CONCENTRATION RELATIONSHIPS IN NEGATIVE OSMOSIS

by

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Introduction

When two liquid solutions are separated by a membrane, the spontaneous flow of any solution constituent through the membrane in the direction of lower fugacity may be called positive osmosis of that constituent. Conversely, the spontaneous flow of any constituent through the membrane in the direction opposed to its fugacity gradient may be called negative osmosis. Since the diffusion of all constituents must be in the direction of lower fugacity, it follows that, in all cases, negative osmosis must involve some secondary mechanism dependent upon the specific nature of the membrane employed.

Negative osmosis was first described in 1835 by Dutrochet (1) and was later studied by Thomas Graham (2). In common with most other matters relating to colloid chemistry, it was little investigated from the time of Graham until the beginning of the twentieth century. Since 1900, the chief investigators have been Flusin (3), Girard (4), Bartell and his associates (5), Freundlich (6), Hamburger (7) and Loeb (8) who have confirmed the reality of the phenomenon and have concentrated upon the explanation of the mechanism or mechanisms responsible for the negative flow. In all cases, these workers
started their experiments with an aqueous solution of an electrolyte on one side of a membrane and pure water on the other. The negative flow was made apparent by an initial increase in volume on the water side, indicating a negative osmosis of water. The case of the negative osmosis of a solute constituent, covered by the general definition, has never been demonstrated. In all known systems which give negative osmosis the membranes have been permeable to both solute and solvent. The membranes employed have usually been those of animal origin. Bartell and his associates used membranes of porous clay and Loeb, membranes of gelatin-treated collodion.

Frequently in such investigations, although the flow has been in the positive direction, the rates observed are not in accord with the results which would be predicted in simple osmosis due to diffusion through a totally inactive membrane. This type of result may be called anomalous positive osmosis. Briefly, it is considered that the causes responsible for anomalous positive osmosis are the same as those operative in negative osmosis. Negative osmosis may be thought of as an extreme case of anomalous osmosis.

Apart from its theoretical interest, the problem of negative osmosis is of great importance because of its potential application in the explanation of biological phenomena. Briefly, the negative osmosis of water is believed to occur in a variety of living systems, notably in the formation of urine by the kidney (9) and in absorption from the intestine (10).
determination of the mechanism or mechanisms involved is a major biological problem and the question immediately arises as to what extent the mechanism involved in the laboratory demonstration is operative in the living system.

All the investigators and numerous authors have remarked upon the importance of the biological side (11), but the fact remains that no single biological negative osmosis has been proved to be due to the same causes as those involved in systems with non-living membranes, or to be due to different causes. Granting the difficulties involved in deciding any case one way or the other, the first step should undoubtedly be to gain a thorough understanding of negative osmosis as it occurs in the laboratory. No doubt, the reason for the lack of success in relating the laboratory and biological negative osmoses is due to the fact that our knowledge of the latter is far from complete. The general purpose of the work to be described herein was to add something to this knowledge.

The problem presented by negative osmosis with non-living systems has two main aspects as suggested by Bancroft and Nugent (12); first, the elucidation of the mechanism or mechanisms involved and secondly, that of concentration relationships. The first has been largely investigated while the second has been practically untouched.

Graham originally suggested that capillary electrical force might be responsible (13). If so, negative osmosis could be thought of as spontaneous electrical endosmose in
which the electromotive force impressed across the membrane was replaced by a difference in potential due to the presence of different solutions on the two sides.

Flusin observed osmosis with pigs bladder membranes, starting with water on one side and various aqueous solutions on the other. (14) Positive osmosis was observed in some cases and negative in others. He also observed the swelling of pig's bladder in pure water and in each of the solutions employed and made the striking observation that, in all cases in which the membrane material swelled more strongly in pure water, the flow was in the positive direction, whereas, when it swelled more strongly in the solution, negative flow of water occurred. This observation was indeed of great importance but lead Flusin to a conclusion which seems to be erroneous, namely; that the differential swelling per se is responsible for the direction of the flow.

Graham's original suggestion that negative flow might be due to capillary electrical forces, and thus essentially spontaneous electrical endosmose, was made in the early days of the knowledge of the latter (15). Later work on electrical endosmose by Quincke (16), Helmholtz (17), Lamb (18), von Smoluchowski (19), Perrin(20) and others lead to further efforts to explain negative osmosis on this basis, which culminated in 1919 in Loeb's classical demonstration of the expected connection (21).

Other conditions remaining the same, the electrical endosmose of solutions of electrolytes exhibit characteristic
variations with concentration showing maxima at concentrations of the order of M/500 and minima at concentrations of the order of M/20. Loeb showed with a series of electrolytes that the curves obtained by plotting concentration against flow, in the case of spontaneous negative and anomalous osmosis, were of the same general shape as in the case of electrical endosmosis, and exhibited maxima and minima in the same concentration ranges. He suggested that negative osmosis occurs when the characteristic minimum in the concentration flow curve falls below the line of zero positive flow. The striking cases of negative osmosis observed by Loeb were obtained with gelatin-treated collodion membranes and solutions of various acids and hydroxides of bivalent metals. In general, maximum negative flow with the various acids was obtained at concentrations of the order of 0.15M. The concentrations for maximum negative flow were of the same order of magnitude as that found by Flusin some years before, using solutions of acetic and tartaric acids and pig's bladder membranes (22). In that work, the degree of negative osmosis increased with the concentration to a maximum and then decreased, the osmosis becoming positive at higher concentrations. The negative osmosis with tartaric acid showed a maximum value at about 0.33M.

Bartell (23) and Bartell and Hocker (24) observed negative osmosis with porous clay membranes, starting with pure water on one side and solutions of various salts on the other. They concluded that, in any case, the magnitude of the negative flow
was closely related to the relative migration velocities of the ions of the particular salt. The greater the difference between the velocities for the positive and negative ions, the greater appeared to be the negative flow. Frequently, the greatest negative flows were associated with the smallest transference of electrolyte to the pure water side, indicating that negative osmosis, in the cases observed, was not purely a diffusion phenomenon.

As a result of the experiments which have been described, the chief purpose of which has been to determine the nature of the mechanism or mechanisms operative in anomalous and negative osmosis, it appears that two mechanisms have been suggested, namely; the imbibition mechanism of Flusin and the electro-capillary mechanism originally suggested by Graham. The latter is undoubtedly operative in most cases. Freundlich (25) suggests that the parallelism between swelling and the direction of osmotic flow observed by Flusin may be due to the fact that the two are brought about by the same mechanism rather than that the flow is due to the imbibition per se. R. L. Nugent (26), as quoted in the next paragraph, goes further in this regard and suggests that Freundlich's "possibility" must represent the actual facts.

"Seifriz has recently summarized the various mechanisms which have been suggested to account for imbibition or swelling of the type observed by Flusin. In addition to the capillary electrical mechanism which has been accounted for above, it
has been suggested that imbibition swelling may be the result of surface tension, osmotic or adsorption forces. It is true that any of the three can account for the taking up of water or an aqueous solution by membrane materials. On the other hand, none of the three is capable of transferring water through a membrane from a solution in which it exhibits a lower fugacity to one in which it exhibits a higher fugacity. Consider a simple negative osmosis system in which the membrane separates a solution from pure water, and suppose the membrane to have been first immersed in the electrolyte solution. The solution now contained in the membrane pores is in equilibrium with the mass of the solution, and the fugacities of all independently diffusible constituents must be equal in the two phases, regardless of whether the membrane has taken up solution due to capillary, osmotic or adsorption forces. Suppose now that the membrane in this condition is made to separate the solution from pure water. The fugacity of the pure water is higher than that of the water in the membrane, which is equal to that of the water in the solution. Capillary, osmotic and adsorption forces tending to take up water from the solution cease abruptly at the interface between the membrane and pure water. The only tendency is for water to flow from the pure water side into the membrane in the direction of the lower fugacity.

"The spontaneous electrical endosmotic mechanism is capable of transferring solution to the pure water side, because it does not depend upon an attraction of water by the material
itself (adsorption), or into the membrane structure by osmotic or capillary forces, but rather upon an electrical pumping mechanism resulting from the selective adsorption of ions by the membrane material and an imposed difference in electrolyte concentrations on the two sides of the membrane. A solution can not be transported through a membrane by forces such as surface tension, osmotic attraction within the membrane and adsorption by the membrane material, which only tend to attract it into the membrane structure.

"The general conclusion is that the only imbibition mechanism capable of producing negative flow of water through a membrane is a capillary-electrical one of the same type as that responsible in spontaneous electrical endosmose. Therefore, an imbibition mechanism for negative osmosis as apart from the spontaneous electrical endosmotic mechanism can not exist on the basis of the present conception of the possible types of imbibition forces."

The contention of Bancroft and Nugent (27) that the study of the concentration changes accompanying negative flow is second in importance only to that of the mechanism itself is based upon the fact that a comparison of concentration relationships may frequently be the most ready means of establishing or disproving the connection between negative osmoses in living and non-living systems. For example, in the formation of urine in the kidney, a negative flow of water is believed to occur from the glomerular filtrate back to the blood through
the walls of the kidney tubules. This flow normally takes place in such a way that the solution from which the flow occurs grows continuously more and more concentrated. If the negative flow in the kidney is to be related to the laboratory phenomenon, the latter must show an increase in the concentration of the solution from which the flow takes place. As has been mentioned, this general question in connection with negative osmosis has been almost completely neglected. The primary purpose of the work described in the present paper was to accomplish an introductory study of the subject along lines originally suggested by Bancroft and Nugent (28).

In addition to the major objective, it was believed that the experimental work involved might lead to an amplification or modification of existing ideas as to the mechanism or mechanisms responsible for the phenomenon.

The consideration of possible mechanisms responsible for negative osmosis lead to the conclusion that the capillary-electrical or spontaneous electrical endosmotic mechanism is the only one proved to be operative. The logical point of departure in any investigation of the concentration relations in a case of negative osmosis, as suggested by Bancroft and Nugent (29), is to postulate that the relationships in this phenomenon are similar to those operative in electrical endosmose and to attempt a direct demonstration of the fact. The outstanding difference between the two appears to be the fact in electrical endosmose, one starts with the same solution on
the two sides of the membrane, whereas in negative osmosis, one starts with pure water on one side and a solution on the other. It would appear that in negative osmosis, effects due to diffusion must be superposed upon those ordinarily present in electrical endosmose. The present state of knowledge with regard to concentration factors in electrical endosmose must be borne in mind in attempting to outline a study of the concentration relationships in negative osmosis.

Electrical endosmose may be defined as the flow of liquid through a membrane or any system of capillary tubes brought about by the application of a difference of potential on the two sides. In the ordinary situation, one starts with solutions of identical composition. The earlier investigators of the phenomenon have already been listed.

It is generally agreed that, in electrical endosmose, an electrical double layer is formed at the solution-capillary wall interface in the capillaries or pores of the membrane. The wall side of the double layer is fixed in position while the solution side is movable. When a difference of potential is applied at the ends of the capillaries, the movable side of the double layer migrates in the direction of the electrode of sign opposite to that of the solution side of the electrical double layer. The transport of liquid is then due to the migration of the ions constituting the solution side of the double layer, together with their water of hydration, and to solution which is carried along with them due to
simple friction. It would be expected that if the capillaries were sufficiently small, the entire solution within the capillary or membrane pore would be carried along with the movable side of the double layer.

On the foregoing basis, it would be expected that the concentration of the transported liquid should be that of the solution within the pores of the membrane. The question then arises as to whether the liquid in the pores has the same composition as the bulk of the solution. Theoretically, this is not necessarily the case. In very small pores, the selective adsorption of solute or solvent might conceivably lead to a more dilute or more concentrated solution within the pores of the membrane. Indeed, Mathieu (30) claimed to have proved the existence of such dilute solutions in the case of fine glass capillaries immersed in certain solutions.

In a consideration of the possible role of the existence of such dilute or concentrated solutions in the pores of filtration membranes, Bancroft and Nugent (31) have pointed out that differential concentration effects of this type cannot affect the composition of the filtrate obtained. Similar considerations apply to the case of selective adsorption in the pores of membranes in electrical endosmose. Briefly, any selective action of the membrane resulting in the taking in of a solution of different composition on one side should theoretically be exactly balanced when solution is given off at the other, with the result that liquid transported across
the membrane should have the same concentration as the solution in bulk regardless of selective effects within the pores. It would thus seem probable that electrical endosmose should involve the transport of solute equal in amount to that contained in a volume of the solution equivalent to that transported by the capillary electrical mechanism.

Concentration effects due to electrode reactions and the transport of ions should be exactly the same as those which would occur in the simple electrolysis of the solutions between the electrodes used, assuming identical conditions of current density, etc., with the extremely important limitation that the transference numbers of the ions within the membrane structure or capillaries be the same as those in the free solution. This latter condition need not always be the case and may be the exception rather than the rule, as evidenced by the work of Hittorf (32), Bethe and Toropoff (33), Michaelis and his co-workers (34). Bethe and Toropoff point out that, in the case of electrical endosmose of sodium chloride with capillaries in which the wall layer is negatively charged, and in which the transference numbers of the cations (the cation of the electrolyte and the hydrogen ion of water) is relatively greater than in the solution in bulk, there should occur a reduction of electrolyte concentration, an increase of hydroxyl ion concentration and a wandering of water to the side of the membrane facing the negative electrode. The opposite result should be obtained in the case of a membrane with the positive
side of the double layer associated with the pore walls.

In addition, the transport numbers within the pores are those which are effective in determining the transference of ions from one side of the membrane to the other, and must be used in the calculation of changes in concentration due to simple transference of ions.

In 1914 Remy (35) pointed out, and has since emphasized a fact which is of obvious importance in any general consideration of the relationships in electrical endosmose, namely; the effect of the transference of hydrated ions across the membrane. It is generally agreed that ions are hydrated in solution and carry their water of hydration with them when they migrate in electrolysis. Obviously then, if we have the transport of highly hydrated cations across a membrane in one direction and that of an equal number of slightly hydrated anions in the other, one would expect a net transfer of water to the negative pole. Remy is further in agreement with the idea of the fundamental importance of the capillary-electrical mechanism of transport and believes that in certain cases, the flow due to the hydration of ions is negligible. On the other hand, he feels that in certain cases, the transport of water is almost entirely due to the transference of hydrated ions across the membrane.

Remy's methods of demonstrating the two extreme effects are interesting. In the case of pure electrical endosmose occurring in accord with the capillary electrical mechanism,
he points out that the total number of ions passing through the membrane is so small that entirely impossible degrees of hydration would have to be assumed in order to explain the observed transport of water on this basis. In the cases where the transport of water is believed to be almost entirely due to the transport of hydrated ions, a membrane is employed which is believed, on reasonably good grounds, to be incapable of transporting a significant amount of water by means of the capillary electrical mechanism. It is obviously to be expected that in many cases of electrical endosmose, the flow of water is due to a combination of the two effects. Remy has suggested no method for the quantitative evaluation of the relative importance of the two factors in such cases.

The simplest type of system for the demonstration of concentration relationships in negative osmosis would apparently be one in which the transport numbers of the ions in the pores of the membrane are the same as in the bulk of the solution and in which the effect of the differential transport of hydrated ions is negligible. In such cases, the net concentration changes on the two sides of the membrane should be expressible as the sum of three theoretical factors: first, the electrical endosmotic transport of solution of the same concentration as that of the solution in bulk; second, concentration effects due to electrode reaction and transference; and third, a volume correction applied to each side to correct for any volume change associated with a concentration change.
of the order of the one observed. The latter can be determined empirically in any case. It would ordinarily be expected to be small in comparison with the first two factors.

It is interesting that the cases of electrical endosmose with porous clay membranes and moderately dilute sulfuric acid and sodium hydroxide appear to approximate this type. Results of Bancroft and Nugent (36), which indicate the concentration of the endosmotic flow to be closely the same as that of the solution in bulk, are given in the following table.

**APPARENT CONCENTRATION OF THE ENDOSMOTIC FLOW IN ELECTRICAL ENDOSMOSE.**

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Diaphragm</th>
<th>Direction of flow</th>
<th>Concen. of the solution in gms. per gm</th>
<th>Apparent concen. of flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$SO$_4$</td>
<td>Porous clay</td>
<td>To cathode</td>
<td>0.00162</td>
<td>0.00175</td>
</tr>
<tr>
<td>NaOH</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0.00209</td>
<td>0.00197</td>
</tr>
</tbody>
</table>

Two other facts with regard to electrical endosmose should be mentioned. The first is the early demonstration by Wiedemann (37) that, other conditions remaining the same, the volume of fluid transported in unit time is directly proportional to the current which flows. The second is the fact that in any case of electrical endosmose, the flow may theoretically be prevented by the application of a definite hydrostatic pressure (38).

At the beginning of the work, it was considered that the primary object should be to study one negative osmotic system as completely as possible before attempting to conduct experiments.
with different systems. This view was adopted, since it seemed that in this way, a general point of view with regard to concentration relationships would be most quickly achieved. It appeared that a study of comparative results, based upon such a general point of view, could be carried out much more effectively.

The study of one of Loeb's systems was decided upon, namely; oxalic acid solution with membranes of gelatin-treated collodion. The decision to use membranes of this type was based upon several reasons. Such membranes can be readily prepared. Variations in their permeability are easily obtained. The conditions of preparation are such that one would expect them to give fairly reproducible results. Animal membranes are somewhat difficult to obtain and to manipulate. It was felt that the order of reproducibility with porous clay membranes would be lower than that obtainable with those of gelatin-treated collodion.

Oxalic acid was chosen as being one of the electrolytes with which Loeb obtained maximum effects and one which could be readily determined by titration with potassium permanganate.
Outline of the Method of Investigation.

In connection with his experiments on negative osmosis, Loeb (39) observed that solute diffused to the pure water side at a more rapid rate than that corresponding to the transport of the solution as such; and that the initial rate of transfer of solute to the water side was proportional to the original concentration of the solution. Using various acids and gelatin-treated collodion membranes, he obtained negative flow in the range of concentration from about 0.01M to 1.0M with maximum negative flow at concentrations of the order of 0.15M.

The first experimental problem was to prepare gelatin-treated collodion membranes and to determine, first, whether negative osmosis occurred when these were set up with water on one side and solutions of oxalic acid on the other, and second, if the phenomenon were obtained, whether the three observations of Loeb with regard to concentration relationships could be checked.

It seemed desirable to work with the combination of membranes and solutions which gave maximum effect. Therefore, in addition to determining the concentration of oxalic acid which gave maximum negative flow, it was decided to try the effect of using gelatin-treated collodion membranes of varying permeabilities, which could easily be obtained by drying a given volume of collodion solution for different lengths
of time before treating with water (40). The thickness of membranes obtained in this way is a fair inverse measure of their relative permeability.

The foregoing experiments obviously required the design and construction of apparatus which would provide a membrane holder, some method of measuring the volume of flow and a means of obtaining solution from both sides of the membrane for analysis. In addition, it was believed that stirring should be provided on both sides—a feature frequently not incorporated by previous workers.

The concentration relationships in any case of electrical endosmose may be said to have been completely determined when the net concentration changes on both sides of the membrane have been quantitatively described as the sum of the several separate effects responsible. Since the general plan of attack was to attempt to determine the extent to which the concentration relationships in electrical endosmose could be applied in describing those obtaining in negative osmosis (41), the next step appeared to be to determine the concentration relationships in electrical endosmose experiments with a system which approximated the negative osmosis system as closely as possible.

It was therefore decided to carry out such experiments, using the gelatin-treated collodion membrane and the oxalic acid solution of the concentration which gave maximum effects in the preliminary negative osmosis runs. The plan was to
observe the net concentration change on the cathode side and to calculate the concentration change on that side which was due to the electrode reaction and transference of ions through the membrane. The difference between the first value and the sum of the last two should be due to the amount of oxalic acid transported by the endosmotic flow itself. This final amount, divided by the volume of the flow which took place, would give the concentration of the endosmotic flow which could then be compared with that of the solution in bulk.

It was apparent that calculation of the electrical endosmotic concentration relationships as described would require a knowledge of the total current which flowed in any experiment and of the transport numbers of the ions within the pores of the membrane. A comparison of these latter with the transport numbers of the ions in the free solution would afford a means of calculating the extent to which effects of the Bethe-Toropoff type must be taken into consideration.

It was believed that the net concentration change on the anode side would be seriously affected by the oxidation of variable amounts of oxalate ions at the electrode (42). It was therefore decided not to examine the concentration relationships on the anode side, since those on the cathode side in themselves seemed to provide a satisfactory basis for comparison with negative osmotic results.

In addition to their importance in connection with the concentration relationships in negative osmosis, the projected
electrical endosmotic experiments appeared to afford a possible means of determining quantitatively to what extent the electrical endosmotic flow was due to the true capillary-electrical transport of solution, and to what extent due to the transference of hydrated ions. It will be remembered that Remy (43) suggested no means of differentiating the two effects in systems where both were operative.

It is apparent that if the flow is due entirely to the electrolytical transport of hydrated ions, the concentration should be zero. If, on the other hand, it is due to true capillary-electrical transport of solution, it should equal the concentration of the solution in bulk for reasons which have been advanced in the previous section. An intermediate result could be assumed to indicate a combination of the two effects and its value would afford a means of calculating the relative magnitudes of the two.

A plan originally suggested by Bancroft and Nugent (44) was adopted for the investigation of the concentration relationships in negative osmosis. This involved conducting straight negative osmosis runs, noting the total volume of flow and the total transference of solute to the water side. Following each such run, a run is made applying just sufficient pressure to the water side to prevent increase in volume there. After a period of time just equal to that taken in the normal run, the total amount of solute transferred to the water side is determined and compared with the correspond-
ing value obtained in the normal negative osmotic run.

The total amount of oxalic acid found on the water side after normal negative osmosis has taken place can be thought of as due to one or more of three theoretical factors: ordinary diffusion, the transportation of oxalic acid by true capillary electrical flow and electrolytic effects. In connection with the latter, Freundlich (45) has pointed out that, in so far as negative osmosis is spontaneous electrical endosmose, it must involve the continuous flow of current through the membrane. This would necessitate concentration effects on the two sides of the membrane associated with the discharge and transference of ions. Effects of the Bethe-Toropoff type would not be expected to enter in negative osmosis since any flow of current is entirely within the membrane structure.

Pressure just sufficient to prevent apparent flow would be expected to prevent true capillary-electrical flow, possibly to accelerate flow of water to the solution side, and to have very little effect upon the diffusion of solute to the water side and the flow of current of the type postulated by Freundlich.

Therefore, if the total amount of oxalic acid appearing on the water side in a run under pressure should just equal that appearing in a normal run, the conclusion would be that the concentration of the flow is zero, and that the flow itself is entirely due to the diffusion of hydrated ions. On the other hand, if the negative osmotic flow is pure endos-
motic flow, it would be expected that the oxalic acid found on the water side in the pressure runs should be less than that found in the normal runs by an amount equal to the oxalic acid contained in a volume of the original solution equivalent to that of the flow.

Here, as in the case of the electrical endosmose results, any intermediate value could be assumed to indicate a combination of the two causes. Incidentally, the question of the pressures required to prevent apparent flow in these experiments is one of considerable interest.

The foregoing general scheme of procedure was adopted as apparently leading to an analysis of the concentration relationships in both electrical endosmotic and negative osmotic experiments with the system under investigation. Upon its successful conclusion, it was hoped to carry out experiments with membranes of animal origin and oxalic acid solutions in order to make a comparison of their behavior with that observed with membranes of gelatin-treated collodion.
Experimental Methods and Results.

The Apparatus for Observing Negative Osmosis, and the Preparation of the Membranes.

The apparatus designed and employed in carrying out all straight negative osmosis runs is shown in Plate A. Figure 1 illustrates the apparatus as a whole and Figure 2, a cross section of the osmotic cell.

Several points of comparison with other set-ups which have been used in the study of anomalous and negative osmosis should be mentioned. Thorough stirring is provided on both sides of the membrane, a feature which has not usually been provided by other workers. The membrane holder provides for the use of flat membranes as opposed to Loeb's experiments in which flask-shaped membranes were employed (46). It is well recognized that flat collodion membranes may be prepared with a higher degree of reproducibility than in the case of those of the flask-shaped type. (47)

All negative osmosis runs with this apparatus were made, starting with pure water in the cell and an oxalic acid solution in the beaker. The large volume of solution employed as compared with the water permits a practically constant composition of solution on one side of the membrane. Under these conditions, the percentage change in concentration on the solution side of the membrane is very small as compared with that which occurs on the pure water side in the interior.
Plate A.

Figure 1.

Standard Apparatus Used.

1. Stirring motor.
2. Outside stirrer.
3. Inside stirrer.
4. Calibrated tube for measuring flow (2 mm. inside diameter).
5. Open tube (9 mm. inside diameter).
6. Meter stick.
7. Beaker (2 liters capacity).
8. Osmosis cell (Shown actual size in Figure 2).
   a. Rubber stopper.
   b. Large glass tube (35 mm.).
   c. Brass membrane holder
   d. Membrane
   h. Difference in capillary rise in small and open tube.

Figure 2.
of the cell. This advantage precludes one met with the type of apparatus used by many workers, namely; that of having (48) capillary tubes in connection with the liquid on both sides. With that arrangement, the measured decrease in volume on one side should equal the increase on the other. In actual cases, they agree closely, and the mean volume change is taken to be the volume of the flow. This precaution does not seem to be necessary, since, if one can eliminate causes of error, an observation on one side should be sufficient.

An important feature of the apparatus is the fact that flow occurs throughout under zero hydrostatic pressure. The measuring tube was parallel to the surface of the liquid. The level of the solution in the open tube, 5, and that of the measuring tube, 4, were adjusted so that their heights above the surface of the liquid in the beaker, 7, were equal to the respective capillary rises of water in the two tubes.

The metal parts of the membrane holder were made of brass, decidedly not the most desirable material for the purpose, but necessitated by the fact that better material was not available. Definite traces of copper may be demonstrated in solutions of oxalic acid which have been in contact with brass for 24-hour periods. To eliminate copper as far as possible, the brass surfaces in contact with the liquid were carefully paraffined at the start of each experiment and, in most cases, fresh solutions of oxalic acid were employed. In spite of these precautions, traces of copper must be considered to have been
present in all cases. It is not felt that these traces influenced the results in any significant way, but their probable presence must, of course, be noted.

At the end of a run, the apparent flow could be determined directly from the movement of the liquid in the tube. This tube was approximately two millimeters in diameter (inside measurement) and a meter in length. Its volume in cubic centimeters per centimeter of length had been determined by calibration with mercury. The calibration procedure was to place mercury in the tube and after noting the length of the column, to weigh the contained mercury. This weight, divided by the density of mercury under the conditions of the calibration, would give the actual volume occupied, which, divided by the length of the column, would give the actual volume per centimeter length of the tube. The average value was found to be 0.02877 cc. per centimeter with no serious deviation throughout. The total solution inside the cell and measuring tube could be removed at the end of the run and titrated with potassium permanganate to determine the net transfer of oxalic acid to the water side.

The oxalic acid solutions were prepared in all cases from Merck's "Reagent Quality" oxalic acid. All determinations of oxalic acid were carried out by titration with potassium permanganate solutions standardized against Merck's "Reagent Quality" sodium oxalate.

The collodion membranes were prepared on a mercury surface
in a crystalizing dish 14 cm. in diameter. (49) Before the preparation of a membrane in every case, the mercury was cleaned by pin-holing through filter paper, followed by spraying through 10 per cent potassium hydroxide solution, 10 per cent nitric acid and distilled water in that order.

The collodion solution employed in all cases was prepared from J. T. Baker's collodion cotton. In preparing the solution, twelve grams of the cotton were soaked in 300 cc of ether for fifteen minutes, and then dispersed by the addition of 100 cc of alcohol and shaking.

25 cc. of the collodion solution were poured on the clean mercury surface and allowed to stand at room temperature, in the open air, for various lengths of time. At the termination of this drying period, 200 cc of distilled water were poured on the membranes. They were then separated from the sides of the dish and further rinsed with distilled water. They were next immersed in a 0.5 per cent solution of Eastman Kodak Company's "ash-free" gelatin in distilled water for four days in an electric oven at a temperature of 38-40 degrees Centigrade, after which they were rinsed with distilled water and immersed therein for a period of 24 hours under the same temperature conditions. They were then stored at room temperature in an oxalic acid solution of the concentration to be employed in succeeding experiments. Three small membranes of the proper size to fit the membrane holder were cut from each large membrane as needed.
Loeb's gelatin-treated collodion membranes were treated with one per cent gelatin solution for about fifteen hours and then used (50). It is felt that the method described here gives better results, although time was not available for a thorough comparison of results obtained by the two methods and confirmation of this assumption.

The Preliminary Demonstration of Negative Osmosis with the System to be Employed.

Bancroft and Nugent (51) observed satisfactory negative osmosis with beef bladder membranes and solutions of oxalic acid in the concentration range from one to ten per cent. Loeb, (52) using gelatin-treated collodion membranes and oxalic acid solutions, observed steadily increased negative flow in the range from about 0.01 molar to 1 molar. He did not use solutions more concentrated than the latter value, but his data indicates that still greater negative flow should have been obtained with somewhat more concentrated solutions in the range employed by Bancroft and Nugent.

On the basis of these previous results, it was decided to start with a solution of oxalic acid between one and ten per cent. An approximately 4.3 per cent solution was taken for the first experiments.

A gelatin-treated collodion membrane was prepared as described in the previous section, using 25 cc. of collodion solution and allowing it to dry for about half an hour,
followed by rinsing with water and treatment with 0.5 per cent gelatin at 38-40 degrees Centigrade for twenty-four hours. A small membrane of the size to fit the membrane holder was cut from the large membrane and placed therein. All conditions were arranged as described in connection with the apparatus, distilled water being placed within the cell and 4.3 per cent oxalic acid solution outside, with stirring on both sides of the membrane.

Under these conditions, an apparent negative flow, as indicated by the movement of the liquid in the tube to the right, was immediately observed. This proceeded at a rate of about a centimeter per minute for half an hour with little or no sign of decrease in rate. Repeated experiments gave strikingly similar results, although no attempt was made at this point to demonstrate quantitative reproducibility.

Having demonstrated negative osmosis with the system, the next question was to decide whether the apparent flow might be due to some accidental factor. Runs were accordingly made, starting respectively with 4.3 per cent oxalic acid on both sides and distilled water on both sides, using the gelatin-treated collodion membrane described above. These runs were conducted at room temperature, and from theoretical considerations were expected to yield zero flow in that time as compared with the flow of 40 to 60 cm. obtained in the original negative osmosis experiment. With 4.3 per cent oxalic acid on both sides, the flow was in the direction of positive
osmosis and amounted to 0.5 cm. per hour, which is considered to be negligible in view of the conduct of the experiment at room temperature. Starting with distilled water on both sides, an apparent positive flow of 0.6 cm. occurred in fifty minutes, again considered negligible.

The next factor to be determined was whether or not the gelatin treatment of the collodion membranes was a vital factor in giving them their negative osmotic properties. A collodion membrane was prepared in exactly the same manner as described for the gelatin-treated collodion membrane used in the foregoing experiments, with the omission of the gelatin treatment. A run was conducted in the usual manner at room temperature for a period of one hour. At that time, a positive flow of 4.5 cm. had occurred. The positive direction of the flow was to be expected if the gelatin treatment were an essential factor in giving the membranes their negative osmotic properties.

In order to observe whether the stirring mechanism might be responsible for the difference in behaviour observed, the run with the straight collodion membrane was repeated, first with the direction of the stirrer reversed and then with no stirring. In the experiment with reversed stirring, a positive flow of 7.7 cm. was noted, while in the experiment with no stirring, the corresponding flow was 0.5 cm., also in the positive direction. Rates of stirring markedly in excess of that used in all runs did not appreciably affect rates of flow.

It was thought that the total transfer of oxalic acid
to the pure water side in the foregoing experiments with straight collodion membranes might be of interest for future reference. Accordingly, the contents of the cell and tube were titrated with potassium permanganate at the conclusion of each of the three runs. In the run with normal stirring, 0.271 grams of oxalic acid were transferred to the water side; in that with stirring reversed, 0.291 grams, and in that with no stirring 0.0051 grams of oxalic were found.

It was determined that the addition of 4.3 per cent oxalic acid solution containing 0.5000 grams of the acid to a volume of distilled water equal to that contained in the osmosis cell produced no significant volume change.

The general conclusions from these preliminary experiments are that, with the apparatus and gelatin-treated collodion membranes as described, it is possible to obtain clear-cut negative osmotic flow, which proceeds at a rate sufficient for accurate observation in experiments requiring not more than two hours time.

The Reproducibility of Results with Membranes Prepared under Standard Conditions.

It was next of importance to determine the reproducibility of the results obtained with gelatin-treated collodion membranes under standard conditions of preparation and use. It was of particular interest to determine the reproducibility of runs using the same identical membrane after varying lengths
of time of storage in oxalic acid, of runs with different small membranes cut from the same large membrane, and finally, in runs with small membranes cut from different large membranes, the latter having been prepared according to the standard directions. Approximately 4.3 per cent oxalic acid solutions were used throughout, as in the case of the preliminary experiments.

Results involving two large membranes are shown in Table I, in which the Roman numerals indicate large membranes and the small letters indicate different small membranes cut from these large membranes.

**TABLE I**

NEGATIVE OSMOTIC FLOW IN SUCCESSIVE RUNS WITH THE SAME SMALL MEMBRANE, WITH DIFFERENT SMALL MEMBRANES FROM THE SAME LARGE MEMBRANE AND WITH SMALL MEMBRANES FROM DIFFERENT LARGE MEMBRANES.

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Storage</th>
<th>4.3% Oxalic Acid in cm.</th>
<th>Grams Oxalic Acid Inside after Run.</th>
<th>Concentration of Outside Solution in Grams of Oxalic Acid per cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>48 hrs.</td>
<td>49.2</td>
<td>0.336</td>
<td>0.0435</td>
</tr>
<tr>
<td></td>
<td>4 days</td>
<td>50.0</td>
<td>0.348</td>
<td>0.0427</td>
</tr>
<tr>
<td></td>
<td>6 days</td>
<td>50.9</td>
<td>0.348</td>
<td>0.0424</td>
</tr>
<tr>
<td></td>
<td>18 days</td>
<td>49.0</td>
<td>0.373</td>
<td>0.0424</td>
</tr>
<tr>
<td></td>
<td>55 days</td>
<td>55.7</td>
<td>0.427</td>
<td>0.0511</td>
</tr>
<tr>
<td>Ib</td>
<td>5 days</td>
<td>50.0</td>
<td>0.332</td>
<td>0.0425</td>
</tr>
<tr>
<td></td>
<td>55 days</td>
<td>56.5</td>
<td>0.416</td>
<td>0.0436</td>
</tr>
<tr>
<td>IIA</td>
<td>3 days</td>
<td>55.0</td>
<td>0.354</td>
<td>0.0430</td>
</tr>
<tr>
<td>IIB</td>
<td>3 days</td>
<td>58.0</td>
<td>0.367</td>
<td>0.0426</td>
</tr>
</tbody>
</table>
The results with membrane Ia indicate that with the same small membrane, with storage periods varying from two days to three weeks, the amount of flow is reproducible to within 5 per cent, with all values grouped well about the mean.

The results with all membranes indicate that the flow obtained with small membranes cut from the same large membrane also agree to within 5 per cent. The variation between results obtained with large membranes prepared according to standard procedure indicate that these may vary by as much as 10 per cent. The agreement between the total amounts of oxalic acid transferred in the various cases is about the same as that between the flows.

The general result is an indication of a fair degree of reproducibility between results with the same small membranes stored for periods of several weeks in oxalic acid, and between different small membranes cut from the same large membrane, with less reproducibility obtainable between small membranes from different large membranes. The variations observed are clearly small as compared with the difference between the results with gelatin-treated collodion membranes and those with straight collodion membranes. It is felt that this may be attributed to some extent to differences in room temperatures and atmospheric conditions during the preparation of the membranes; to the fact, as determined later, that in no case are the membranes of uniform thickness over the entire area; and perhaps to certain obscure variables, whose nature could
not be determined. In connection with the experimental results to be described and the conclusions drawn from these, the order of reproducibility of results obtained with different membranes and with successive runs with the same membrane, as indicated by these experiments, is borne in mind.

The Variation of the Rate of Negative Osmosis with the Time of Drying Used in the Preparation of the Membrane.

As is well known, the permeability of collodion membranes may be varied over wide limits by varying the time of exposure of the collodion to air, previous to the treatment with water. The permeability decreases with time of drying, presumably due to a decrease in the diameter of the membrane pores, as does the thickness of the membrane, for a given volume of collodion and a given membrane area.

Since the half hour drying period used in the preliminary experiments was arbitrary, it was felt that some other time of drying might lead to greater negative osmotic effect. It was therefore decided to investigate the effect of time of drying on the rate of negative osmotic flow. 20 cc. of collodion solution were used in each case. The drying and negative osmotic runs were conducted at room temperature. In certain cases, the actual thickness of the membrane was determined by the use of a micrometer. The centimeters of flow along the tube were noted in each case, as were the total amounts of oxalic acid transferred to the water side, both
with the thought that this information might be important in connection with later work. The results are shown in Table II, together with the concentration of the external oxalic acid solution in each case. With the thinner membranes, it was found necessary to use vaseline between the membrane and the holder to prevent leaks.

**TABLE II**

**THE EFFECT OF TIME OF DRYING OR THICKNESS UPON NEGATIVE OSMOTIC FLOW.**

<table>
<thead>
<tr>
<th>Time of Drying</th>
<th>Thickness of Membrane in mm.</th>
<th>Flow in one Hour, in Cm.</th>
<th>Grams Oxalic Acid Inside after Run.</th>
<th>Concentration of Outside Solution in Grams of Oxalic Acid per cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 min.</td>
<td>0.17</td>
<td>66.7</td>
<td>0.376</td>
<td>0.0432</td>
</tr>
<tr>
<td>20 &quot;</td>
<td></td>
<td>64.0</td>
<td>0.362</td>
<td>0.0425</td>
</tr>
<tr>
<td>30 &quot;</td>
<td>0.165</td>
<td>57.0</td>
<td>0.442</td>
<td>0.0432</td>
</tr>
<tr>
<td>50 &quot;</td>
<td></td>
<td>52.5</td>
<td>0.474</td>
<td>0.0414</td>
</tr>
<tr>
<td>60 &quot;</td>
<td>0.125</td>
<td>40.0</td>
<td>0.366</td>
<td>0.0450</td>
</tr>
<tr>
<td>75 &quot;</td>
<td></td>
<td>31.5</td>
<td>0.348</td>
<td>0.0450</td>
</tr>
<tr>
<td>85 &quot;</td>
<td></td>
<td>3.5</td>
<td>0.0256</td>
<td>0.0411</td>
</tr>
<tr>
<td>100 &quot;</td>
<td></td>
<td>0.0</td>
<td>0.000</td>
<td>0.0450</td>
</tr>
<tr>
<td>120 &quot;</td>
<td></td>
<td>2.0</td>
<td>0.000</td>
<td>0.0450</td>
</tr>
<tr>
<td>.10</td>
<td>48.5</td>
<td>0.298</td>
<td>0.0456</td>
<td></td>
</tr>
<tr>
<td>.055-.085</td>
<td>30.0</td>
<td>0.316</td>
<td>0.0411</td>
<td></td>
</tr>
<tr>
<td>.05-.08</td>
<td>1.4</td>
<td>0.155</td>
<td>0.0456</td>
<td></td>
</tr>
<tr>
<td>.06-.07</td>
<td>1.9</td>
<td>0.148</td>
<td>0.0456</td>
<td></td>
</tr>
<tr>
<td>.05-.07</td>
<td>3.2</td>
<td>0.0731</td>
<td>0.0456</td>
<td></td>
</tr>
<tr>
<td>.05-.07</td>
<td>1.0</td>
<td>0.193</td>
<td>0.0456</td>
<td></td>
</tr>
</tbody>
</table>
The minimum time of twenty minutes represents the least time of drying which results in the formation of a membrane when water is added. With shorter times, the collodion film disintegrated on the addition of water. The practical absence of flow with times of drying greater than eighty-five minutes indicates that the membranes have become fairly impermeable (53).

The conclusion of immediate importance is that the maximum flow is clearly obtained with the minimum time of drying. Accordingly, twenty minutes was taken as the standard time of drying for succeeding experiments.

The Effect of Variation of the Concentration of the Oxalic Acid Solution upon the Rate of Negative Osmosis.

Reasons for believing that maximum flow might be obtained with an oxalic acid solution in the concentration range from 1 to 10 per cent have been given, based upon previous results.

In searching for maximum effect, it was of importance to determine the point definitely. Accordingly, membranes were prepared in the usual manner with the optimum time of drying of twenty minutes. Runs were made with the same gelatin-treated collodion membrane at room temperature for periods of one half hour, starting with distilled water within the cell and solutions of oxalic acid outside varying in concentration from 7.15 to 0.0035 per cent. In each case, the flow in centimeters, the total amount of oxalic acid transferred to the pure water side and the concentration of the outside solution
in grams of oxalic acid per cubic centimeter were noted, the latter two being for future reference. The results are given in Table III in the order in which the experiments were performed.

**TABLE III**

NEGATIVE OSMOTIC FLOW, USING THE SAME MEMBRANE WITH OXALIC ACID SOLUTIONS OF DIFFERENT CONCENTRATION.

<table>
<thead>
<tr>
<th>Approximate Concentration of Acid in Per Cent</th>
<th>Flow in 1/2 Hr., in Cm.</th>
<th>Grams Oxalic Acid Inside after Run.</th>
<th>Concentration of Outside Solution in Grams per cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.2</td>
<td>19.0</td>
<td>0.293</td>
<td>0.0729</td>
</tr>
<tr>
<td>5.7</td>
<td>21.9</td>
<td>0.199</td>
<td>0.0537</td>
</tr>
<tr>
<td>4.3</td>
<td>23.2</td>
<td>0.145</td>
<td>0.0408</td>
</tr>
<tr>
<td>2.85</td>
<td>25.0</td>
<td>0.117</td>
<td>0.0271</td>
</tr>
<tr>
<td>1.43</td>
<td>22.0</td>
<td>0.0625</td>
<td>0.0136</td>
</tr>
<tr>
<td>0.71</td>
<td>22.5</td>
<td>0.0350</td>
<td>0.00673</td>
</tr>
<tr>
<td>0.36</td>
<td>20.0</td>
<td>0.0187</td>
<td>0.00337</td>
</tr>
<tr>
<td>0.36</td>
<td>22.0</td>
<td>0.0199</td>
<td>0.00337</td>
</tr>
<tr>
<td>0.036</td>
<td>8.0</td>
<td>0.00292</td>
<td></td>
</tr>
<tr>
<td>0.018</td>
<td>4.4</td>
<td>0.00160</td>
<td>0.000170</td>
</tr>
<tr>
<td>0.0036</td>
<td>3.1</td>
<td>0.000486</td>
<td>0.0000369</td>
</tr>
<tr>
<td>0.18</td>
<td>19.0</td>
<td>0.00982</td>
<td>0.00166</td>
</tr>
</tbody>
</table>

For the present purpose, it is sufficient to note that a maximum flow was observed with 2.85 per cent and that 4.3 per cent gave a value close to this maximum. It was decided to continue the use of the latter, since it had been used in all previous experiments and approximated the maximum.
The Relationship of Results Obtained to Previous Observations of J. Loeb.

Figure 3 illustrates the variation of negative osmotic flow and transference of oxalic acid to the water side with variations in the initial concentration of the oxalic acid solution over the range from about 0.2 to 7.2 per cent. The data are taken from Table III.

The maximum negative flow at a concentration of about 2.85 per cent is in general accord with Loeb's results. The total transference of oxalic acid to the water side is seen to be quite closely proportional to the oxalic acid concentration, a result entirely in accord with those of Loeb.

If the figures in the second column of Table III are multiplied by 0.02877, the results are the cubic centimeters of flow in each case. If the figures in the third column are divided by the cc. of flow, the results represent the grams of oxalic acid per cc. of flow, which may be called the "net concentration of the flow" in each case. These net concentrations are shown, together with the concentration of the outside solution and the ratio of the first to the second in each case.

In accord with Loeb's observation, the net concentration of the flow in every case is much greater than that of the original solution, varying here from 5.3 to 136 times this value. This means that ordinary diffusion of oxalic acid to the water side, in each case studied, is the chief cause for
Figure 5.

0.025 0.050 0.070

CONCENTRATION IN GRAMS PER CC.
its transfer through the membrane and that the relative impor-
tance of this factor becomes greater as the concentration of
the oxalic acid decreases; reaching very high values with
solutions of the order of 0.02 per cent.

TABLE IV.

THE NET CONCENTRATION OF THE NEGATIVE OSMOTIC FLOW WITH
VARIOUS OXALIC ACID SOLUTIONS.

<table>
<thead>
<tr>
<th>Concentration of Oxalic Acid in Grams per cc. (1)</th>
<th>Net Concentration of the Flow in Grams per cc. (2)</th>
<th>Ratio, (2) to (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0729</td>
<td>0.438</td>
<td>6.02</td>
</tr>
<tr>
<td>0.0537</td>
<td>0.328</td>
<td>6.11</td>
</tr>
<tr>
<td>0.0408</td>
<td>0.217</td>
<td>5.32</td>
</tr>
<tr>
<td>0.0271</td>
<td>0.164</td>
<td>6.05</td>
</tr>
<tr>
<td>0.0136</td>
<td>0.0985</td>
<td>7.25</td>
</tr>
<tr>
<td>0.00673</td>
<td>0.0541</td>
<td>8.05</td>
</tr>
<tr>
<td>0.00337</td>
<td>0.0324</td>
<td>9.64</td>
</tr>
<tr>
<td>0.00337</td>
<td>0.0313</td>
<td>9.29</td>
</tr>
<tr>
<td>0.00166</td>
<td>0.0179</td>
<td>10.30</td>
</tr>
<tr>
<td>0.000170</td>
<td>0.0126</td>
<td>135</td>
</tr>
<tr>
<td>0.0000369</td>
<td>0.00545</td>
<td>136</td>
</tr>
</tbody>
</table>

The general conclusion of this section is that the nega-
tive osmosis results with the system employed are in general
agreement with Loeb's conclusions with regard to concentra-
tion relationships.
The Transport Numbers of the Ions of Oxalic Acid in Free Solution and in the Pores of Gelatin-Treated Collodion Membranes.

It has been pointed out that, in order to calculate the concentration relationships in the projected electrical endosmotic experiments with oxalic acid solutions and gelatin-treated collodion membranes, it would be necessary to employ values for the transport numbers of the ions of oxalic acid within the structure of these membranes. The method adopted for these determinations was that of measuring the total potential of a concentration cell in which hydrogen electrodes dipped into differently concentrated oxalic acid solutions separated by one of the membranes in question (54). The transport numbers of the ions within the membrane structure may be assumed to be those which are effective in determining the liquid-liquid junction potential and, on the basis of this assumption, the latter may be calculated from the total potential observed and the respective concentrations of hydrogen ion in the two solutions. The total potential of an exactly similar cell with no membrane at the liquid-liquid junction was also determined for the purpose of comparison.

All potential measurements were carried out at room temperature with observation of the solution temperature being made periodically during the determination. The hydrogen electrodes were of the needle type, platinized according to the directions of Popoff, Kunz and Snow (55). The hydrogen electrode-saturated
calomel electrode cell set-ups were checked against standard phosphate buffer solutions. The hydrogen employed was tank hydrogen, purified by bubbling through alkaline potassium permanganate, alkaline pyrogallol and distilled water. No correction was made for the variation of atmospheric pressure from 760 mm.

In the cells without membranes, two methods were used in establishing the liquid-liquid junctions. In the first, one solution was contained in a beaker and the other in a calomel electrode vessel. The liquid levels were adjusted so as to eliminate hydrostatic flow at the boundary, and the total potential was measured with a hydrogen electrode dipped in each solution. In the other, the N/10 solution was contained in a separatory funnel with a short stem below the stop-cock (closed and ungreased) dipping into the N/100 solution contained in the beaker. Here again, hydrogen electrodes dipped into each of the beakers.

The cell with the membrane at the boundary was established by placing the N/100 solution inside the membrane holder used in the osmosis experiments, with the membrane in place, and putting the other side of the membrane in contact with the other solution, again with hydrogen electrodes dipping into each.

The hydrogen ion concentration of the N/100 oxalic acid solution was determined to be $4.24 \times 10^{-3}$ and that of the
N/10 solution, $3.14 \times 10^{-2}$.

The second dissociation constant of oxalic acid, $4.9 \times 10^{-5}$ (56), indicates that, in N/10 solution, the concentration of divalent oxalate ion is small as compared with that of the monovalent bi-oxalate ion. Assuming, therefore, that the ionized portion of the oxalic acid is completely split into hydrogen and bi-oxalate ions, the total EMF of the concentration cells is given by the expression

$$E = 2N_a \frac{RT}{F} \ln \frac{C_i}{C_a}$$

where $E$ is the total potential; $N_a$ is the transference number of the bi-oxalate ion; $R$, $T$ and $F$ have their usual significance; and $C_i$ and $C_a$ are respectively the concentrations of hydrogen ion in gram-ions per liter in the concentrated and dilute solutions respectively (57).

The results of the potential measurements and the calculations are shown in Table V.

<table>
<thead>
<tr>
<th>Cell used</th>
<th>Total Potential in Millivolts</th>
<th>Transference number of the Bi-oxalate Ion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without Membrane</td>
<td>9.2</td>
<td>0.0913</td>
</tr>
<tr>
<td>With Membrane</td>
<td>11.3</td>
<td>0.113</td>
</tr>
</tbody>
</table>

Both values are in fair agreement with the value of the transport number of the bi-oxalate ion calculated from the
results of Bein (58), namely; 0.107.

The small change in the value for the transference number obtained with the membrane at the liquid-liquid boundary indicates that the transference numbers within the membrane structure vary very little from those in the free solution. The value 0.113 is used in succeeding calculations and the reasonable assumption is made that effects of the Bethe-Toropoff type may be neglected in the electrical endosmotic experiments. Appreciable effects of this type would only occur if a considerable difference existed between the values for the transference numbers under the two conditions.

Concentration Relationships in Electrical Endosmose with 4.3 Per Cent Oxalic Acid and Gelatin-Treated Collodion Membranes.

Since the outstanding theory of the mechanism involved in negative osmosis is that it is spontaneous electrical endosmose, it was decided, as previously mentioned, to examine the concentration relationships in electrical endosmose with oxalic acid solutions and gelatin-treated collodion membranes prior to attempting to determine the relationships in the negative osmosis with a similar system. 4.3 per cent oxalic acid and one "maximum effect" membrane were used throughout the experiments which were carried out with the apparatus and circuit as shown in Plate B, Figures 4 and 5. In each case, the total flow of electricity was determined by means of a
Plate B

Apparatus Modified for Electrical Endosmose Runs. Remainder of cell same as Plate A. (Actual size)

1. Wire from coulometer.
2. Brass connector
3. Platinum spiral cathode
4. Small funnel, to deliver gas formed.
5. Rubber stopper to fit cell shown in Plate A.

Figure 4.

Wiring Diagram

1. Potential difference from 12 volt generator.
2. Variable resistance.
3. Copper coulometer.
4. Osmosis cell.
5. Membrane.
6. Vessel containing oxalic acid.
7. Platinum electrodes.
copper coulometer placed in series, and the volume of flow and change in concentration in the cell were noted. Flow was to the anode, showing that the membrane was positively charged with respect to the solution. The cathode was placed inside the cell, since it was necessary to observe the concentration changes on the cathode side, due to oxidation of oxalate at the anode, as proved by the presence of carbon dioxide in the anode gases.

Employing the value, 0.113, as the transference number of the bi-oxalate ion, it is a simple matter to calculate the amount of electrolyte which should leave the cell due to transference. Subtracting this value from the observed loss of electrolyte in the cell gives the amount which may be assumed to have been associated with the negative flow as such. This value, divided by the cubic centimeters of flow, gives the apparent concentration in grams per cubic centimeter, and this, divided by the concentration of the original solution, gives its ratio thereto. The results are shown in Table VI.

**TABLE VI.**

<table>
<thead>
<tr>
<th>Total Loss of Oxalic by the Cell, in Gms.</th>
<th>Calculated Loss Due to Transference, in Grams.</th>
<th>Associated Loss with Endosmotic Flow, in Grams.</th>
<th>Concentration of Endosmotic Flow, Grams per cc.</th>
<th>Ratio of Concentration of Endosmotic Flow to Concentration of Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.137</td>
<td>0.130</td>
<td>0.007</td>
<td>0.00750</td>
<td>0.172</td>
</tr>
<tr>
<td>0.185</td>
<td>0.154</td>
<td>0.031</td>
<td>0.0324</td>
<td>0.740</td>
</tr>
<tr>
<td>0.152</td>
<td>0.143</td>
<td>0.009</td>
<td>0.0109</td>
<td>0.245</td>
</tr>
</tbody>
</table>
Lack of time has prevented the continuation of this series of experiments to the point where an accurate conclusion can be reached. This will be done at the first opportunity. The preliminary indication is that the concentration of the flow lies between zero and that of the concentration of the solution, and is probably less than half of the latter. In accord with the discussion in a previous section, this is taken as an indication that the flow is a composite effect of true capillary-electrical flow and flow due to the differential transfer of hydrated ions through the membrane.

The Concentration Relationships in Negative Osmosis with 4.3 Per Cent Oxalic Acid Solution and Gelatin-Treated Collodion Membranes.

The experiments done under the above heading each involved two parts. The first was entirely similar to an ordinary negative osmosis run with "maximum effect" membrane and 4.3 per cent oxalic acid solution, with the exception that sufficient pressure was applied to the water side to prevent an increase in volume.

The details of the pressure apparatus used in conjunction with the regular set-up are shown in Plate C, Figures 6 and 7. The arrangement permitted an easy adjustment of pressure, with continuous stirring of the solution within the cell.

It was expected that a run under pressure would stretch the membrane somewhat, and probably alter their permeability.
Plate C

Figure 7. Pressure Application System.

1. Bottle (5 gallon carboy).
3. Stopcock to release pressure.
4. Source of pressure.
5. End of Calibrated tube of standard apparatus.

P. Pressure.

Apparatus Modified for Pressure Runs.

Fig. 6. Mercury Seal for Stirring Under Pressure (Actual Size).

1. Inside stirrer
2. Open tube in standard apparatus (No. 5)
3. Tube sealed to stirrer by stopper at top (a)
4. Outer tube containing mercury, sealed to central tube by stopper at bottom (b)
5. Tube used to measure flow.
6. Mercury levels

P. Difference in levels or pressure on system.
This was the reason for making the pressure run before the other portion of each experiment, which consisted of a normal negative osmosis run with the same membrane used in the pressure run, and carried out immediately following the latter. All runs of both types were for a period of one hour. In every case, the total amount of oxalic acid entering the cell in this time was noted, as was the exact concentration of the external solution. The volume of the flow was also noted in the runs without pressure.

Briefly, the scheme, as outlined in a preceding section, was to compare the amounts of oxalic acid which entered the cell in the pressure and non-pressure runs. It was expected that the amount in the non-pressure runs in each case equal or exceed that entering in the pressure runs. The excess would be the amount of oxalic acid associated with the flow, which was prevented in the pressure runs. The excess in the non-pressure runs, divided by the cubic centimeters of flow would give the actual concentration of the osmotic flow in grams per cc. This latter could then be compared with the concentration of the solution used, and this final result, compared with those obtained in the electrical endosmotic runs. The results are shown in Table VII.

The conclusion from the ratios in the last column of the table is clearly that the concentration of the negative osmotic flow is either zero, or a small fraction of the concentration of the outside solution. This fact leads to the further con-
clusion that the flow is largely or entirely due to the transfer of hydrated ions by simple diffusion and either does not involve true capillary-electrical transport of liquid, or involves this to a comparatively small degree.

TABLE VII.

THE CONCENTRATION OF THE OSMOTIC FLOW IN NEGATIVE OSMOSIS.

<table>
<thead>
<tr>
<th>Total Oxalic Acid Transferred</th>
<th>Vol.: Excess</th>
<th>Concentration</th>
<th>Ratio of Flow to Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Pressure Run</td>
<td>in Non-Run</td>
<td>in Pressure</td>
<td>in Gms. of Flow, Concentration</td>
</tr>
<tr>
<td>0.420</td>
<td>0.418</td>
<td>1.75</td>
<td>0.002</td>
</tr>
<tr>
<td>0.401</td>
<td>0.441</td>
<td>2.05</td>
<td>0.030</td>
</tr>
<tr>
<td>0.435</td>
<td>0.456</td>
<td>2.04</td>
<td>0.021</td>
</tr>
<tr>
<td>0.402</td>
<td>0.423</td>
<td>1.59</td>
<td>0.021</td>
</tr>
<tr>
<td>0.351</td>
<td>0.352</td>
<td>1.21</td>
<td>0.001</td>
</tr>
</tbody>
</table>

This point of view is supported by the fact that, although we should have to postulate flow of current through the membrane of the order of amounts which flowed in the electrical endosmotic experiments if the entire flow were due to true capillary-electrical flow, no sign of gas evolution is observed at the two sides of the membrane.

The maximum pressures required in the pressure runs are of interest. These varied from 60 to 90 mm. of mercury and represent maximum values which appeared during the course of the runs.
A comparison of the negative osmotic results with those obtained from the electrical endosmotic experiments indicates agreement in so far as both show that at least a portion of the flow in each case is due to the transfer of hydrated ions.
Summary and Conclusions.

The history of present knowledge concerning negative osmosis has been reviewed and the importance of two factors has been emphasized, namely; the question of the mechanism or mechanisms responsible for the phenomenon and that of the concentration relationships involved. The presumptive relationship of the phenomenon to electrical endosmose is emphasized throughout.

An experimental program is described, pointing toward the elucidation of these concentration relationships in one outstanding case;--that of negative osmosis observed with gelatin-treated collodion membranes.

The conclusion is reached that, under the experimental conditions employed, the negative flow is entirely, or to a great extent, due to the water of hydration associated with the ion or ions and molecules of oxalic acid which diffuse to the water side. This is the first experimental indication of the importance of this type of flow in negative osmosis.

In negative osmosis with this system, agreeing with the previous observation of J. Loeb, the net concentration of the flow is always greater than that of the solution, the initial rate of flow from the oxalic acid side to the water side of the membrane is proportional to the initial oxalic acid concentration, and maximum negative flow is obtained at a concentration of the order of 0.15 molal.
A method is suggested for quantitatively determining the extent to which the differential transfer of hydrated ions and true capillary-electrical flow enter in a case of electrical endosmose. Experiments with this method are encouraging; and further experiments will be carried out. The electrical endosmotic flow with a 4.3 per cent oxalic acid solution and a gelatin-treated collodion membrane appears to be a combined effect of the two mechanisms.

The pressure required to prevent apparent negative osmotic flow with a 4.3 per cent oxalic acid solution and a gelatin-treated collodion membrane has been observed to be as high as 89 mm. of mercury. This is believed to be the highest pressure ever observed in connection with negative osmosis. It is noteworthy that this value far exceeds that of the osmotic pressure of the serum proteins, a fact which may prove to be of importance in physiology.
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