relation between the membrane potential and the NaCl concentration.

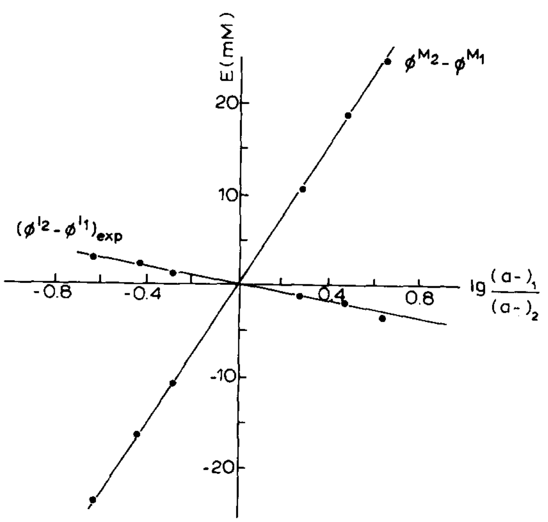

Fig. 5. Relation between the membrane potential and the KCl concentration.

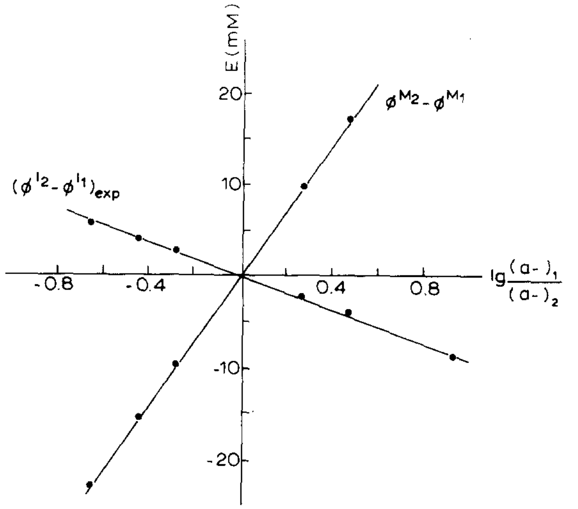

Fig. 6. Relation between the membrane potential and the NaCl concentration. On negative side the KCl solution (0.10 M) was placed.

ion has been confirmed by the experimental demonstration [9].

Using the method described in Principle of the method, the concentration of negative ionogenic groups within the membrane was found to be in the order of 10^{-4} to $5 \cdot 10^{-4}$ M. In this range the calculated values are in close agreement with the experimental ones. The results are given in Tables I–IV where the value of concentration of negative groups in the membrane ($\overline{X}$) was chosen equal to $2.5 \cdot 10^{-4}$ M. This fact indicates that in the basement membrane, the density of ionogenic groups is low. This result was also obtained by direct titration of heparan sulfate from basement membrane [31,32]. But the role of heparan sulfate proteoglycan is known as very important due in particular to its internal anionic layer which exists specially in this type of biomembrane [30,31]. Its importance can be related undoubtedly to the membrane potential behaviour.

Another test was undertaken with KCl and NaCl separated by the membrane and at the same concentrations for every one in the series of experiments (0.01 : 0.01 M, 0.02 : 0.02 M, $\cdots$,0.50 : 0.50 M). These measurements show that the membrane potential keeps a constant value (approx. 5.5 mV). Membrane potential for this system is called a bi-ionic potential [33–35]. In Table IV we should consider the system as a bi-ionic system. But in this case, the effect due to the difference in mobilities of the two distinct cations can be assumed as negligible, compared to the effect due to the concentration difference on the two sides of a membrane. For this reason we take the value 5.1 mV, where the concentrations of two electrolytes KCl and NaCl are equal (0.10 M), as the origin. This value was also found in this test. The general expression of the bi-ionic potential is given [36] by

$$\phi^{l_2}-\phi^{l_1}=\frac{RT}{F}\left(\frac{\overline{D}_B-\overline{D}_A}{\overline{D}_A Z_A-\overline{D}_B Z_B}\ln\frac{\overline{D}_B Z_B}{\overline{D}_A Z_A}+\frac{1}{Z_A Z_B}\ln K_{BA}\right.$$
$$\left.+\frac{Z_A-Z_B}{Z_A Z_B}\ln\frac{\overline{C}}{(C_A)_1}+\frac{1}{Z_B}\ln\frac{(C_A)_1}{(C_B)_2}+\ln\frac{(\gamma_A^{1/z_A})_1}{(\gamma_B^{1/Z_B})_2}\right) \tag{22}$$

where the $\overline{D}$ terms are the diffusion coefficients of two different electrolytes, in our system KCl and NaCl. $\overline{C}=Z_A\overline{C}_A+Z_B\overline{C}_B$ is the total counterion concentration, and $(C_A)_1$, $(C_B)_2$ are the bulk concentrations of solutions on the two sides of the membrane. K_{BA} is the corrected molar selectivity coefficients for the ion exchange reaction between the two electrolytes: $\overline{B}+A=\overline{A}+B$. If we assume some conditions such as $\overline{D}_A/\overline{D}_B$, K_{BA}, $\overline{C}$ are constants, there are neither co-ions nor convection in the membrane, and the boundary condition $(\overline{C}_B/C_B)_2=(\overline{C}_A/C_A)_1=(\overline{C}/C)$ holds. In the case of our experiments $Z_A=Z_B=1$ for Na^+ and K^+, $a_A \simeq a_B$ (if the Ag-AgCl electrode is taken as metal electrode), and if $\bar{\gamma}_B=\bar{\gamma}_A$, Eqn. 17 becomes [37,38,39]

$$\phi^{l_2}-\phi^{l_1}=\frac{RT}{F}\ln\frac{\overline{D}_A}{\overline{D}_B} \tag{23}$$

In the preceding paper [9], we gave values of the diffusion coefficient within the membrane for some salt solutions under the same conditions as in our mathematical model. These values are $1.188 \cdot 10^{-7}$ cm^2/s for KCl and $0.983 \cdot 10^{-7}$ cm^2/s for NaCl at 37 °C. If we introduce these values into Eqn. 23, we get the membrane potential for this bi-ionic system as 5.1 mV. This result affords a proof of validity for our mathematical model in its applications to the basement membranes.

From these results, we notice that in basement

TABLE I *

SALT: NaCl. $\bar{X} = 2.5 \cdot 10^{-4}$ M.

C(M)	$\Delta\phi^M$(mV)	$\log\frac{(a_-)_1}{(a_-)_2}$	$\Delta\phi^l_{exp}$(mV)	$\Delta\phi^l_{cal}$(mV)
0.01	35.85	0.934	−21.61	−24.63
0.02	22.15	0.649	−17.79	−17.03
0.03	16.80	0.483	−12.92	−12.65
0.05	9.45	0.277	−7.62	−7.25
0.10	0.00	0.000	0.00	0.00
0.20	−9.60	−0.276	7.35	7.18
0.30	−15.15	−0.439	11.86	11.43
0.50	−24.80	−0.641	14.66	16.73

TABLE II

SALT: KCl. $\bar{X} = 2.5 \cdot 10^{-4}$ M.

C(M)	$\Delta\phi^M$(mV)	$\log\frac{(a_-)_1}{(a_-)_2}$	$\Delta\phi^l_{exp}$(mV)	$\Delta\phi^l_{cal}$(mV)
0.02	34.95	0.646	−4.77	−4.12
0.03	26.54	0.479	−2.93	−3.04
0.05	15.15	0.275	−1.78	−1.73
0.10	0.00	0.000	0.00	0.00
0.20	−15.03	−0.270	1.58	1.68
0.30	−23.45	−0.430	3.01	2.67
0.50	−34.26	−0.625	4.20	3.88

TABLE III

SALT: $CaCl_2$. $\bar{X} = 2.5 \cdot 10^{-4}$ M.

C(M)	$\Delta\phi^M$(mV)	$\log\frac{(a_-)_1}{(a_-)_2}$	$\Delta\phi^l_{exp}$(mV)	$\Delta\phi^l_{cal}$(mV)
0.01	28.90	0.851	−23.46	−23.41
0.02	20.20	0.585	−15.81	−16.00
0.03	15.11	0.432	−11.48	−11.74
0.05	8.80	0.246	−6.32	−6.68
0.10	0.00	0.000	0.00	0.00
0.20	−9.15	−0.260	6.84	7.04
0.30	−14.55	−0.420	11.27	11.36
0.50	−21.70	−0.634	17.28	17.14

TABLE IV

SALT: KCl (CONCENTRATION FIXED AT 0.10 M, NEGATIVE SIDE).
SALT: NaCl (VARIABLE CONCENTRATION, POSITIVE SIDE). $\bar{X} = 2.5 \cdot 10^{-4}$ M.

C(M)	$\Delta\phi^M$(mV)	$\log\frac{(a_-)_1}{(a_-)_2}$	$\Delta\phi^l_{exp}$(mV)	$\Delta\phi^l_{cal}$(mV)
0.01	44.92	0.929	−12.26	−11.95
0.03	24.02	0.479	−5.43	−6.07
0.05	13.62	0.273	−3.17	−3.45
0.10	0.00	0.000	0.00	0.00
0.20	−13.58	−0.280	3.65	3.52
0.30	−21.88	−0.443	5.41	5.57
0.50	−31.98	−0.646	7.76	8.10

membrane, ionogenic groups such as encountered in heparan sulfate proteoglycan, despite of their weak concentration, play an important role, particularly in electrochemical behaviour. The results of membrane potential measurements can be analyzed on the basis of the T.M.S. model, the basement membrane being considered as an ion exchange membrane bearing fixed negative charges. The study has shown that in the membrane phase the ratios of the mobilities between chloride ion and alkaline cation are different to those in water. In this membrane, the mobility of Cl^- is higher than that of K^+, Na^+ and Ca^{2+}. This leads us to understand the phenomena according to which cations diffuse at a slower rate than do the anions, just as the non-equilibrium system in the biomembranes. Other experimental methods are used in order to afford further proof to support our hypotheses. Furthermore, in order to operate under conditions approaching those encountered in physiological media, we are run-

TABLE V

TRANSPORT NUMBER VALUES IN WATER AND INSIDE THE BASEMENT MEMBRANE

Electrolyte	In water		Inside membrane	
	t_+	t_-	$\bar{t}_+$	$\bar{t}_-$
NaCl	0.39	0.61	0.29	0.71
KCl	0.49	0.51	0.45	0.55
$CaCl_2$	0.58 [a]	0.42 [a]	0.28	0.72

[a] Values at 35 °C and 0.05 equiv. g/l.

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* Note: In Tables I–V, $\Delta\phi^M = \phi^{M_2} - \phi^{M_1}$, $\Delta\phi^l_{exp} = (\phi^{l_2} - \phi^{l_1})$ calculated with Eqn. 3, and $\Delta\phi^l_{cal} = (\phi^{l_2} - \phi^{l_1})$ the membrane potential calculated with T.M.S. theory (Eqn. 9). In Table IV, when $C_{NaCl} = C_{KCl} = 0.10$ M, $\Delta\phi^M = 5.1$ mV. This value was taken as the origin.

ning experiments in which Krebs solution is used as a solvent instead of water. The results will be published in future papers.

References

1 Schwartz, M.L. and Veis, A. (1980) Eur. J. Biochem. 103, 29–37

2 Vuento, M. (1979) Biochem. J. 183, 331–337

3 Cotta-Pereira, G. and Rodrigo, F.G. (1978) in Biology and Chemistry of Basement Membranes, (Kefalides, N.A., ed.), pp. 111–119, Academic Press, New York

4 Farquhar, M.G. (1978) in Biology and Chemistry of Basement Membranes (Kefalides, N.A., ed.), pp. 43–80, Academic Press, New York

5 Foellmer, H.G., Madri, J.A. and Furthmayer, H. (1983) Lab. Invest. 48, 639–649

6 Ciba Foundation Symposium 108 (1984) Basement Membranes and Cell Movement, Pitman, London

7 Yurchenco, P.D. and Furthmayr, H. (1984) Biochemistry 23, 1839–1850

8 Timpl, R., Wiedemann, H., Van Delden, V. et al. (1981) Eur. J. Biochem. 120, 203–211

9 Fan, B.T. and Lapluye, G. (1986) J. Chim. Phys. 83, 41–61

10 Abrahamson, D.R. (1985) J. Cell. Biol. 100, 1988–2000

11 Vracko, R. (1982) New Trends in Basement Membrane Research, (Kuehn, K. et al., eds.), pp. 1–8, Raven Press, New York

12 Ingber, D.E., Madri, J.A. and Jamison, J.D. (1981) Proc. Natl. Acad. Sci. USA, 78, 3901–3905

13 Kefalides, N.A. (ed.) (1978) Biology and CHemistry of Basement Membranes, Academic Press, New York

14 Madri, J.A., Pratt, B.M. et al. (1984) Basement Membranes and Cell Movement, pp. 6–18, Pitman, London

15 Rosenzweig, L.J. and Kanwar, Y.S. (1982) Lab. Invest. 47, 177–184

16 Kanwar, Y.S., Linker, A. and Farquhar, M.G. (1980) J. Cell Biol. 86, 688–693

17 Katz, S.I. (1984) in Basement Membranes and Cell Movement, pp. 243–255, Pitman, Lond

18 Trelstad, R.L., Hayashi, K. and Toole, B.P. (1974) J. Cell Biol. 62, 815–830

19 Lakshminarayanaiah, N. (1969) Transport phenomena in membranes, Academic Press, New York

20 Harris, E.J. (1960) Transport and Accumulation in Biological Systems, Academic Press, New York

21 Koryta, J. (1972) Anal. Chim. Acta 61, 329

22 Moelwyn-Hughes, E.A. (1961) Physical Chemistry, p. 1106, Pergamon Press, Oxford

23 Teorell, T. (1935) Proc. Soc. Exp. Biol. Med. 33, 282

24 Teorell, T. (1935) Proc. Natl. Acad. Sci. USA 21, 152

25 Teorell, T. (1951) Z. Elektrochem. 55, 460

26 Meyer, K.H., Sievers, J.F. (1936) Helv. Chim. Acta 19, 649, 665

27 Teorell, T. (1953) Progr. Biophys. Chem. 3, 305

28 Kanwar, Y.S. and Farquhar, M.G. (1979) Proc. Natl. Acad. Sci. USA 76, 4493–4497

29 Stanley, J.R., Woodley, D.T. et al. (1982) J. Invest. Dermatol. 79, 69s–72s

30 Kanwar, Y.S., Hascall, V.C. and Farquhar, M.G. (1981) J. Cell Biol. 90, 527–532

31 Hassell, J.R., Robey, P.G. et al. (1980) Proc. Natl. Acad. Sci. USA 77, 4494–4498

32 Timpl, R., Fujiwara, S. et al. (1984) in Basement Membranes and Cell Movement, pp. 25–37, Pitman, London

33 Wilson, J.R. (1960) Demineralization by Electrodialysis, Butterworth, London and Washington

34 Helfferich, F. (1956) Discuss. Faraday Soc. 21, 83

35 Helfferich, F. (1962) Ion exchange, McGraw-Hill, New York

36 Laksminarayanaiah, N. (1976) Membrane electrodes, Academic Press, New York, San Francisco, London

37 Wyllie, M.R.J. and Kanaan, S.L. (1954) J. Phys. Chem. 58, 73

38 Andelman, J.B. and Gereqor, H.P. (1966) Electrochim. Acta 11, 869

39 Ilani, A. (1966) Biophys. J. 6, 329

40 Yuasa, M., Kobatake, Y. and Fujita, H. (1968) J. Phys. Chem. 72, 2871–2877