

STUDIES ON PERMEABILITY OF MEMBRANES.

VIII. THE BEHAVIOR OF THE DRIED COLLODION MEMBRANE TOWARD BIVALENT CATIONS.

BY A. A. WEECH AND L. MICHAELIS.

(From the Laboratory of Research Medicine, Medical Clinic, The Johns Hopkins
University, Baltimore.)

(Accepted for publication, August 22, 1928.)

Our previous studies of the behavior of the dried collodion membrane toward electrolytes have been made chiefly with the monovalent cations, H^+ , Li^+ , Na^+ and K^+ . We have reported the results of electric transfer experiments, conductivity measurements, potentiometric studies and measurements of the total electrolyte content of the membrane pores. During the course of these various studies a few observations were made concerning the behavior of some membranes with bivalent cations, chiefly Ca^{++} . The results of these observations were somewhat different from what we had expected and at the same time we did not feel able to explain them satisfactorily. Now, however, in the light of more fully developed ideas concerning the structure of the membranes the results begin to have a definite meaning and indeed provide additional evidence in favor of these ideas. We shall give first the experimental results and then discuss briefly their significance.

Experimental Results.

1. *The Potential Difference of Concentration Chains with the Chlorides of Bivalent Cations.*—In measuring the p.d. established across the dried collodion membrane with 0.1 N and 0.01 N solutions of calcium chloride, the results are at first exceedingly inconstant and only after a period of 3 to 4 days, during which time the solutions are frequently renewed, is it possible to obtain readings which are in any way constant. One must also be sure that the calcium chloride used is not contaminated with salts of any of the monovalent cations

as the relatively slight potential effects due to the former might be totally hidden by the effect of the latter. It is advisable to use a system of intermediary bridges such as has been previously described in order that the solutions in direct contact with the membrane may not be contaminated with KCl from the agar bridges. When these precautions are taken it is possible after some days to obtain successive readings which agree within 2 or 3 millivolts. This degree of accuracy is sufficient for our purposes although less than one can obtain with salts of the monovalent cations. The slight fluctuation of the results is due probably in part to the presence of small amounts of impurities, in part to the fact that the much higher resistance of the membrane in calcium chloride solution lowers the sensitivity of the galvanometer and in part to the lowered permeability of the membranes to the Ca^{++} itself, a feature which will be discussed later. In one membrane which had a previously determined *CoP* value of 49 millivolts (p.d. between 0.1 N and 0.01 N KCl solutions) a final result of about -15 millivolts was obtained. The negative sign is used because the more dilute of the solutions was negative instead of positive as in the case of the monovalent cations. This membrane exhibited essentially the same potential difference when placed between 0.1 N and 0.01 N solutions of barium chloride, namely about -14.5 millivolts. In another membrane whose *CoP* value was 46 millivolts the calcium concentration potential was about -13 millivolts. These figures are almost in agreement with that of -18 millivolts reported by Fujita and Michaelis for the same concentration chain without membrane. So the effect of the membrane upon the potential difference appears negligible in this case.

2. *Electric Transfer Experiments.*—The method of performing these experiments has been previously described. When the solutions on the two sides of the membrane are calcium nitrate and sodium chloride the current can be so arranged that the only ions within the membrane are Ca^{++} and Cl^- , migrating in opposite directions. In our experiments the chlorine entering the anode compartment was determined by titration with 0.01 N silver nitrate in the presence of potassium chromate as previously described and the calcium entering the cathode chamber estimated by the Kramer-Tisdall blood technique. In our first experiments performed with electrolytes in concentration of

0.02 N we were not able to obtain a satisfactory agreement between the total transfer as calculated from the current passed and that actually found by analysis. For this reason these figures cannot be used for a critical analysis but it was evident in all of the experiments that more of the current was transported by Cl^- than by Ca^{++} , this result being the reverse of that obtained with the monovalent cations. Here it will be recalled that the transfer number of the anion in all dilutions was less than 0.5 and that the greater the dilution the smaller it became.

The results of our experiments in 0.1 N concentration were more satisfactory because of a relatively good agreement between the total

TABLE I.

Experiment No.	Current intensity	Time	Total transfer by calculation	Chlorine transferred	Calcium transferred	Total transfer by analysis	Transfer number of anion
	milliamperes	min.	milliequivalents	millimols	milliequivalents	milliequivalents	$\frac{\text{Cl}^-}{\frac{1}{2}\text{Ca}^{++} + \text{Cl}^-}$
89	2	30	0.0373	0.0190	0.0180	0.0370	0.513
90	2	30	0.0373	0.0170	0.0156	0.0326	0.521
91	2	30	0.0373	0.0187	0.0146	0.0333	0.561
92	2	30	0.0373	0.0210	0.0146	0.0356	0.590
93	2	30	0.0373	0.0218	0.0152	0.0370	0.589

Results of electric transfer experiments with calcium; 0.1 N calcium nitrate in anode compartment; 0.1 N sodium chloride in cathode compartment.

transfers as calculated and as determined by analysis. These results have been listed in Table I. It will be seen that in every instance the transfer number, expressed in equivalents, of chlorine is close to 0.5 and in general tends to be a little greater than this. All of these experiments were performed with a single membrane (C-1) with a *CoP* of value of about 49 millivolts.

3. *Conductivity Measurements.*—The difficulties of obtaining reliable measurements of the conductivity of the dried collodion membrane and the limited range of concentration which provides figures suitable for reasonable interpretation have been discussed in a previous communication. It will be recalled that our comparative experiments were performed in 0.5 N concentration. The membrane resistance in

HCl was at least 15 times less than in KCl, the order of resistances being $\text{HCl} < \text{KCl} < \text{NaCl} < \text{LiCl}$. With CaCl_2 an exceptionally high resistance was encountered and the results obtained cannot be regarded as having any absolute meaning. Suffice it to say that the resistance of a membrane in CaCl_2 is much greater even than in LiCl.

4. *Total Electrolyte Content of Pores.*—The method of estimating the total electrolyte content of the membrane pores by making repeated extractions with the electric current has already been described. The results obtained by this method indicated that a membrane has essentially the same electrolyte content when immersed in a solution of KCl as in one of HCl, but that in a solution of the same concentration of LiCl this content is only half as large. The same sample of membrane used in these previously reported experiments was also tested in 0.5 N solution of CaCl_2 . Here a much lower total electrolyte content was obtained than with any of the chlorides of monovalent cations. It was found that the same membrane in contact with the CaCl_2 solution contained only 20 per cent as much chloride ion as when in contact with LiCl and only 10 per cent as much as when wetted with a KCl solution.

DISCUSSION.

By viewing together the several experimental items just outlined it is possible to obtain an understanding of the behavior of the dried collodion membrane toward the calcium ion and probably toward the bivalent ions in general. The exceedingly small electromotive forces arising from concentration chains indicate that in the case of CaCl_2 the equivalent mobilities of the anion and cation within the membrane pores are essentially the same. As this is the same condition which exists in a free aqueous solution when no membrane is present we may state that the membrane exerts no effect in altering the relative mobilities of the anion and the cation. The same conclusion may be drawn from the results of the electric transfer experiments in which the transfer number of the anion, expressed in terms of equivalents (not molarity), 0.5 or slightly greater corresponds closely with the slightly negative potential arising in concentration chains. A diffusion chain of $m/10$ and $m/100$ CaCl_2 should have, on

the assumption of the equivalent mobility of Ca to equalize the one of Cl, an E.M.F. of -18 millivolts (the more dilute solution being negative). The experimentally found value of -14 millivolts in presence of the collodium membrane shows that a statement saying that the equivalent mobilities of Ca and Cl are equal within the membrane, is legitimate to a rather fair degree of accuracy. So the effect of the membrane, as to lower the relative mobility of the anion, vanishes when the cation is bivalent. Here any membrane effect disappears. The question at once arises as to which of the two actions is responsible for the equality. (1) Has the membrane acted so as to decrease the mobility of calcium to the same extent as it decreases the mobility of chlorine in the case of the chlorides of the monovalent cations? If this explanation be the correct one we need suppose no unusual behavior of the anion. (2) In the presence of calcium, is the specific effect of the membrane in retarding the mobility of chlorine abolished? If this were the proper explanation equality of transfer numbers would result because the chloride ion would move faster in the presence of calcium than in the presence of monovalent cations. The extremely high electrical resistance of a membrane in equilibrium with a solution of calcium chloride, as measured in our conductivity experiments, indicates that the mobilities of both calcium and chlorine are very much decreased within the membrane and that the main action is to decrease the mobility of calcium rather than to increase that of chlorine.

The results of experiments in which the total electrolyte content of a membrane immersed in a solution of CaCl_2 was compared with the content when immersed in KCl and LiCl indicate that the main reason for the low mobility of calcium is not that the membrane exerts any specific action on individual cations and decreases their mobility within the pores but that the membrane is highly impermeable to the calcium ion itself. The explanation for this impermeability is undoubtedly the same as that which we have already advanced to explain the slow rate of diffusion of glucose as compared with glycerol and acetone. Only a small fraction of the total pore area is distributed among pores large enough to permit the passage of the calcium ion. This concept as applied to electrolytes has already been elaborated in connection with the discussion of the difference in membrane

conductivities in HCl, KCl, NaCl and LiCl. We pointed out that the volume of an ion must be regarded as including the surrounding water shell and that the number of membrane pores able to transport an electrolyte is determined by that one of its ions which has the greater volume: Inasmuch as the hydrated calcium ion is considerably larger than the hydrated ions of either hydrogen, potassium, chlorine, sodium or lithium it follows not only that the quantity of electrolyte (CaCl_2) able to penetrate the membrane will be determined by the volume of the hydrated calcium ion and be independent of the chlorine ion but also that the conductivity and total electrolyte content must be less than with any of the other electrolytes mentioned.

In a previous paper we have given our reasons for believing that a certain range of pore sizes exists throughout which there is a rapid change in the actual mobility of the anion. All experiences have tended to show that when the pores are relatively large in size the specific effect of the membrane in retarding the mobility of anions is abolished or rendered so small that it escapes observation. Now, in the case of calcium chloride it would be seen that the large size of the hydrated calcium ion allows the salt to pass through the membrane only by way of the largest pores and that these pores are too large to exert any appreciable effect on the mobility of the chloride ion. For this reason large potential differences in concentration chains are not observed and electric transfer experiments show that both the anion and the cation have transfer numbers close to 0.5, when expressed in equivalents.

SUMMARY.

A study of the behavior of the dried collodion membrane toward the bivalent calcium ion showed that:

1. There is almost no potential difference established across a membrane separating two calcium chloride solutions of 0.1 and 0.01 N concentrations.
2. The transfer numbers of chlorine and calcium, as measured in electrical transfer experiments, are both close to 0.5.
3. A sample of membrane in equilibrium with a solution of calcium chloride has an extremely high electrical resistance, greater than is

observed with solutions of the chlorides of any of the monovalent cations.

4. The total electrolyte content of a membrane in equilibrium with a solution of calcium chloride was only 20 per cent of that observed when the solution was lithium chloride and 10 per cent of that found when the solution was potassium chloride.

In explaining these various results it is supposed that (1), (2) and (3) are all the result of (4), that is, of the inability of the calcium ion to penetrate any but the largest of the membrane pores. As the total quantity of electrolyte able to penetrate the membrane is very small the electrical conductivity must also be very small. Moreover, the few larger pores that are large enough to transport the hydrated calcium ion are too large to exert any appreciable effect in decreasing the mobility of the anion. Thus the membrane has no effect in modifying the potentials established across concentration chains with CaCl_2 and the transfer numbers determined experimentally are what one would expect if no membrane were present.