

A QUANTITATIVE ELECTROCHEMICAL THEORY OF THE ELECTROLYTE PERMEABILITY OF MOSAIC MEMBRANES COMPOSED OF SELECTIVELY ANION-PERMEABLE AND SELECTIVELY CATION-PERMEABLE PARTS, AND ITS EXPERIMENTAL VERIFICATION

II. A QUANTITATIVE TEST OF THE THEORY IN MODEL SYSTEMS WHICH DO NOT INVOLVE THE USE OF AUXILIARY ELECTRODES

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The theory of mosaic membranes which are composed of ideally cation- and ideally anion-selective parts (1) has been tested (see part I of this paper (2)) by means of model systems which involve the use of auxiliary electrodes. The purpose of this communication is to present analogous model experiments which do not involve the use of auxiliary electrodes—in other words, a test of the theory in truly all-electrolytic systems without the presence of metallic electrodes. Such direct proof of the applicability of the theory in all-electrolytic model systems seems desirable particularly as a contribution to the elucidation of bioelectric phenomena and as an incentive to further electrochemical model studies in this field.

The plan of the present work is developed most concisely by reference to a sequence of drawings.

Fig. 1 *a* illustrates schematically a system in which a mosaic membrane separates a lower compartment (of invariable volume) filled with 0.1 N KCl from an upper compartment filled with 0.01 N KCl. The electronegative, cation-permeable parts of the membrane (indicated by minus signs) and the electropositive, anion-permeable parts (indicated by plus signs) are assumed to be of ideal ionic selectivity.

In the system illustrated in Fig. 1 *a*, cations pass through the electronegative parts of the membrane and anions through the electropositive parts, neutralizing each other electrically. Thus a continuous movement of the electrolyte occurs across the membrane which does not cease until equal concentrations of electrolyte are established in the two compartments.

To correlate the rate of this movement of electrolyte with the electrical characteristics of the system and to formulate the outlined qualitative concept

in a manner which is amenable to a quantitative test, a system is considered in which the cation-permeable and anion-permeable parts of the membrane are separated from each other as shown in Fig. 1 *b*. A U tube contains in its left arm an electronegative, exclusively cation-permeable membrane and in its right arm an electropositive, exclusively anion-permeable membrane. The upper and lower parts of the system contain the same solutions as before. The

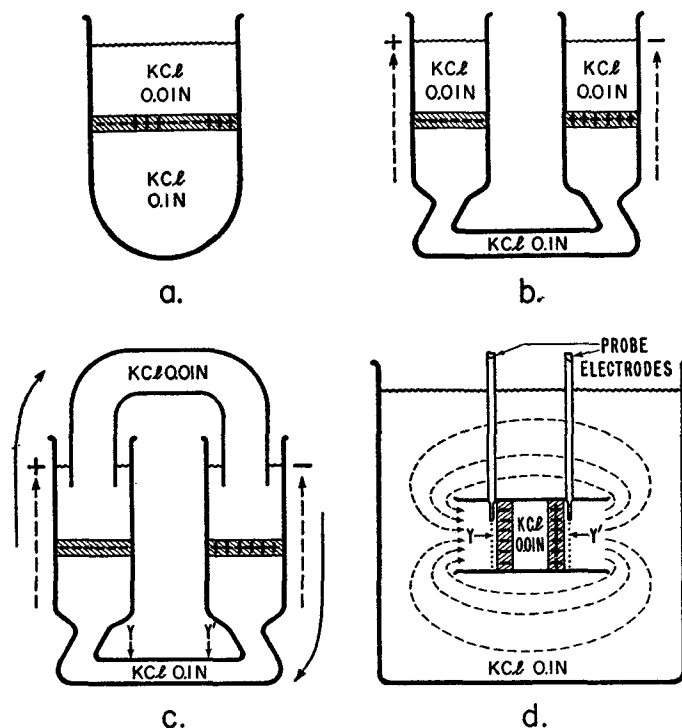


FIG. 1. Pictorial development of the idea of a mosaic membrane model without auxiliary electrodes.

only processes which can occur in this system are the establishment of (1) a hydrostatic pressure in the lower compartment and (2) two static membrane potentials, the magnitude of which is determined by the activities of the electromotively active ions in the two solutions. The directions of these membrane potentials are indicated in Fig. 1 *b* by broken line arrows pointing to a plus and a minus sign, respectively.

In order to reestablish in Fig. 1 *b* the essential features of the situation represented in Fig. 1 *a* it is necessary to connect the two dilute solution compartments by a conduit filled with 0.01 N KCl as shown in Fig. 1 *c* (in which the

two arrows Y and Y' should be disregarded at present). The system of Fig. 1 *c* is an all-electrolytic electrical circuit (1, 2); a (positive) electrical current flows in a clockwise direction through the system as indicated by the solid line arrows of Fig. 1 *c*. The total E.M.F. in the system E is the sum of the two membrane potentials. The strength of the current in the system, I , (provided there is no membrane polarization) is E/R , in which R is the total resistance of the circuit.

The current is transported through the negative membrane in the left arm of the system exclusively by cations which move in a clockwise direction, and through the positive membrane in the right arm by an equivalent quantity of anions which move counter-clockwise (the counter-clockwise movement of anions in electrical terms being a clockwise movement of positive current). Accordingly, the quantity of electrolyte (in equivalents) which moves in a given time in the model system in Fig. 1 *c* from the concentrated to the dilute solution must be numerically identical with the number of faradays which flow during the same period. The system may be considered as two membrane-concentration cells arranged in series in a short-circuited state, the two membranes acting as machines for the reversible transfer between the two solutions of cations and anions, respectively.

The quantity of electrolyte which moves during the experimental period into the dilute solution can be determined readily by chemical analytical methods or conductometrically. Thus the main experimental problem is to measure exactly the quantity of electricity which flows in the system during the experimental period. The method chosen in the preceding paper consisted in cutting the model of Fig. 1 *c* at a suitable point, either in the concentrated or the dilute solution, and connecting the open ends of the interrupted circuit to two reversible symmetrical electrodes which make it possible to conduct the current through a conventional (low resistance) current-measuring instrument. In the present paper we have chosen an approach in which the current is determined in an all-electrolytic circuit without being directed through such electrodes and the concomitant auxiliary devices. This can be done if the circuit contains an element of known and constant resistance across which potential measurements can be made at frequent intervals during the experimental period. For this purpose, as indicated schematically in Fig. 1 *c*, probe electrodes are inserted at the planes Y and Y' , the resistance of the volume element between the planes Y and Y' being known and constant.

The potential difference between the probe electrodes is measured immediately on closing the circuit and repeatedly thereafter at short intervals until the end of the experiment when the flow of the current is interrupted. From each potential reading and the known constant resistance, the value of the corresponding current is calculated; the quantity of electricity which flows during the experimental period is equal to the area under the curve of strength of

current plotted against time. Thus, this method of determining the current is valid regardless of changes in the current which may be caused by the decrease in resistance of the dilute solution, the concomitant changes in membrane resistance and decrease in electromotively effective concentration difference, by membrane polarization, etc.

The geometrical arrangement of an experimental model, the types and resistances of the membranes, the electrolyte used, and the absolute concentration of the two solutions and the concentration ratio may be freely chosen provided that the essential features of the theoretical model are retained (2). From the practical point of view, however, it is necessary to balance carefully the variable factors of the system with one another so that the experiments may be carried out under optimum conditions (2).

A suitable geometrical arrangement of a model system is shown schematically in Fig. 1 *d*. It consists essentially of two flat membranes, one positive and one negative, which are mounted some small distance apart, parallel to each other in the middle of a tube made of insulating material. The space between the two membranes forms the dilute solution compartment. The whole assembly is immersed in a large container filled with the concentrated solution. The extensions of the tube to the left and the right of the membranes serve the purpose of making the planes *Y* and *Y'* equipotential planes. The part of the circuit through the concentrated solution between *Y* and *Y'* is the constant resistance element across which the potential, E_R , is measured at frequent intervals while the model system is in operation.

EXPERIMENTAL

The membranes were cut from the cylindrical parts of test tube-shaped permselective, electronegative, sulfonated polystyrene collodion membranes (3) and electropositive protamine collodion membranes (2, 4). Membranes with a characteristic concentration potential of less than 54.5 ± 0.1 mv. (corrected for the asymmetry of the KCl bridges used in the measurement) were not considered potentially useful for the model studies.

Fig. 2 *a* shows a cross-sectional view of the experimental cell. It consists essentially of five plastic rings clamped by means of six plastic bolts with plastic nuts. The circular membranes, *M* and *M'*, were located between the flat, mirror-smooth faces of the lucite ring *A* and the ebonite rings *B* and *B'*. Clamped to the latter were two bakelite rings *C* and *C'* which carried sections of glass tubing with the same inside diameter as the rings *A*, *B*, and *B'*. A side view of ring *A* is shown in Fig. 2 *b*. Ring *A* carried a conductance cell *F* (which also served as an inlet for filling the dilute solution compartment) and a vertical glass tube which served as an air outlet while the dilute solution compartment was filled and as an opening that admitted a glass stirrer *E*.

The rings *B* and *B'* carrying thin rubber gaskets cemented to their inside faces were provided with one hold each, into the upper, wider part of which a section of glass tubing was cemented, through which movable saturated KCl-agar bridges, used as the probe electrodes, were admitted. The tubular extensions attached to the rings

C and C' were of a length that assured that the planes Y and Y' (where the tips of the probe electrodes, D and D' , were located) were equipotential planes.

The glass jar in which the assembly was immersed during the experiments was filled with 15 liters of the more concentrated solution (a volume so large that no detectable change of concentration occurred during an experiment). The assembly was supported approximately in the center of the jar by vertical glass tubes into which the pegs in rings B and B' fitted. The concentrated solution, vigorously stirred, served as a constant temperature bath of $25.00 \pm 0.05^\circ\text{C}$. The stainless steel stirrer was covered with an insulating varnish, so that no metallic or conducting non-metallic materials (except for the membranes) came anywhere into contact with the solutions.

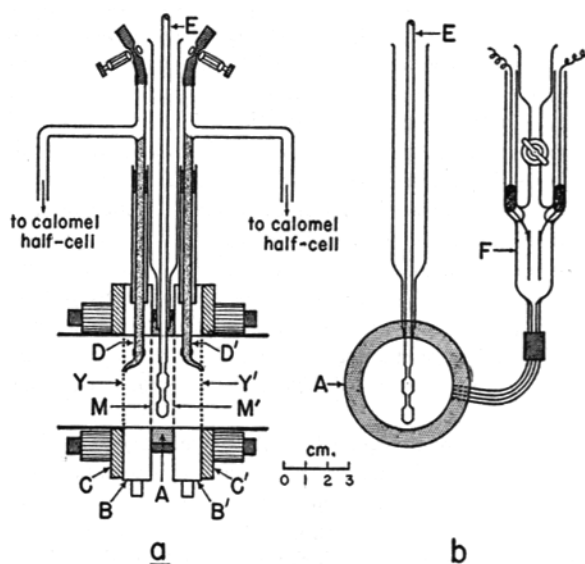


FIG. 2 *a*. Cross-sectional view of the experimental cell; Fig. 2 *b*. Side view of the ring which forms the dilute solution compartment and of the attached conductance cell.

For the measurement of the *resistance of the constant resistance element of the circuit* platinum disk electrodes of virtually the same diameter as the openings of the various plastic rings were mounted in the planes Y and Y' between rings B and C , and B' and C' . The outer faces of the electrodes were lightly platinized, and the backs painted with an insulating varnish. Insulated wire leads welded to the electrodes led out of the assembly through the glass tubes in plates B and B' from which the probe electrodes had been removed. With sheet rubber disks in place of the membranes and the dilute solution compartment left empty, the apparatus was placed on its support in the jar filled with the electrolyte solution to be used in the subsequent model experiment. The resistance was measured by the Kohlrausch method (1000 cycles A.C.) with an accuracy of better than one part in a thousand. When the apparatus was repeatedly disassembled and reassembled the resistance agreed consistently

within ± 0.5 per cent, this variation being the limit of the error in the determination of the resistance of the constant resistance part of the circuit in the operating model. No detectable changes in the resistance were observed on moving the apparatus even as much as 5 cm. away from the center of the jar.

The potential, E_R , across the constant resistance part of the circuit was measured by means of a Leeds and Northrup type K-2 potentiometer. The electrolytic bridges D and D' consisted of glass tubes mounted movably by means of thin rubber strips in the glass support tubes cemented into the rings B and B' . They were provided with side arms leading to calomel half-cells. Their upper ends were fitted with short pieces of rubber tubing and screw clamps, their lower ends were cemented to small glass tips in alignment with the side arms of the bridges which thus acted as indicators of their position, the open ends of the tips being located in the planes Y and Y' .

The electrolytic bridges contained saturated KCl-agar gel from the end of the tip to the junction of the side arm, as is indicated in Fig. 2 *a* by the shaded area. The side arms were filled with saturated KCl solution. The tips of the bridges were prevented from drying out when not in use by covering them with small rubber caps. During use in the experiments the lower one-half to two-thirds of the calomel electrodes were immersed in the concentrated solution at constant temperature.

The degree to which the potential was uniform in the two planes Y and Y' was tested during the early part of the closed circuit period of each experiment. First one then the other of the two probe electrodes was moved up and down, while the other was kept stationary. There was never more than ± 0.5 per cent variation in the potential readings. During the experiments the probe electrodes were set in a position which gave a median potential. The probable error in each potential reading was not more than ± 0.15 mv.

The raw potential data were corrected for the asymmetry potential of the calomel electrodes. The error due to this factor is in no case more than 0.05 mv.

Since the potential, E_R , across the constant resistance part of the circuit continuously diminishes during the course of an experiment, an appropriate number of potential/time measurements were made during the closed circuit period of each experiment. The total number of electrochemical equivalents of electricity, N_F , transferred by the current is computed from the sum of the products of the average strength of the current during the individual time intervals and their duration (2). The probable error in N_F varies from less than ± 1.0 per cent to about ± 2.0 per cent, depending on the electrical characteristics of the several systems.

The quantity of electrolyte moving across the membranes from the concentrated to the dilute solution, either on open or on closed circuit, was calculated from the known volume of the dilute solution and its increase in concentration.

The measurement of the change in concentration of the dilute solution was made by means of a conductance cell F of about 4 ml. capacity attached to ring A (Fig. 2 *b*). The electrodes were kept wet continuously to prevent any changes in them. The volume of adhering liquid, estimated to be 0.05 ml., was taken into account. During use, the conductance cell was surrounded by the concentrated electrolyte solution which served as thermostatic liquid ($25.00 \pm 0.05^\circ$).

To make a concentration measurement the cell F was filled and emptied three times; after filling the cell a fourth time the resistance of the solution was determined.

The corresponding concentration of the solution was evaluated from a large scale empirical resistance/concentration chart. Concentrations determined in this way were reproducible within less than ± 0.1 per cent.

TABLE I

A Typical Model Experiment without Auxiliary Electrodes

Electrolyte: KCl

Concentration of concentrated solution: 0.0500 N

Volume of concentrated solution: 15 liters

Volume of dilute solution: 19.00 ml.

 Resistance of constant resistance part of circuit: 106.3 Ω

<i>t</i>	E_R	<i>I</i>	Quantity of electricity	Concentration of dilute solution	Leak
<i>min.</i>	<i>mv.</i>	<i>amp.</i> $\times 10^{-3}$	<i>amp.-min.</i> $\times 10^{-3}$	<i>m.eq. liter</i> ⁻¹	<i>m.eq. liter</i> ⁻¹ <i>min.</i> ⁻¹
0				4.953	0.00013
15				4.955	
30				4.957	
Circuit closed					
0	28.40	0.2672		4.959	
10	28.25	0.2658	2.6650		
20	28.05	0.2639	2.6485		
30	27.95	0.2629	2.6340		
40	27.65	0.2601	2.6150		
50	27.55	0.2592	2.5965		
60	27.30	0.2568	2.5800		
70	27.20	0.2559	2.5635		
80	27.00	0.2540	2.5495		
90	26.90	0.2531	2.5355		
100	26.80	0.2521	2.5260		
110	26.65	0.2507	2.5140		
115	26.60	0.2502	1.2522	5.933	
Circuit opened					
0				5.933	0.00017
15				5.936	
30				5.938	
			29.68		$L_{avg.} = 0.00015$

$$N_p = \frac{\text{ampere-minutes} \times 60}{F} = \frac{29.68 \times 10^{-3} \times 60}{96,494} = 18.46 \times 10^{-6} \text{ equivalents.}$$

$$N_{\text{conc.}} = (5.933 - 4.959 - 0.00015 \times 115) 19.00 \times 10^{-6} = 18.18 \times 10^{-6} \text{ equivalents.}$$

$$\Delta = +1.5 \text{ per cent.}$$

The quantity of electrolyte entering the dilute solution on closed circuit in a mosaic model with non-ideal, leaky membranes is composed of two parts, the electrolyte whose movement is physically identical with the current flow and the electrolyte which leaks across the two membranes. This leak of electrolyte in the model systems

was determined from the rate at which electrolyte entered the dilute solution compartment on open circuit. The model was assembled and a known volume of dilute solution pipetted into the chamber between the membranes. With the tips of the electrolytic bridges D and D' covered by small rubber caps the whole assembly was placed in the more concentrated solution. The circuit was interrupted immediately by pressing rubber stoppers holding L-shaped glass tubes into the end of each of the two extension tubes of the rings C and C' , the upper ends of the L-shaped tubes projecting above the surface of the solution in the jar. Each of the membranes was thus in contact with about 45 ml. concentrated solution while no electrical current could flow. The dilute solution was continuously stirred. The conductance of the

TABLE II

A Comparison of the Numbers of Electrochemical Equivalents of Electricity and of Electrolyte Transferred in some Representative Mosaic Membrane Models without Auxiliary Electrodes

Electrolyte	Concentrations on closing of circuit	Increase in concentration of dilute solution	Correction for leak	Electrolyte transferred $N_{conc.}$	Faradays moved N_F	Δ
	<i>eq. liter⁻¹</i>	<i>m.eq. liter⁻¹</i>	<i>m.eq. liter⁻¹</i>	<i>eq. $\times 10^{-4}$</i>	<i>F $\times 10^{-4}$</i>	<i>per cent</i>
KCl	0.0500/0.004959	0.974	0.017	18.18	18.46	+1.5
KCl	0.0500/0.005006	0.933	0.009	17.56	17.76	+1.1
KCl	0.0500/0.001554	1.308	0.046	23.98	24.62	+2.6
KCl	0.0500/0.001532	1.134	0.033	20.92	21.48	+2.6
KCl	0.100 /0.004990	2.004	0.040	37.32	37.63	+0.8
LiCl	0.0500/0.005171	0.694	0.021	12.79	12.97	+1.4
LiCl	0.0500/0.004898	0.657	0.009	12.31	12.15	-1.3
LiCl	0.0500/0.004983	1.049	0.019	19.57	19.37	-1.0
KIO ₃	0.0500/0.005034	0.540	0.030	9.69	9.53	-1.7
KIO ₃	0.0500/0.004982	0.628	0.040	11.17	11.54	+3.2
KIO ₃	0.0500/0.004938	0.435	0.019	7.90	8.08	+2.2

dilute solution was measured at successive intervals of 15 or 20 minutes, the increase in conductance per unit of time being a measure of leak of electrolyte across the membrane. A similar determination of the leak was also made at the end of the closed circuit period of each experiment. The average of the rates of leak before and after the open circuit period was used as the correction factor.

In the experiments reported below, the error in the *number of equivalents of electrolyte moved on closed circuit corrected for leak*, $N_{conc.}$, may be estimated to vary from about ± 0.5 per cent to ± 2.5 per cent depending on the magnitude of the per cent increase in concentration of the dilute solution.

Data on a *typical experiment* are shown in Table I. The negative membrane had a characteristic concentration potential of +54.6 mv., and a standard electrical resistance in 0.1 N KCl solution of 285 Ω cm.²; corresponding figures for the positive membrane are -54.6 mv. and 200 Ω cm.². Table II summarizes several more representative experiments, Δ in both tables indicating the per cent deviation between $N_{conc.}$ and N_F .

DISCUSSION

The experimental data show that the number of faradays moved, N_F , and the number of equivalents of electrolyte transported, $N_{\text{conc.}}$, agree satisfactorily. The mean deviation of the ratios from the theoretically predicted 1:1 ratio is ± 1.8 per cent—well within the limits of the accuracy of the measurements. Thus, the quantitative theory of the electrolyte permeability of mosaic membranes composed of ideally cation-selective and ideally anion-selective parts is now verified in model systems which do not rely on the use of auxiliary electrodes through which the current flows. The experiments presented here confirm the previous test in model systems which did involve the use of such auxiliary electrodes.

The experimental confirmation of the theory of the electrolyte permeability of mosaic membranes demonstrates that complex membrane systems are amenable to a detailed theoretical study and also to quantitative experimental investigation by means of models. One may hope now to attack successfully various problems in the fields of bioelectricity and of general physiology which heretofore have defied complete physicochemical analysis. Thus, for instance, the theoretical investigation of certain other composite membrane systems involving anion- and cation-selective parts has recently led to the construction of all-electrolytic membrane systems in which both anions *and* cations are accumulated simultaneously against concentration gradients, thus duplicating *in vitro* the phenomenon of electrolyte accumulation which *in vivo* constitutes one of the most important problems of physicochemical biology (5).

SUMMARY

The theory of the electrolyte permeability of mosaic membranes composed of ideally anion-selective and ideally cation-selective parts in juxtaposition is tested in a model which consists of an all-electrolytic cyclic arrangement of four component parts: dilute solution/anion-selective membrane/concentrated solution/cation-selective membrane/dilute solution. In this system cations move from the concentrated to the dilute solution across the cation-selective membrane and an equivalent number of anions move through the anion-selective membrane. This movement of ions corresponds to a flow of current in the system. According to the theory, the number of equivalents of electrolyte which penetrate in any given time across the membranes must be identical with the number of faradays of electricity which flow during the same period. The system is essentially a combination of two membrane-concentration cells arranged in series in a short-circuited state without the presence of electrodes.

Experimentally the magnitude of the current was determined by measuring with probe electrodes the potential across an element of the circuit whose resistance was known and constant. The number of faradays of electricity (de-

terminated from time-current data) flowing in the system during a measured time was compared with the analytically determined number of equivalents of electrolyte which moved across the membranes during the same period. In a variety of experimental systems the two values show a 1:1 ratio with a mean deviation of ± 1.8 per cent.

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