EXPERIMENTAL STUDIES ON BI-IONIC POTENTIALS ACROSS PERMSELECTIVE MEMBRANES

by

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I. INTRODUCTION

Biological membrane systems for the most part are nonequilibrium systems. For this reason, the study of membrane potentials in nonequilibrium, dynamic model systems should contribute to the basic understanding of *in vivo* membrane systems to an even greater extent than the consideration of Gibbs-Donnan membrane potentials.

The magnitude of the membrane potentials which arise in equilibrium systems, as well as in numerous closely related quasi-equilibrium systems, are defined according to the thermodynamic (Gibbs-Donnan) theory of membrane equilibria by the concentration of the various species of permeable and impermeable ions in the two solutions without reference to any specific mechanism of the restraining action of the membrane. Classical thermodynamics, however, is not applicable to dynamic, nonequilibrium membrane systems; the understanding of the origin and of the magnitude of dynamic membrane potentials requires a knowledge of the specific molecular processes which take place across the membrane.

Out of the conceivable multitude of dynamic membrane systems (of an electrolytic nature), the systems with membranes of ideal ionic selectivity lend themselves particularly well to physicochemical analysis since the only ionic processes which can occur across such membranes are either all-anionic or all-cationic. The simplest possible systems in this group are those in which the so-called bi-ionic potential arises[1].

The bi-ionic potential, B.I.P., was defined as the *dynamic* membrane potential which arises across a membrane separating the solutions of two electrolytes at the same concentrations having different "critical" ions, which are able to exchange across the membrane, and the same "noncritical" ion species for which the membrane is (ideally) impermeable[1].

The general scheme of a cell in which a B.I.P. arises can be represented in the following manner:

$$\begin{array}{c|c|c} \text{Solution 1} & & \text{Solution 2} \\ c_1\, A^+\, X^- & \longleftarrow\!\oplus\!\longrightarrow & c_1\, B^+\, X^- \end{array}$$

where A^+ and B^+ represent (univalent) cations, the "critical" ions, which are able to exchange freely across the exclusively cation-permeable (electro-negative) membrane, $| \leftarrow\!\oplus\!\rightarrow |$, and X^- the noncritical anions which are unable to penetrate through it. Systems of this type are unstable and spontaneously degrade toward equilibrium; nevertheless they may be investigated in their original, essentially undegraded state under suitable experimental conditions[2,3].

The study of B.I.P. systems can be considered as a first and necessary step in the quantitative investigation of dynamic membrane systems with membranes of ideal or almost ideal ionic selectivity in particular, and thus also of the dynamics of membrane systems in general[1].

Bi-ionic potentials have been investigated experimentally only in a limited number of instances[2–7]. According to the nature of the membrane and the combinations of critical ions, the B.I.P. may be as high as 150 mV or more.

The sign and magnitude of the B.I.P. depend on the relative ease with which the two species of critical ions can penetrate across the membrane[1–4,6]. The more readily permeable species of critical ions impresses its sign of charge on the other solution.

The magnitude of the B.I.P. is a function of the relative contributions of the different species of ions in the system toward the virtual transportation of electricity across the membrane,

$$E_{\mathrm{BIP}} = + \frac{RT}{F} \ln \frac{\tau_{\mathrm{A}^+}}{\tau_{\mathrm{B}^+}} \tag{1}$$

where τ_{A^+} and τ_{B^+} represent the transference numbers of critical ions A^+ and B^+ within the membrane, the sign of the potential value referring to the charge of solution 2. There is no simple correlation between the ratio of the transference numbers of the two species of critical ions within the membrane and the ratio of their mobilities in free solution[1].

An explanation of the origin of the B.I.P. has been based on a consideration of the membrane as ion-exchange bodies according to the fixed charge theory[1]. Membranes of ion-exchange character carry either acidic or basic dissociable groups in the microsurfaces of their pore structure, these fixed charged groups on the pore walls being an integral, invariable, and immovable part of the membrane structure. The counter ions of the fixed charged groups, the so-called "critical" ions, are dissociated off into the liquid in the pores where they are freely movable, available for diffusion and the transportation of electrical current. In membranes of ideal ionic selectivity, ions of the same sign of charge as that of the fixed charged groups, the so-called "noncritical" ions, are prevented by electric repulsion from penetrating across the membranes. The only ions which can diffuse and transport electricity across such membranes are the critical ions[8].

In B.I.P. systems two coexisting species of exchangeable (critical) ions compete for positions as counter ions of the fixed dissociable groups; in general, as in ion-exchange systems, the two competing species of ions are adsorbed to a different extent. The sequences of the relative adsorbabilities of the various ions are the two Hofmeister series of cations and anions (unless steric hindrance and related effects come into play as complicating factors). The relative abundance of the two species of critical ions in the pores multiplied by their relative diffusion velocities determine their relative contributions toward the virtual transportation of electricity across the membrane, in other words, the ratio of their transference numbers.

One interesting conclusion can be drawn from this physical picture of the origin of the B.I.P. which previously has not been stated explicitly. From the literature on ion exchangers it is known that the relative adsorbed quantities of two competing species of ions of the same sign of charge are in many instances fairly independent of the absolute concentration. To the extent that this holds true with membranes one would expect the bi-ionic potential to be independent of the absolute concentration of the two solutions separated by the membrane. This prediction is of considerable importance in the further treatment of dynamic membrane potentials in all types of polyionic systems with membranes of an extreme degree of ionic selectivity[1,9,10].

The experimental study of membrane systems in which bi-ionic potentials arise has been facilitated in recent years by the availability of the "permselective" collodion-matrix membranes. These membranes under a wide variety of experimental conditions behave as membranes of ideal or almost ideal ionic selectivity. Depending on the mode of their preparation they yield B.I.P.'s which, with the same pair of critical ions, may differ in magnitude by a factor of two, and have conductances which differ by several orders of magnitude[3,11,12].

This paper is the first in a planned series of studies on polyionic potentials across membranes of extreme ionic selectivity[9,10]. After exploring some of the theoretical and experimental limitations for the meaningful measurements of B.I.P.'s, we present here the results of a potentiometric study of B.I.P. systems with a greater variety of electrolytes and permselective membranes than heretofore used, including data on the influence of concentration on the magnitude of the B.I.P.

II. SOME LIMITATIONS OF THE POTENTIOMETRIC STUDY OF DYNAMIC MEMBRANE SYSTEMS

The potential difference between two solutions separated by a membrane is determined by using either specific or nonspecific electrodes to measure the potential of the *whole cell* and then evaluating on the basis of *nonthermodynamic assumptions* the contributions of the electrode systems to this measured value.

If specific electrodes are used, the potentials at the electrode/solution interfaces are calculated by the Nernst equation on the basis of extrathermodynamic assumptions concerning single ion activities. In many instances, as for most of the cells in the present study, specific electrodes are lacking and nonspecific electrodes with salt bridges must be used. In this case the asymmetry of the liquid junction potentials at the two salt bridge/solution interfaces can be calculated by the Planck or Henderson equations, which also involve nonthermodynamic concepts. With such nonspecific electrodes, *e.g.* calomel cells with saturated KCl-agar bridges, the asymmetry of the two liquid junction potentials in most instances is considered insignificant compared to the measured potential of the whole cell and may be ignored.

The interpretation of the potential across the membrane (obtained by either of the above methods) in terms of the ionic processes occurring across the membrane also requires nonthermodynamic assumptions concerning the single ion activities in the two solutions; these uncertainties are least with dilute solutions[13-15].

The determination and interpretation of membrane potentials is thus restricted by the same factors as the determination of liquid junction potentials, of pH, and of analogous quantities[13-15].

These limitations apply generally, both to equilibrium and dynamic membrane systems. With the dynamic membrane systems some additional factors have to be considered.

In the theoretical treatment of the B.I.P. the assumption was made that the layers of solution immediately adjacent to the membrane have the same composition as the bulk solutions; in other words that the membrane itself is the entire transition zone between the two solutions. This, however, is not necessarily the case in experimental systems. Even with the most thorough stirring of the bulk solutions, a layer of liquid—the so-called *diffusion layer*—exists adjacent to each surface of the membrane through which the removal of ions from the membrane/solution interface is limited by diffusion[16-20]. The layers of solution immediately adjacent to the membrane contain ions which have exchanged across the membrane. Thus, they are of a composition different from that of the bulk solutions. Experimentally, the membrane plus the two diffusion layers rather than the membrane alone comprise the transition zone.

If the rates of exchange of ions across the membrane were high compared to the rates of diffusion across the diffusion layers, then the concentration of exchanged ions at the two membrane/solution interfaces would be high. Conversely, the concentrations of exchanged ions in the layers of solutions immediately adjacent to the membrane would be low if the rates of exchange of ions across the membrane were small compared with the rates of removal of those ions by diffusion across the diffusion layers.

The rates of the exchange of critical ions across permselective oxidized collodion and protamine collodion membranes are known to be at least qualitatively correlated to the reciprocal resistance of these membranes[3]*. Accordingly, systems with membranes of high resistance compared with that of the diffusion layers are the most suitable ones for the study of dynamic membrane potentials.

The definition of the B.I.P. and the molecular theory of its origin outlined above are based on the assumption that a *stationary state* has been established across the membrane which is maintained during the course of the measurements.

In order to establish a stationary state, sufficient time must elapse after initial contact of the membrane with the solutions so that the diffusing ions have attained their proper, definite, uneven distribution along the axis of the pores, which is characteristic for each system. This well-defined, time-invariant, stationary state may best be maintained by continuously flowing two solutions of constant composition past the two sides of the membrane at such rates that an increase in the flow rates does not alter in any detectable way the processes taking place across the membrane.

Experimentally, the time required to reach a stationary state across the membrane will vary with different membranes and with the nature and concentration of the various electrolytic solutions. The time will also vary with the nature of the critical ions originally present within the membrane[11, 24]. The lower the resistance of the membrane and the higher the concentrations of the solutions, the shorter will be the time required to reach the stationary state**.

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* This is in agreement with the fact that the quantities of ions which diffuse across any zone of an electrolytic system are closely correlated to the electric conductance of that zone provided the mechanism underlying diffusion is the same as that responsible for electrical conductance[21-23].

**It might be added that fully meaningful potential measurements in dynamic membrane systems can be made under a variety of well-defined conditions other than the stationary state. This is true particularly of membranes of ideal ionic selectivity. With such membranes under

In summing up this discussion, it should be noted that the conditions most favorable for the establishment of the stationary state within a reasonable length of time, for a minimal effect of the diffusion layers, and for minimal uncertainties due to the nonthermodynamic assumptions are somewhat antagonistic. The most suitable conditions for the study of dynamic membrane potentials would be those in which these antagonistic requirements are balanced.

III. EXPERIMENTAL TECHNIQUES

Selectively cation-permeable (electronegative) and selectively anion-permeable (electropositive) permselective membranes were prepared as described previously[3,12]. These membranes have the shape of test tubes, are about 30 μ thick, and have an active surface of approximately 50 cm^2. They were tested by our standard methods (a) for their electrical resistance (in 0.1 N KCl) as an indicator of the rates at which ions diffuse across them, and (b) for their electromotive properties in concentration cells 0.1 N KCl | membrane | 0.01 N KCl as an indicator of their ionic selectivity[3,12]. These potentials, corrected for the asymmetry of the liquid junction potentials, were in the range of 53.6 to 54.8 mV, the theoretical maximum potentials being 55.1 mV.

The potential measurements were made by the Poggendorff method using two saturated calomel half-cells, the asymmetry of which was taken into account. For each potential measurement, the membrane was filled with one solution and immersed in a vessel containing the other solution. The whole assembly was placed in a thermostat (25.00 ± 0.10° C); the KCl-agar bridges (after first wiping their tips with filter paper) were inserted, and both solutions stirred with filtered air. Immediately a potential reading was taken, the reading being repeated with the KCl-agar bridges in the reversed position. This whole procedure took approximately 1.5 minutes. With dilute solutions, the potential readings were reproducible within ± 0.30 mV; with more concentrated solutions, within ± 0.10 mV. Each potential value recorded below is the mean of at least two such potential measurements. Measurements made within a few days of each other gave identical results; after several weeks, changes of up to ± 1.0 mV occurred.

The most desirable experimental technique to establish the stationary state across the membranes would be to flow one solution past one side of the membrane and the other solution past the other side until a time-invariant potential unchanged by increased flow rate is obtained. In extended routine experiments, it is more practical first to immerse the membrane for several days in a solution containing the one or the other or, preferably, a mixture of the two species of critical ions to be studied; thereafter the membrane is filled with the one solution and immersed in the other; both solutions are stirred and frequently changed until a constant potential is reached.

Table I shows that with a representative membrane of medium resistance a constant, reproducible potential was reached in this manner within one hour (which did not change even after more prolonged periods than those shown in Table I) independent of the previous state of equilibration of the membrane with KCl, LiCl, or a mixture of KCl and LiCl***. The final potential is reached more quickly and is more precisely reproducible with the more concentrated solutions.

Except with membranes of very high resistance, the measurements were routinely carried out after the membrane had been interposed between the two solutions for at least one hour.

Since in our experiments the two solutions were not continuously replaced, the systems at the time of measurement were slightly degraded. To ascertain the magnitude of the error which is caused thereby, the rates were determined at which the potentials in the systems shown in Table I decreased when the solutions were no longer replaced. Extrapolating back to zero time, the error in the potential measured 1.5 minutes after the solutions were changed was found

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proper nonstationary state conditions, potential readings can be obtained which are practically identical with those obtained under the corresponding stationary state conditions. (Nonstationary state systems, however, could not be used readily for a meaningful study of the kinetics of the exchange of ions across membranes.) The situation is analogous to that arising with liquid junctions which can be studied under various well-defined states other than the stationary state, such as states with a free diffusion boundary or a constant mixture boundary between two bulk solutions. In simple cases the measured liquid junction potentials are virtually independent of the nature of the boundary layer.

*** Studies on the kinetics of the rate of the exchange of ions across permselective membranes which are currently carried out in this laboratory furnish additional, independent proof that stationary state conditions prevail under the conditions chosen for our work.

TABLE I

THE TIME REQUIRED BY A REPRESENTATIVE PERMSELECTIVE SULFONATED POLYSTYRENE COLLODION MEMBRANE FRESHLY INTERPOSED BETWEEN THE TWO SOLUTIONS TO REACH A CONSTANT AND REPRODUCIBLE BI-IONIC POTENTIAL (STANDARD RESISTANCE OF MEMBRANE IN 0.1 N KCl: 890 Ωcm^2)

(T = 25.0 ± 0.1° C)

Membranes previously saturated with	*Soln. 1* KCl	*Soln. 2* LiCl	*Minutes* 1.5	10	30	60	120	240
			Potential					
	eq./l	eq./l	mV	mV	mV	mV	mV	mV
0.10 N KCl	0.0200	0.0200	+ 33.2	+ 40.4	+ 42.9	+ 43.7	+ 43.6	+ 43.6
0.10 N KCl	0.200	0.200	+ 38.3	+ 39.1	+ 39.5	+ 39.5	+ 39.5	+ 39.5
0.10 N LiCl	0.0200	0.0200	+ 40.3	+ 41.6	+ 42.5	+ 42.6	+ 42.5	+ 42.5
0.10 N LiCl	0.200	0.200	+ 38.8	+ 38.7	+ 38.9	+ 39.3	+ 39.4	+ 39.3
0.05 N KCl 0.05 N LiCl	0.0200	0.0200	+ 37.1	+ 41.0	+ 43.2	+ 43.1	+ 43.2	+ 42.8
0.05 N KCl 0.05 N LiCl	0.200	0.200	+ 39.0	+ 39.1	+ 39.4	+ 39.4	+ 39.4	+ 39.4

to be less than ± 0.2 to ± 0.3 mV, that is, less than ± 0.5 to ± 0.7%. A correction for this effect has not been applied to the data presented below.

The theory of the B.I.P. implicitly assumes that a reversal of the position of the solutions with respect to the membrane does not change the magnitude of the potential, in other words, that the membranes are functionally symmetrical. Preliminary tests showed that most permselective collodion-matrix membranes in combination with strong electrolytes having univalent critical ions are essentially symmetrical. In most systems presented below the asymmetry was less than 0.5 mV; in some, particularly those with membranes of very high resistance and with critical ions of greatly different hydrated size, the asymmetry was as much as 3 mV.

IV. EXPERIMENTAL RESULTS

Table II gives representative bi-ionic potentials with various pairs of critical ions across cation-permeable oxidized collodion and anion-permeable protamine collodion permselective membranes of different standard electrical resistances. Columns 1 and 2 indicate the electrolytes used; columns 3 to 6 give the corresponding B.I.P.'s with four membranes of different resistance. Column 7 gives the ratio of the transference numbers within the membrane of column 5 calculated from equation 1, referred to the K$^+$ and Cl$^-$ ions as unity. In order to visualize more easily the specific influence of the membrane on the magnitude of the transference numbers within the membrane, column 8 presents the ratio of the mobilities of the critical ions in free solution referred also to the K$^+$ and Cl$^-$ ions as unity.

Table II shows that the B.I.P.'s across permselective oxidized collodion and protamine collodion membranes differ widely with the nature of the critical ions. The B.I.P.'s with the same pair of critical ions differ for different membranes. If for each membrane the various cations or anions are arranged (as in Table II) in the order of their relative electromotive efficacy, the resulting sequence is a Hofmeister series. Analogous data obtained with other types of permselective membranes (including polyacrylic acid and sulfonated polystyrene collodion membranes) yielded

TABLE II

BI-IONIC POTENTIALS WITH VARIOUS PAIRS OF CRITICAL IONS ACROSS CATION-PERMEABLE AND ANION-PERMEABLE PERMSELECTIVE MEMBRANES OF DIFFERENT STANDARD ELECTRICAL RESISTANCES

($T = 25.0 \pm 0.1°$ C)

a. Oxidized collodion membranes

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
		Resistance in 0.1 N KCl					
Solution 1 0.0100 *N*	*Solution 2* 0.0100 *N*	*Ω cm²* 160	*Ω cm²* 320	*Ω cm²* 1550	*Ω cm²* 2180	*Ω cm²* 1550	$\frac{u^0_{B+}}{u^0_{K+}}$
		Bi-ionic potential				$\frac{\tau_{B+}}{\tau_{K+}}$	
		mV	mV	mV	mV		
KCl	CsCl	− 7.5	− 7.7	− 8.7	− 8.4	1.41	1.07
KI	NH_4I	− 4.7	− 5.5	− 6.8	− 6.8	1.30	1.00
KCl	RbCl	− 4.5	− 4.8	− 5.6	− 5.6	1.25	1.04
KCl	KCl	± 0.0	± 0.0	± 0.0	± 0.0	1.00	1.00
KCl	NaCl	+25.5	+29.0	+35.5	+37.0	0.251	0.682
KCl	LiCl	+45.0	+51.2	+63.7	+66.3	0.084	0.526
KI	$(CH_3)_4NI$	+55.5	+68.2	+81.6	+82.5	0.042	0.611

b. Protamine collodion membranes

		Resistance in 0.1 N KCl					
Solution 1 0.0250 *N*	*Solution 2* 0.0250 *N*	*Ω cm²* 155	*Ω cm²* 500	*Ω cm²* 1250	*Ω cm²* 5400	*Ω cm²* 1250	$\frac{u^0_{Y-}}{u^0_{Cl-}}$
		Bi-ionic potential				$\frac{\tau_{Y-}}{\tau_{Cl-}}$	
		mV	mV	mV	mV		
NaCl	NaCNS	+22.2	+29.5	+32.0	+36.4	3.48	0.864
NaCl	$NaNO_3$	+14.4	+21.0	+23.1	+26.9	2.46	0.937
NaCl	NaI	+10.4	+11.9	+12.2	+12.0	1.61	1.01
NaCl	NaBr	+ 5.3	+ 7.2	+ 7.2	+ 7.9	1.32	1.03
NaCl	NaCl	± 0.0	± 0.0	± 0.0	± 0.0	1.00	1.00
NaCl	$NaBrO_3$	− 4.0	− 2.6	− 2.2	− 2.2	0.918	0.708
NaCl	NaFormate	−11.2	− 9.1	− 7.6	− 5.3	0.744	0.715
NaCl	NaBenzoate	−34.9	−39.2	−38.6	−34.3	0.222	0.423
NaCl	$NaIO_3$	−34.7	−41.4	−45.1	−48.5	0.173	0.538
NaCl	NaAcetate	−40.5	−44.2	−46.2	−46.4	0.165	0.536
NaCl	NaPropion.	−51.8	−57.1	−59.3	−58.4	0.099	0.469
NaCl	NaButyrate	−57.4	−62.5	−65.9	−63.6	0.077	0.427

the same general result. In all these respects the various types of permselective membranes act in B.I.P. systems in the same manner as other membranes of high ionic selectivity[4–7].

With all pairs of critical cations and with the majority of pairs of critical anions, membranes of higher standard resistance yielded B.I.P.'s of higher absolute magnitude. In the two systems with the bromate and formate anions, the B.I.P. is lower with membranes of higher standard resistance; in the three systems with the benzoate, propionate, and butyrate anions no definite correlation appears. A comprehensive explanation of these results is outside the scope of this paper. It would have to be based on much more extensive data concerning the structure of the membranes than are available at present, particularly on their pore-size spectrum and charge density*.

The theory of the B.I.P. predicts implicitly that bi-ionic potentials are independent of the nature of the noncritical ions. In order to determine the degree to which this assumption is correct, the B.I.P.'s were compared across the same permselective membrane in several cells with a particular pair of critical ions but with each cell having a different species of noncritical ions. Data representative of numerous such experiments, given in Table III, show that the B.I.P. within the limits of experimental error is independent of the nature of the noncritical ions.

TABLE III

THE LACK OF INFLUENCE OF THE NATURE OF THE NONCRITICAL IONS ON THE BI-IONIC POTENTIALS ACROSS PERMSELECTIVE MEMBRANES ($T = 25.0 \pm 0.1°$ C)

		Resistance in 0.1 N KCl					*Resistance in 0.1 N KCl*		
Soln. 1 *0.0500 N*	*Soln. 2* *0.0500 N*	*Ω cm²* *200*	*Ω cm²* *950*	*Ω cm²* *1940*	*Soln. 1* *0.0500 N*	*Soln. 2* *0.0500 N*	*Ω cm²* *145*	*Ω cm²* *1300*	*Ω cm²* *4460*
		Bi-ionic potential					*Bi-ionic potential*		
		mV	mV	mV			mV	mV	mV
KCl	LiCl	+ 45.5	+ 59.2	+ 64.1	KNO_3	KAc	− 54.6	− 66.6	− 73.4
KNO_3	$LiNO_3$	+ 44.4	+ 57.5	+ 62.0	$NaNO_3$	NaAc	− 56.0	− 68.0	− 74.2
KI	LiI	+ 44.9	+ 58.0	+ 63.3	$LiNO_3$	LiAc	− 56.5	− 68.3	− 74.8
K_2SO_4	Li_2SO_4	+ 45.3	+ 59.1	+ 64.1	$Ba(NO_3)_2$	$Ba(Ac)_2$	− 56.3	− 67.3	− 73.6

The influence of the concentration on the magnitude of the bi-ionic potential was determined by comparing the potentials which arise across the same permselective membrane in systems with the same pair of critical ions at various concentrations. Such experimental data obtained with representative cation-permeable and anion-permeable permselective membranes of various standard electrical resistances and with different pairs of critical ions at concentrations from 0.00100 *N* to 0.500 *N* are presented in Figs. 1 and 2. At the higher concentrations, the error of the potential determinations due to the asymmetry of the liquid junction potentials may be as much as 1 to 2 mV.

The potential versus concentration curves of Figs. 1 and 2 are characterized by long horizontal or nearly horizontal branches extending from concentrations of a

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* In the case of permselective oxidized collodion membranes of the same degree of oxidation but with different degrees of drying, the cause of the higher B.I.P.'s with the more thoroughly dried membranes may be surmised to be primarily a steric effect; the larger critical ions are screened out selectively by the narrower pores. For example, a membrane was dried stepwise for different periods in air; the standard resistance and the B.I.P. were measured after each drying. The standard membrane resistances (in 0.1 *N* KCl) were 1250, 22,500, 50,000, and 100,000 Ωcm²; the B.I.P.'s in the system 0.0500 *N* KCl $|\longleftarrow\oplus\longrightarrow|$ 0.0500 *N* LiCl were + 60, + 64, + 77, and + 79 mV, respectively.

In another experiment, permselective oxidized collodion membranes differing in their preparation only by differences in drying time had standard electrical resistances of 150, 1560, 22,800, and 205,000 Ωcm² and gave B.I.P.'s in the system 0.0500 *N* K_2SO_4 $|\longleftarrow\oplus\longrightarrow|$ 0.0500 *N* Na_2SO_4 of + 26.0, + 35.0, + 42.6, and + 47.4 mV. In the system 0.0500 *N* K_2SO_4 $|\longleftarrow\oplus\longrightarrow|$ 0.0500 *N* Li_2SO_4, the B.I.P.'s were + 44.7, + 63.5, + 80.2, and + 89.9 mV.

References p. 352.

few hundredths normal up to half normal and a more or less sharp downward curvature at the lower concentrations. In most instances there is a very flat maximum in the region of 0.05 to 0.1 *N*.

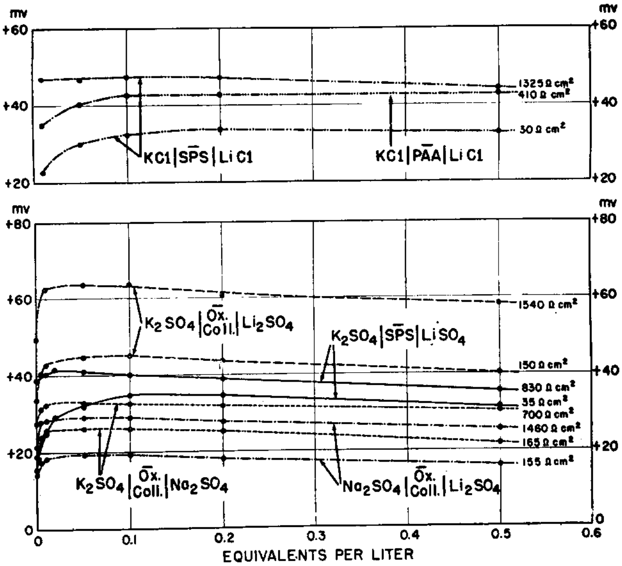

Fig. 1. The variation with concentration of the bi-ionic potential across representative cation-permeable permselective membranes ($T = 25.0 \pm 0.1°$ C). Ox. Coll. = Oxidized collodion membrane; SPS = Sulfonated polystyrene collodion membrane; PAA = Polyacrylic acid collodion membrane.

The decrease in potential from the maximum in the range of low concentrations is most pronounced with the membranes of low resistance and may be as much as 20 millivolts in 0.001 *N* solution. This effect is due to the above-discussed influence of diffusion layers. In this region one of the basic assumptions concerning the nature of B.I.P. systems is not fulfilled, namely, identity of the bulk concentration in the two solutions with the concentration of the two liquid layers in contact with the membrane.

The long horizontal or virtually horizontal parts of the curves indicate that the B.I.P.'s across the various permselective membranes (in the concentration range where an influence of diffusion layers is absent) is nearly constant up to concentrations of at least 0.5 *N*. At this concentration the potentials are about 1 to 6 millivolts lower than at the respective maxima. This latter effect is approximately the same for the systems with univalent as with bivalent noncritical ions. Since it is known that the leak of bivalent noncritical ions even in 0.5 *N* solutions is insignificantly small, the decrement in potential cannot be attributed to the higher leak of noncritical ions at the higher concentrations[3, 8, 12, 25]. The probable cause of the decrement in potential at the higher concentrations is discussed below.

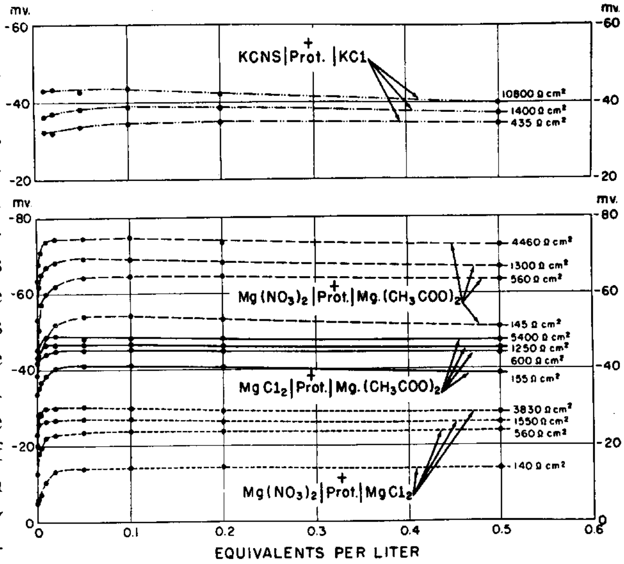

Fig. 2. The variation with concentration of the bi-ionic potential across representative anion-permeable permselective protamine collodion membranes ($T = 25.0 \pm 0.1°$ C).

V. DISCUSSION

The demarcation of the experimental conditions under which B.I.P.'s can be measured in a fully meaningful manner and with a high degree of accuracy was presented explicitly in this paper because the same basic factors must be considered in any quantitative study of dynamic membrane systems in general.

The identity of the regularities in the magnitude of the B.I.P.'s with different pairs of critical ions across the various types of permselective collodion-matrix membranes and across other previously investigated types of membranes of high ionic selectivity indicates that a great variety of membranes can be expected to act essentially in the same manner. The well defined, fast reacting permselective membranes can, therefore, be utilized for the investigation of the B.I.P. and related phenomena without the danger that their specific character plays a disturbing role.

The importance to biology of the B.I.P. stems to a large extent from the physical meaning of the ratio of the transference numbers in the membrane, τ_{A^+}/τ_{B^+} as a quantitative measure of intrinsic ionic specificity in permeability, a fact which becomes fully clear in the study of dynamic systems with more than two species of critical ions[9, 10]. In this connection it is worth while noting that minor changes in the mode of preparation of permselective membranes have a profound effect on the ratio of the transference numbers in the membrane. The slight changes brought about in the membrane by drying at lower humidities can be presumed to bring about reductions in the diameter of the pores and a somewhat denser packing of dissociable groups. Similarly, seemingly minor changes in living membranes due to minor changes in molecular arrangement of their undoubtedly highly organized structure (caused, for instance, by changes in pH, the release of strongly adsorbable ions, *etc.*) might bring about great changes in the relative permeability of competing species of ions.

The observation that the bi-ionic potential is fairly constant over a wide range of concentration confirms the theoretical prediction and is, as was indicated above, an important factor in the development of the theory of polyionic potentials[9, 10].

The observed slight decline of the B.I.P. at the higher concentrations is readily understood on the following basis: The B.I.P.'s were measured between solutions of equal concentrations of the critical ions rather than between solutions of equal activities as would be required by a physico-chemically more appropriate definition of the B.I.P. At concentrations below 0.05 *N* the ratio of the activities of the critical ions is practically unity. At higher concentrations this ratio tends to deviate significantly from unity depending on the particular pair of electrolytes under consideration. For example, in the systems presented in Fig. 1, where the decline of the B.I.P. at higher concentrations is fairly conspicuous, the ratio of the activities of the critical ions in solution 1 to that of solution 2 is significantly less than unity; this deviation becomes greater as the concentration increases. Thus, the observed decline of the B.I.P.'s at higher concentrations is accounted for at least qualitatively. The quantitative evaluation of this effect involves the theory of the polyionic potentials which we plan to present in detail at a later date[9, 10].

In principle another approach to the study of B.I.P. systems is also of interest, namely, the independent determination of the mobilities and adsorbed quantities of the critical ions within the membranes of B.I.P. systems and the correlation of these quantities to the magnitude of the B.I.P.[1]. However, a meaningful quantitative correlation can be expected only if the membranes are closely similar in the geometrical and electrical structure to the idealized theoretical membrane. The available membranes show great heterogeneities in their electrical and geometrical structure; their permeability and electromotive properties depend primarily on those portions of the pathways across the membrane which by virtue of small diameter and presence of charged groups determine the quality and the intensity of the ionic processes which occur[1,26,27]. Investigations of the above-indicated nature will become more worth-while and meaningful as membranes of a higher degree of structural regularity will become available.

At present a more fruitful test of the theory of the B.I.P., particularly of the basic concept of transference numbers, is the study of the kinetics of the exchange of two or more species of critical ions across permselective membranes, a topic which is actively pursued in this laboratory.

SUMMARY

1. The theoretical and experimental limitations are discussed of the measurement of the dynamic bi-ionic membrane potential, B.I.P., which arises across a membrane separating the solutions of two electrolytes at the same concentration having different "critical" ions which are able to exchange across the membrane and the same "noncritical" ion species for which the membrane is impermeable. Particular attention is given to the influence of the "diffusion layers" adjacent to each surface of the membrane.

2. The B.I.P.'s with a variety of pairs of critical ions across several types of cation-selective and anion-selective permselective collodion-matrix membranes show that the electromotive efficacy of the various cations and anions corresponds to their position in the Hofmeister series. The absolute magnitude of the bi-ionic potentials depends on minor differences in the mode of preparation of the permselective membranes.

3. The B.I.P. with a given pair of critical ions across a given membrane is independent of the nature of the noncritical ions.

4. The B.I.P. with a given pair of critical ions is nearly constant within the range of concentration in which the diffusion layers do not play a disturbing role.

5. Some problems of membrane specificity and their biological implications are briefly discussed.

RÉSUMÉ

1. Les auteurs discutent les limitations théoriques et expérimentales à la mesure du potentiel bi-ionique dynamique de membrane, B.I.P., qui prend naissance à travers une membrane séparant les solutions de deux électrolytes à la même concentration ayant des ions "critiques" différents qui peuvent s'échanger à travers la membrane et la même espèce d'ions "non critiques", auxquels la membrane est imperméable. L'influence des "couches de diffusion" adjacentes à chaque face de la membrane est particulièrement mise en lumière.

2. Les B.I.P. obtenus avec un certain nombre de paires d'ions critiques à travers divers types de membrane à support de collodion, permosélectives pour les cations ou pour les anions, montrent que l'efficacité électromotrice des différents cations et anions correspond à leur position dans les séries de Hofmeister. La valeur absolue des potentiels bi-ioniques dépend de petites différences dans le mode de préparation des membranes permosélectives.

3. Le B.I.P. obtenu pour une paire d'ions critiques donnée et pour une membrane donnée est indépendant de la nature des ions non critiques.

4. Le B.I.P. pour une paire d'ions critiques donnée est pratiquement constant dans le domaine de concentration à l'intérieur duquel les courbes de diffusion n'occasionnent pas de perturbations.

5. Quelques problèmes posés par la spécificité des membranes et leurs conséquences biologiques sont brièvement discutés.

ZUSAMMENFASSUNG

1. Die theoretischen und experimentellen Beschränkungen der Messungen des dynamischen, biionischen Membranpotentials (B.I.P.) werden erörtert; dieses Membranpotential entsteht zwischen Lösungen von 2 Elektrolyten derselben Konzentration, mit verschiedenen "kritischen", durch die Membran vertauschbaren Ionen, und den gleichen "nicht-kritischen" Ionenarten, welchen gegenüber die Membran undurchlässig ist. Der Einfluss der "Diffusionsschichten" an beiden Seiten der Membranoberfläche wird besonders beachtet.

2. Das mit verschiedenen kritischen Ionenpaaren beobachtete biionische Potential, durch mehrere Typen von Kollodium-Matrix-Membranen mit selektiver Kationen- und Anionendurchlässigkeit, bewies, dass die elektromotorische Wirksamkeit der verschiedenen Kationen und Anionen ihrer in der HOFMEISTER-Serie eingenommenen Position entspricht. Der absolute Wert des biionischen Potentials hängt von geringfügigen Unterschieden in der Herstellungsweise der permselektiven Membranen ab.

3. Das für ein gegebenes Paar von kritischen Ionen gefundene B.I.P. durch eine bestimmte Membran ist unabhängig von der Natur der nicht-kritischen Ionen.

4. Das für ein gegebenes Paar von kritischen Ionen festgestellte B.I.P. ist binnen des Konzentrationsgebietes, in welchem die Diffusionsschichten keine störende Rolle spielen, nahezu unabhängig von der Konzentration.

5. Einige Probleme der Membranspezifität und ihre biologische Bedeutung werden kurz erörtert.

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