THE CONTRIBUTIONS OF NORMAL AND ANOMALOUS OSMOSIS TO THE OSMOTIC EFFECTS ARISING ACROSS CHARGED MEMBRANES WITH SOLUTIONS OF ELECTROLYTES

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INTRODUCTION

The term "anomalous osmosis" is commonly used to denote all those osmotic phenomena arising with solutions of electrolytes which seem to be contrary to the common experience that the flow of liquid across a membrane which separates a solution from pure solvent (or a more dilute solution) occurs ordinarily toward the side of the (more concentrated) solution, and at a rate roughly proportional to the concentration difference. In the relatively rare instances of "anomalous negative osmosis" a flow of liquid occurs toward the side of the pure solvent (or the more dilute solution). In the not infrequent instances of "anomalous positive osmosis" the rate of movement of liquid towards the side of the solution depends in an involved manner on the concentration difference across the membrane, the flow rates in a medium range of concentrations being much higher than with more concentrated solutions. Anomalous osmotic effects occur across swelling membranes such as rubber and, more commonly, across non-swelling, porous membranes. This latter effect, anomalous osmosis across porous membranes, the topic treated in this paper, is an electrochemical phenomenon; its magnitude is closely correlated with the product of the electrokinetic charge of the membrane and the dynamic membrane potential which results from the diffusion of electrolyte across the membrane. The details of the mechanism of anomalous osmosis are still controversial and the subject of continuing investigation (1–4).

Anomalous osmosis has been of interest to physiologists since the early reports of Dutrochet because it appears to offer some basis for an explanation of the translocation of liquids across living membranes which cannot be explained on the basis of the concept of normal Pfeffer-van't Hoff osmosis (5–

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10). However, its true role in physiology is still not clear. The published data do not demonstrate clearly that anomalous osmosis can occur to a significant extent under conditions which are at least superficially similar to those most frequently encountered in physiology, particularly in animal physiology. Past experiments have dealt almost exclusively with systems in which a membrane separates a solution of a single electrolyte from distilled water or an extremely dilute solution of the same electrolyte. Also, with rare exceptions, these experiments have been restricted to the measurement of pressure differ-

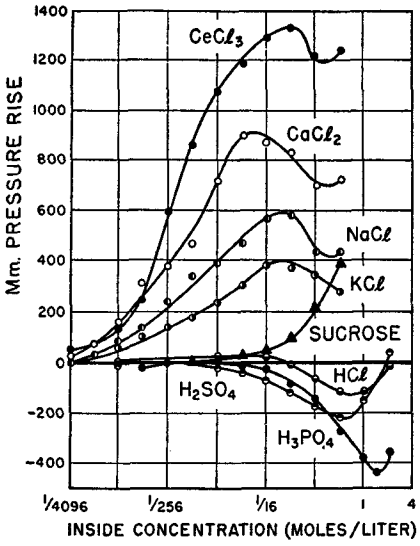

FIG. 1

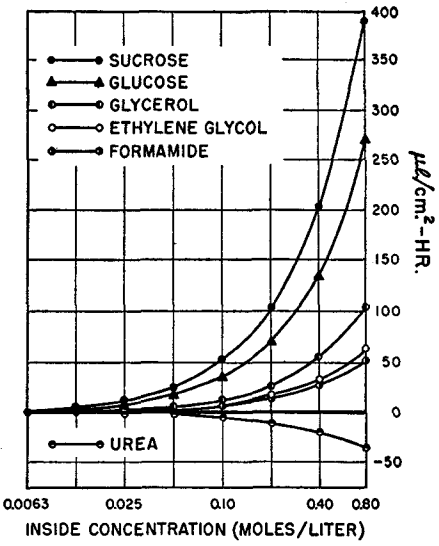

FIG. 2

FIG. 1. Anomalous osmosis across a protamine collodion membrane (data by Abrams and Sollner (12)).

FIG. 2. The rates of movement of liquid across a collodion membrane separating solutions of several non-electrolytes at different concentrations from distilled water.

ences developed across the membrane after an arbitrary time interval (11). While such experiments may be pertinent to certain aspects of plant physiology, information of greater value to animal physiology would be obtained from experiments with systems in which the membrane separates two solutions of different composition, both of which are within the physiological concentration range. Furthermore, information on the rate at which liquid is transported across the membrane against a relatively small pressure would be of greater significance.

A major difficulty in the evaluation of the physiological significance of anomalous osmosis, as well as in its detailed physicochemical study, stems

from the fact that with few exceptions the conditions in systems in which anomalous osmosis arises are such that the simultaneous occurrence of normal osmosis must be presumed. For any detailed investigation of anomalous osmosis, it is therefore necessary to find a method which permits a quantitative distinction between the contributions of normal and anomalous osmosis toward an over-all osmotic effect. The present paper deals with this problem.

Theoretical Considerations

In past studies on anomalous osmosis the contribution of normal osmosis to the over-all osmotic effect arising with a solution of an electrolyte was estimated by comparing the magnitude of this effect with that brought about by a solution of equal concentration of some arbitrarily chosen non-electrolyte. This is illustrated in Fig. 1, in which, following the example of Loeb, sucrose was used as the non-electrolytic solute. The membrane used was an electropositive, preferentially anion-selective protamine collodion membrane (12).

Fig. 1 shows that the osmotic effects with the non-electrolyte sucrose, given by the curve connecting filled triangles, are directly proportional to the concentration of the solute, as would be expected for normal osmosis (13–15), (the curvilinear relation in the figure being a consequence of the semilogarithmic nature of the plot). With the electrolytes, however, no semblance of such proportionality between concentration and osmotic efficacy exists; in several instances anomalous negative osmosis, a transfer of liquid from the side of the solution to the side of the solvent occurs (12).[1] In the past the deviations of the curves found with electrolytes from those found with the non-electrolytic reference substance were assumed to represent the extent of true anomalous osmosis.

Whether or not any arbitrarily chosen non-electrolyte can conceivably be presumed *a priori* to represent a meaningful reference substance for the determination of the contribution of normal osmosis to over-all osmotic effects with electrolytes, is readily decided by comparing the osmotic behavior of different non-electrolytes. Fig. 2 presents the results of some typical osmotic experiments with a collodion membrane of about the same porosity as those used customarily in the study of anomalous osmosis. The experimental data in Fig. 2 are the initial rates of transport of liquid across the membrane, expressed in microliters per square centimeter and hour. (The method used in these experiments is described below.)

[1] The pressure rises with the solutions of neutral salts and of sucrose recorded in Fig. 1 were measured 20 minutes after the test tube–shaped membrane, fitted with a capillary manometer tube and filled with solution, was suspended in a large volume of distilled water. In the experiments with acids, the membrane was filled with water, the acid solution being at the outside; the pressure rises were recorded after 10 minutes.

It is evident from Fig. 2 that the osmotic effects caused by the solutions of different non-electrolytes at the same concentrations differ greatly. Also, it is worthwhile to note that with urea the osmosis is negative; liquid is transported from the side of the solution towards the side of the pure solvent (14, 15). The data prove that it is impossible to find a non-electrolyte which could be used as a universal reference substance for all electrolytes, and likewise that it is impossible to choose, *a priori*, a proper non-electrolytic reference substance for any one specific electrolyte.

A solution of this difficulty lies in the use of each electrolyte as its own reference solute. All existing theories of the mechanism of anomalous osmosis lead to the conclusion that this end could be accomplished by abolishing the electrokinetic charge of the membrane, so that even with electrolytes only normal osmotic effects could occur. The change of the membranes from the charged to the uncharged state, or *vice versa*, must be accomplished without any essential change in the geometrical structure of the membrane; otherwise the osmotic effects observed with the membrane in the two states would not be strictly comparable. This means that the membrane must be non-swelling or must not show any significant change in its state of swelling when its state of charge is altered. From the experimental point of view it would be advantageous if the change in charge could be brought about reversibly.

A satisfactory membrane of this nature is suggested by some of the classical experiments on anomalous osmosis by Loeb (16). With proteinized membranes in contact with electrolyte solutions having a pH equal to the isoelectric point of the protein, the typical N-shaped curves characteristic of anomalous osmosis, such as those shown in Fig. 1, were not obtained. With isoelectric membranes the curves obtained with electrolytes were similar to those obtained with non-electrolytes. Further, proteinized amphoteric membranes, when charged positively or negatively in solutions having pH values significantly different from that of the isoelectric point of the protein used, yielded anomalous osmotic effects like those shown in Fig. 1 for a protamine collodion membrane in the positively charged state.

It is reasonable to assume that with the proper amphoteric, non-swelling membranes the osmotic effect obtained with the membrane in the isoelectric, uncharged[2] state is equal to the contribution of normal osmosis to the over-all osmotic effect obtained with the membrane in the charged state; that is, normal osmotic effects with electrolytes are dependent only upon the geometrical structure of the membrane and are independent of its state of charge. It follows, then, that the contribution of anomalous osmosis towards the over-all osmotic effect with the membrane in a charged state would be the difference

[2] The isoelectric proteinized collodion membrane is not, of course, truly uncharged. It is more accurately described as a balanced mosaic of negative and positive charges.

between the osmotic effects with the membranes in the charged and in the isoelectric state.

The amphoteric material of choice would be one with the isoelectric point near pH 7. With such material, the osmotic effects with the membrane in the isoelectric state can be determined with solutions which require only the most minute additions of acid or alkali to adjust them to the proper pH; more important, the membrane will be positively and negatively charged in solutions containing acid and alkali of such low concentrations that the osmotic effects due to these additives will be insignificant. A material known to be suitable for the preparation of collodion matrix membranes which fulfills very closely the requirement of an isoelectric point near pH 7 is oxyhemoglobin with an isoelectric point of pH 6.75.

EXPERIMENTAL

The purpose of the experimental part of this paper is (*a*) to demonstrate that the contributions of normal and anomalous osmosis toward the over-all osmotic effects which arise with solutions of electrolytes across charged membranes can be determined by the suggested method with the aid of amphoteric membranes; and (*b*) to determine the extent of "true" anomalous osmosis for a variety of electrolytes with positively as well as with negatively charged membranes.

In order to have systems which are better defined and of greater potential physiological significance than those studied in the past, we have measured the osmotic effects arising not between the solution of an electrolyte and pure water but between two solutions of the same electrolyte, the concentrations of which are both of the same order of magnitude, a 2:1 concentration ratio being chosen. These experiments were carried out over a wide range of concentrations including the range of interest in mammalian physiology. The solutions were stirred to maintain the concentration difference across the membrane as nearly as possible identical with that between the bulk of the two solutions, a feature which is missing in nearly all earlier studies.

Preparation of the Membranes.—Collodion membranes of high porosity, of the dialyzing type, were made by a method similar to that used extensively in recent years in this laboratory: A 4 per cent solution of collodion (parlodion Mallinckrodt or Baker collodion cotton, U.S.P.) in a mixture of equal volumes of ethyl ether and absolute ethanol was poured over rubber-coated test tubes (25 × 100 mm.) rotating at a constant speed (18 to 20 R.P.M.) in the horizontal position.[3] The film thus formed was

[3] The immersion in the water shrinks the membranes slightly, making it extremely difficult to remove them intact from the casting tubes. To obviate this difficulty several procedures were attempted: swelling of the membrane in dilute ethanol solutions, prior coating of the casting tubes with starch, powdered talc, caramelized sugar, or rubber. The last procedure was found to be most suitable. A rubber coating is

allowed to dry for several minutes; when thicker membranes were desired, a second layer of collodion solution was applied 3 minutes after the first. Thereafter the tubes were immersed in distilled water and washed for several hours. Finally the skins of rubber covering the test tubes, together with the membranes were slipped off the test tubes and the rubber bags removed from inside the membrane by gentle traction. The membranes were mounted on tightly fitting glass rings of about 20 to 25 mm. length, to which they were secured with linen thread.

The porosity of membranes so prepared depends greatly on the drying time prior to their immersion in water and on the ambient temperature and relative humidity. In general, the porosity is greater the shorter the drying time, the lower the temperature, and the greater the relative humidity. Conditions that yielded one-layer membranes which in the proteinized state produced maximal anomalous osmotic transport rates were for instance: drying time, 4 minutes; temperature, 22°C.; relative humidity, 20 per cent. At the same temperature and a relative humidity of 45 per cent, the drying time had to be lengthened to 6 minutes. Membranes with satisfactory characteristics could not be obtained at relative humidities greater than 50 per cent.

These membranes were proteinized with oxyhemoglobin by the method of Loeb (16). Whole horse blood was centrifuged and the serum decanted. The erythrocytes were three times resuspended in 0.17 M sodium chloride solution, centrifuged, and the supernatant liquid decanted. After the last decantation, distilled water of approximately four times the volume of the erythrocytes was added to hemolyze the cells. Into the oxyhemoglobin solution thus obtained the collodion membranes were immersed for 24 hours. After thorough washing in distilled water, they were ready for use. These membranes could be stored in water to which a crystal of thymol had been added as a preservative for as long as 6 months without undergoing significant changes in their osmotic properties. They were 50 to 100 μ thick; their water content was 60 to 75 volume per cent; and their active area when mounted on the glass rings was about 50 cm^2.

With these membranes, one would expect no significant change in porosity on altering their state of charge. To test this point, the rate of filtration of water was measured with a typical oxyhemoglobin collodion membrane at pH 6.75, 4.0, and 10.0, the membrane being isoelectric, positively charged, and negatively charged, respectively, at these pH values. The rates of filtration were 12.1, 12.5, and 12.4 $\mu l./cm^2.$-hr. under a pressure head of 10 cm. of water; this is constant well within the limits of experimental error. Furthermore, osmotic experiments with solutions of non-electro-

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formed on the casting tubes by dipping them in a 60 per cent solution of prevulcanized latex and curing the adhering layer of latex at 60–80°C. for several hours. Though the rubber-covered tubes cannot be washed before the collodion membranes are cast on them, they do not impart to the collodion membranes any material which influences the electrochemical activity of the membranes. Membranes prepared in this manner, produced with electrolytes osmotic effects identical with those obtained with membranes cast over glass test tubes not covered with rubber.

For the latex solution used, prevulcanized natural latex compound, Gl-15-C, we wish to thank the General Latex and Chemical Corporation, Cambridge, Massachusetts.

lytes yielded essentially identical results with such a membrane in the charged and uncharged states. Any differences in the state of swelling of the adsorbed oxyhemoglobin at the different pH values are obviously too small to be of significance in the fairly large pores of the membranes used.

The arrangement for the *measurement of the osmotic effects* consisted of a rubber stopper (fitting the glass rings of the membranes) which carried a short calibrated glass tube of about 8 mm. diameter, a narrow bore glass tube with a stopcock, and two magnetic stirrers, small alnico permanent magnets covered with lucite and mounted on glass axles held by a strip of plastic. For the experiments, the rubber stopper was inserted firmly into the ring of the membrane which had been filled with solution, and the position of the meniscus in the glass tube adjusted by means of the stopcock, to a level just above the stopper. The volume of solution in the assembled apparatus was about 35 ml. This assembly was then immersed in a beaker containing the "outside"

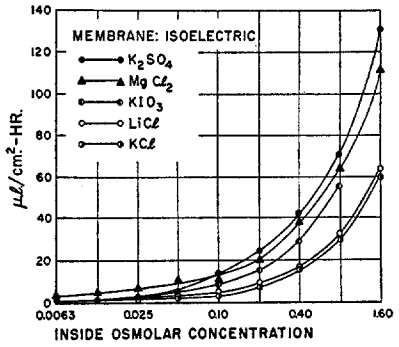

FIG. 3. Rates of movement of liquid across an oxyhemoglobin membrane in the isoelectric state separating solutions of various electrolytes with concentration ratios of 2:1. (The indicated concentrations refer to the more concentrated solutions.)

solution, (350 ml. in the case of solutions and 1000 ml. in the case of pure water), up to a level identical with that of the meniscus in the calibrated tube. The inside solution was stirred by rotating the magnets with a regularly interrupted magnetic field supplied by a large, externally mounted electromagnet. The outside solution was stirred vigorously by a motor-driven propeller.

A 5 minute period was allowed to elapse before the volume change of the inside solution was read; the liquid transport rates in microliters per square centimeter and hour were calculated from such readings. In 5 minutes, the concentration difference across the membrane in most instances decreased by about 5 to 10 per cent. Thus, the calculated liquid transport rates are on the average less than 5 per cent lower than initial rates at zero time. The use of shorter experimental periods would have decreased this discrepancy, but would have increased the error of measurement in the case of systems producing small volume changes. As routine, each measurement was repeated with fresh solutions until succeeding results agreed within 2 μl./cm.^{2}-hr. The changes in hydrostatic pressure which accompanied the changes in volume of the

inside solution were in all instances too small to cause a significant error due to filtration.

All experiments were carried out at room temperature which remained virtually constant during any given experiment and did not vary to a significant degree during any series of experiments with a given solute.

For the experiments with the membrane in the isoelectric state the solutions were adjusted to a pH of 6.75 by the addition of traces of HNO_3 or KOH as required. The

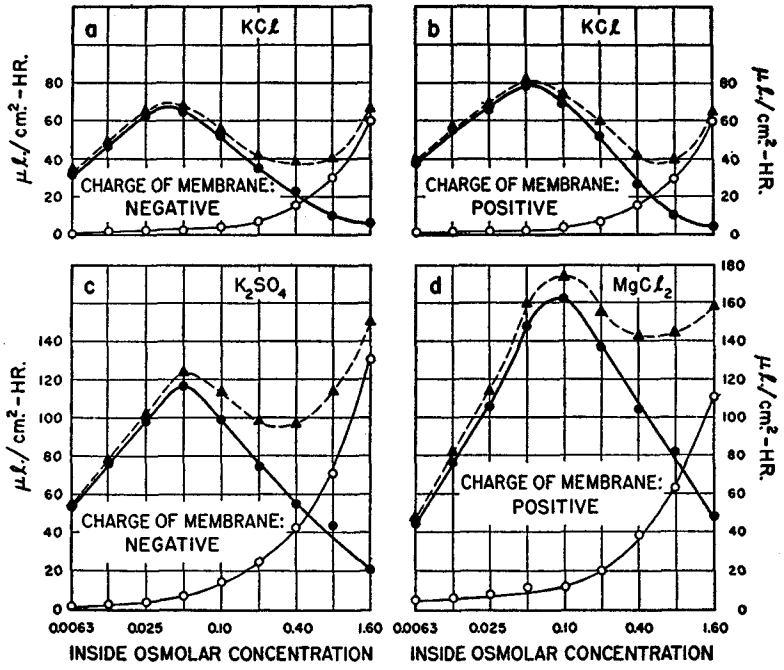

FIG. 4. Rates of movement of liquid across an oxyhemoglobin membrane in the isoelectric and in a charged state separating solutions of an electrolyte with a concentration ratio of 2:1. Four instances in which anomalous osmosis is positive at all concentrations. —○—, membrane in the isoelectric state; —▲—, membrane in a charged state; —●—, true anomalous osmosis.

solutions for the experiments with the membrane in the positively charged and the negatively charged states were similarly adjusted to a pH of 4.0 and 10.0.

The representative experiments reported below were all performed with the same oxyhemoglobin membrane (having a filtration rate of 10 μl./cm.²-hr. at 10 cm. water pressure) and thus are strictly comparable. The electrolytes used were chosen to furnish examples of both positive and negative anomalous osmosis, with the membrane in both the positively and the negatively charged

state (1–3, 12). The concentration of the inside solution was in all the experiments twice that of the outside solution.

Fig. 3 shows the results of some experiments with the membrane in the isoelectric state. The concentrations of the electrolytes are expressed as osmolarities, defined here as the products of the molar concentrations and the number of ions formed on complete dissociation. (Accordingly, osmolarity and molarity of non-electrolytes are numerically identical.)

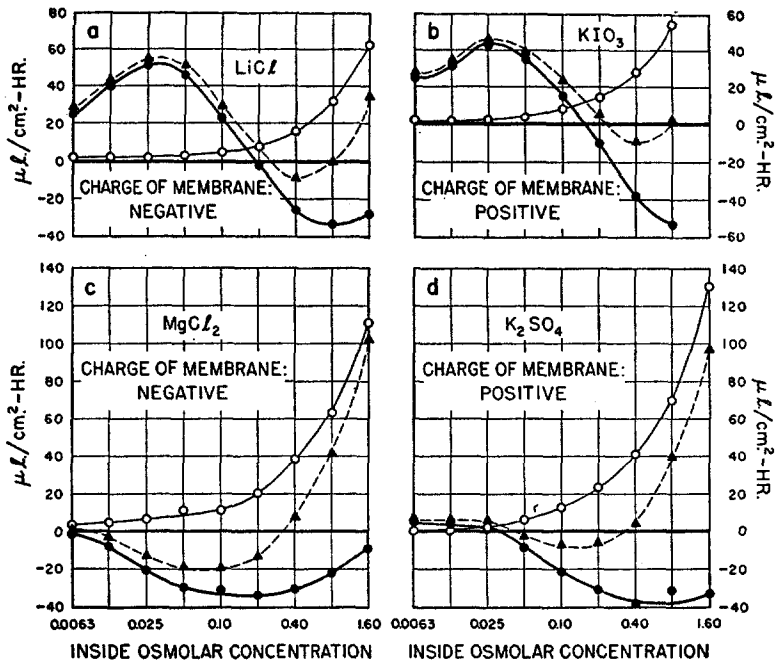

FIG. 5. Rates of movement of liquid across an oxyhemoglobin membrane in the isoelectric and in a charged state separating solutions of an electrolyte with a concentration ratio of 2:1. Four instances in which anomalous osmosis is negative in the whole or in a part of the tested range of concentrations. —○—, membrane in the isoelectric state; —▲—, membrane in a charged state; —●—, true anomalous osmosis.

The curves of Fig. 3 show that the osmotic effects with an isolectric membrane and a given electrolyte are proportional to the concentration difference across the membrane, as are the osmotic effects with non-electrolytes shown in Fig. 2 (the apparent curvilinear relations in Figs. 2 and 3 being a consequence of the conventional semilogarithmic plot). This demonstrates that with isoelectric membranes electrolytes behave osmotically in a "normal" manner, indistinguishable from non-electrolytes.

Fig. 4 (*a–d*) gives the osmotic effects obtained with four systems in which the anomalous osmosis that arises is positive throughout the whole concentration range tested. Fig. 5 (*a–d*) presents four systems in which the anomalous osmosis is negative either over the whole or a part of the tested range of concentration.

The data with the membrane in the isoelectric state are represented by unfilled circles connected by thin solid lines; those obtained with the membrane in a charged state, positively or negatively as the case may be, are represented by triangles connected by thin broken lines. The differences between the two,

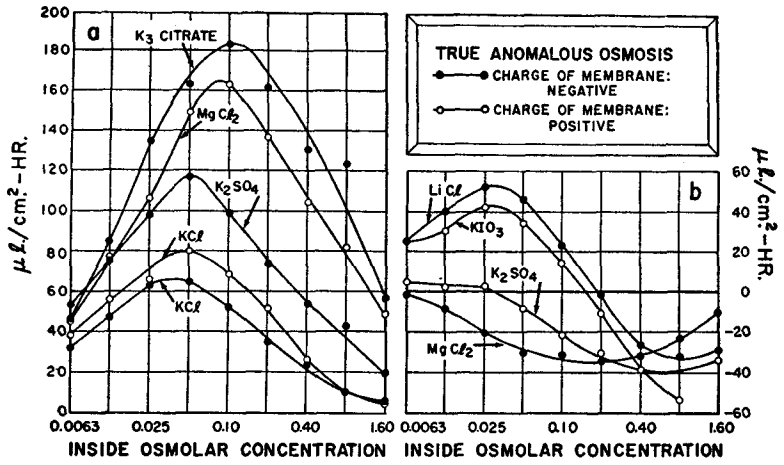

FIG. 6. True anomalous osmosis across an oxyhemoglobin membrane. Fig. 6 *a*, five instances of electrolytes with which the true anomalous osmosis is positive at all concentrations; Fig. 6 *b*, four instances of electrolytes with which the true anomalous osmosis is negative in the whole or in a part of the tested range of concentrations.

the "true" anomalous osmoses, are indicated by filled circles connected by heavy solid lines. In Figs. 4 and 5 the osmolar concentrations of the inside solutions which are the more concentrated, are given on the abscissa, and the rates of transport of liquid in microliters per square centimeter and hour on the ordinate. Negative values indicate a net movement of liquid into the outside, more dilute solution.

To facilitate the comparison of the behavior of the different electrolytes, the data on true anomalous osmotic transport rates are assembled in Fig. 6. Fig. 6 *a* presents the data on the systems which showed only true positive anomalous osmosis (with the results of a series of experiments with potassium citrate added); 6 *b*, the data on the systems which showed negative anomalous osmosis at least at some concentrations.

DISCUSSION

From the physicochemical point of view, the data presented do not require extensive comment. Those in Fig. 3 on the osmotic behavior of several electrolytes with an isoelectric membrane show a linear relation between osmotic effect and concentration difference for each electrolyte, and, with regard to the differences in the osmotic behavior of the various electrolytes at the same concentration, a direct relation between osmotic effect and ionic size. These regularities are good evidence that with isoelectric membranes, normal osmotic effects alone are produced by solutions of electrolytes. Since the porosity of the membrane is unchanged by bringing it to a charged state, the osmotic effects obtained with the isoelectric membrane can be assumed to be equal to the normal osmotic components of the total osmotic effects obtained with the membrane in a charged state. Thus, the use of amphoteric membranes seems to be a valid and practicable way to determine meaningful reference values for estimating the extent of true anomalous osmosis in systems in which normal and anomalous osmoses occur simultaneously.

The shape and relative heights of the curves representing true anomalous osmosis in Figs. 4 and 5 demonstrate that the results presented here are in agreement with those of earlier more qualitative studies. True positive anomalous osmosis is greatest in a medium concentration range and is greater with electrolytes having multivalent ions of the same sign of charge as the membrane than with electrolytes having univalent ions of the same charge (and the same counter ions). The maximal rates obtained were 80 μl./cm.^{2}-hr. with 0.05 osmolar KCl and 165 μl./cm.^{2}-hr. with 0.1 osmolar $MgCl_2$ and the membrane in the positive state, and 185 μl./cm.^{2}-hr. with 0.1 osmolar K_3-citrate and the membrane in the negative state. Maximal true negative anomalous osmosis occurs at higher concentrations and is smaller, 30 to 55 μl./cm.^{2}-hr. at concentrations of 0.2 to 0.8 osmolar.

The main purpose of this paper has thus been achieved; namely to demonstrate a method which allows the determination of the extent of true anomalous osmosis, and to present a selection of quantitative data on this effect.

The described method for the determination of the normal and of the anomalous osmotic components of the over-all osmotic effects arising with solutions of electrolytes may, with caution, be applied also to those non-amphoteric membranes which can be discharged to a sufficient extent by the addition of low concentrations of some electrolyte other than that to be tested for its osmotic efficacy. This should be possible with low-charge density membranes of weak acid or weak base character, as is borne out by some of Loeb's experiments with collodion membranes of, as we know now, low-charge density in their pore system (17, 18). Such membranes were discharged adequately at pH 3.0. High charge density membranes, particularly those of strong acid and strong base character such as the now widely used polyelectrolyte-impregnated collodion

matrix and ion exchanger membranes, cannot be discharged by concentrations of acids or bases which would be compatible with the purpose at hand (19, 20). The discharge of such membranes with polyvalent ions, while feasible, may introduce a variety of complications. Here the extent of anomalous osmosis with any given electrolyte may be estimated best by reference to an amphoteric membrane of the same structural character, which shows a similar osmotic behavior with non-electrolytes and also, when in the charged state, with the electrolyte under consideration.

From the physiological point of view it is of considerable significance that fairly large true anomalous osmotic effects occur in the range of concentrations of mammalian body fluids. In the past, several prominent investigators have doubted that anomalous osmosis, as an electrokinetic effect, can occur at concentrations above 0.05 M, and thus were inclined to the conclusion that osmotic effects arising at higher concentrations must be due solely to normal osmosis (10, 21–23). The data presented here demonstrate definitely that the general electrochemical conditions at the physiological concentration level are such that anomalous osmosis does occur to a considerable extent with suitable proteinized membranes, a result that might have been anticipated on the basis of the known high electrophoretic mobilities of proteins in solutions of fairly high ionic strength.

It also might be worth noting that at concentration levels below those occurring in the mammalian organism, anomalous osmosis is an even more effective mechanism, a fact which might be of particular interest in plant physiology.

The experiments reported here are not as pertinent to mammalian physiology as would be desirable since they were carried out with systems in which the membrane separated two solutions of different concentration of the *same* electrolyte. For the physiologist it would be more valuable to know whether or not anomalous osmotic effects of significant magnitude arise if a membrane separates two solutions of different composition but of the same osmolar concentration. This question will be taken up in a subsequent paper.

SUMMARY

The osmotic effect arising across a porous membrane separating the solution of an electrolyte from water (or a more dilute solution) is ordinarily due to both normal osmosis, as it occurs also with non-electrolytes, and to "anomalous" osmosis. It is shown that the normal osmotic component cannot be measured quantitatively by the conventional comparison with a non-electrolytic reference solute. Anomalous osmosis does not occur with electroneutral membranes. Accordingly, with membranes which can be charged and discharged reversibly (without changes in geometrical structure), such as many proteinized membranes, the osmotic effects caused by an electrolyte can be measured both when only normal osmosis arises (with the membrane in the electroneutral

state) and when normal as well as anomalous osmosis occurs (with the membrane in a charged state). The difference between these two effects is the true anomalous osmosis. Data are presented on the osmotic effects across an oxyhemoglobin membrane in the uncharged state at pH 6.75 and in two charged states, positive at pH 4.0 and negative at pH 10.0, with solutions of a variety of electrolytes using a concentration ratio of 2:1 over a wide range of concentrations. The rates of the movement of liquid across the membrane against an inconsequentially small hydrostatic head are recorded instead of, as conventional, the physiologically less significant pressure rises after a standard time.

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