THE MECHANISM OF NEGATIVE OSMOSIS WITH PROTEIN MEMBRANES

by

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THE PREVENTION OF ECONOMIC DEPRESSION

M. WALTER STEPHENSON

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INTRODUCTION

Osmosis is the passage of liquids or materials in solution through a membrane. Now known to be of the utmost importance in the metabolism of all living organisms, the phenomenon was discovered in 1748 by the Abbé Nollet, who observed the results of filling a vessel with spirits of wine, closing it with a bladder, and immersing it in pure water. The volume on the alcohol side promptly increased with stretching of the bladder, indicating that water had passed through into the alcohol. The result was confirmed in 1814 by Sommering. Between 1825 and 1850, Dutrochet and Vierordt carried out the first quantitative experiments; both finding that, when a membrane separates pure water from a salt solution, the rate of the resulting osmosis depends upon the nature and concentration of the salt and upon the nature of the membrane. Dutrochet, who used porous inorganic membranes as well as organic membranes such as the bladder membrane of the Abbé Nollet, pointed out that flow of material always occurred in both directions; while water was passing through the membrane into the solution, solute was passing in the opposite direction into the water; he called the flow of water, or, in general, the flow of solvent, the endosmotic current and the flow of solute in the opposite direction, the exosmotic current. Starting
about 1854, Graham carried out experiments using a variety of membranes and solutions.

The experiments of Dutrochel and Vierordt were of the type which has since become the usual one -- that is, experiments in which at the start the membrane separates pure water from an aqueous solution. It is clear that their membranes were permeable to both solvent and solute, and that, as a first hypothesis at least, Dutrochel's endosmotic and exosmotic currents could be thought of as the diffusion through the membrane respectively of water to the solution side and solute to the water side. Between 1880 and 1890 (6) the theoretical conclusions of van't Hoff brought into great prominence the concept of the osmotic pressure of solutions, and that of the semipermeable membrane. A semipermeable membrane is permeable to solvent but not to solute. When such a membrane separates a solution from pure solvent, there can only be an endosmotic current; solvent tends to diffuse through into the solution, but solute cannot pass in the opposite direction. The osmotic pressure of a solution is the pressure which when placed upon it is just sufficient to prevent the diffusion into it of pure solvent through a semipermeable membrane. The phenomena with which the present study is concerned do not involve semipermeable membranes, the membrane being in every case permeable to both solvent and solute.

Osmosis which results in an increase in volume on the
solution side and a corresponding decrease on the water side
is called positive osmosis; when the increase in volume is
on the solvent side, it is called negative osmosis. In
terms of Dutrochet's currents, it is apparent that the osmo-
sis is positive when the volume of the endosmotic current is
greater than that of the exosmotic current, and vice versa.
The fundamental process in any osmosis is diffusion, diff-
fusion of solvent to the solution side and of solute to the
solvent side; in this connection it is apparent that with a
membrane permeable to both solvent and solute, diffusion
will continue until the concentration of solute is the same
on both sides of the membrane, and that, when this condition
is reached, further osmotic flow is impossible. Osmotic
flow is considered to be normal when it is due entirely to
diffusion, and anomalous when, in addition to diffusion,
some other factor is operative.

With a given membrane and solutions of varying concen-
trations of a given solvent normal osmosis is indicated if
the initial volume of flow increases regularly and contin-
uously with the concentration of the solution along a curve
symbatic with the one for the variation of osmotic pressure
with concentration. The reason is that diffusion increases
regularly and continuously with osmotic pressure along a
similar curve. In practice, on the basis of this criterion,
it has been found that with concentrations up to one molar,
the concentration range with which this study is concerned,
normal osmosis is obtained with non-ionized solutes, and
that this normal osmosis is always positive. Curve I in
Fig. 1, which was obtained by Loeb in osmotic experiments
with solutions of cane sugar separated from initially pure
water by means of a collodion membrane, indicates normal
osmosis. The fact is that the investigators in this imme-
diate field have ordinarily not attempted to check curves of
this type quantitatively against the predictions of osmotic
theory; they clearly represent osmosis which is normal with-
in the necessary limits of accuracy, and the anomalous na-
ture of other curves is measured by their departure from
this type.

Curves II, III, IV, and V in Fig. 1 are also taken from
Loeb and clearly represent cases of anomalous osmosis. The
flow indicated by curve II is seen to have been positive
and greater than normal; throughout it indicates anomalous
positive osmosis greater than normal. Curve III indicates
the same type of anomalous osmosis up to the point between
sixteenth and eighth molar where it crosses the normal
curve; at higher concentrations the osmosis is seen to have
been less than normal and may be taken as an example of
anomalous positive osmosis less than normal. Curves IV and
V are also due to Loeb, and indicate clearcut negative
osmosis; negative osmosis is always anomalous and there-
fore need not be designated as such.

It has been indicated that with aqueous solutions of
FIGURE 1.

- Sucrose
- $\text{Na}_2\text{SO}_4$
- NaCl
- HCl
- $\text{H}_2\text{SO}_4$
non-electrolytes in the concentration range up to one molar, osmosis is normal and positive. On the other hand with solutions of electrolytes in this concentration range it is as a rule anomalous and may be either positive or negative. If positive, it may be either greater or less than normal. The great question in this connection, and one which has been the object of much study from the time of Dutrochet until the present, is that of the determination of the nature of the mechanism or mechanisms which are superposed upon normal diffusion in such a way as to produce anomalous effects. Dutrochet realized that some factors other than normal diffusion must be involved in certain cases and, as early as 1827, offered an electrical theory in explanation of this factor. His conclusion was that the two sides of the membrane developed different degrees of electricity, but that this difference could not be measured with a galvanometer. (10) Graham considered the possibility of an electrical basis for the anomalous results observed by him, but was inclined to focus his attention upon normal cases. The theories of Dutrochet and Graham are now chiefly of historical interest.

Most of the modern work has been directed toward showing that anomalous osmotic effects are due to spontaneous electroosmosis, and the discussion of this work requires a knowledge of this phenomenon. Electroosmosis is the passage of liquid through a membrane when a difference of electrical potential is impressed across the membrane. (11) The
arrangement for the typical experimental demonstration differs from that for the typical demonstration of osmosis in that instead of having initially pure water on one side of the membrane with a solution on the other, the same solution is present on both sides; and that, instead of proceeding spontaneously, the flow through the membrane is brought about by introducing an electrode on each side of the membrane and impressing an electrical potential between the two. According to Helmholtz, an electrical double layer is set up at the interface between the solution and the pore-wall in the pores of the membrane; the wall side of the double layer is relatively fixed, the solution side relatively movable; and when a difference of electrical potential is impressed across the membrane, the solution side of the double layer moves toward the electrode of opposite sign, carrying with it by friction the rest of the liquid in the pore. Bartell later suggested with good reason that, especially with larger pores, the liquid carried along by friction might be essentially that contained in an annular cylinder within the pore rather than the liquid in the pore as a whole. In a series of papers Remy and his associates have urged that in any case of electroosmosis there must be considered, in addition to the mechanism suggested by Helmholtz, the volume changes brought about by the ordinary electrolytic transport of ions through the membrane in response to the impressed potential; they claim to show that
in certain cases with solutions in the higher concentration range the apparent flow is due practically entirely to this cause. To date all attempts to demonstrate that anomalous osmosis is spontaneous electroosmosis have been concerned with the Helmholtz mechanism to the complete neglect of the Remy mechanism. A general summary of the results of these attempts prior to 1925 was given by Bartell, and one of results prior to 1930 by Süllner.

In 1904 Perrin carried out an extensive investigation of electroosmosis from the point of view of the Helmholtz mechanism. Girard, with considerable success, applied Perrin's results in an investigation of anomalous osmosis. In particular, Girard measured the potentials acting across the membrane, finding these to be functions of the mobilities of the ions just as are boundary potentials between solutions of electrolytes. By simple diffusion, ions from a more concentrated solution move into a less concentrated one, or ions from a solution move into water; the faster moving ion causes the dilute solution or the water to assume the sign of charge borne by the ion. On the other hand the difference of potential between two solutions separated by a permeable membrane, while presumably due to the same causes as one between the same two solutions in direct contact, need not, and usually does not, have the same values as the latter potentials. As shown particularly by Michaelis and his associates the potential with a membrane
present tends to differ from that between the same two solutions in immediate contact because the transport numbers of the ions within the pores tend to differ from those in free solution. In general the transport number of an ion which forms the wall side of an electrical double layer is decreased.

Between 1914 and 1923 a series of investigations (21) carried out by Bartell and his associates did much to establish electroosmotic flow of the Helmholtz type as of fundamental importance in anomalous osmosis. Söllner considers that the chief contribution of this work was the qualitative establishment of the fact that in all cases the anomalous flow was in the direction predicted by the theory. The orientation of the electrokinetic potential within the pores was determined as was that of the potential across the membrane; the anomalous flow, whether reinforcing or opposing the normal flow, was always in the direction of the side of the membrane of sign of charge opposite to that of the movable side of the electrical double layer.

The investigations of Loeb carried on between 1919 and (22) 1924 rank in importance with those of Bartell and his associates. One of Loeb’s outstanding contributions was to show in three cases with salt solutions and colloidion membranes that not only were the three rates of anomalous osmotic flow for a given concentration in the same order as the three rates of electroosmotic flow for the same concentra-
tion, but also that in each case the curves showing the variation of anomalous osmotic flow with concentration exhibited maxima at closely the same concentrations as those at which maxima were exhibited in the corresponding curves for electroosmosis; this demonstration, although involving only three solutes in one concentration range and with one type of membrane, when taken with the results of Bartell and other results of Loeb confirming those of Bartell, is recognized as establishing beyond a reasonable doubt a qualitative relationship between anomalous osmosis and electroosmosis.

Loeb also succeeded in making a quantitative demonstration of a relationship between anomalous osmosis and electroosmosis in showing in a number of cases that, with series of solutions of different concentrations of the same solute, maximum anomalous osmotic flow occurs in the concentration range in which the product of the electrokinetic potential within the pores and the potential across the membrane has a maximum value; this result is predictable from the theoretical equation for electroosmotic flow.

So far as they go, the conclusions of Bartell and his associates and of Loeb have not to date been seriously questioned. They did, however, leave one important point in a rather unsatisfactory condition; this point was the nature of the electric current which, as pointed out by Freundlich in 1916, must accompany anomalous osmosis if it is
indeed spontaneous electroosmosis. Throughout, the thought has been that small local circuits must exist; the question has been as to the nature of these circuits. Freundlich suggested that current might flow in one direction through a pore and return through the material of the membrane; the difficulty here is that the membrane materials which have been employed are non-conducting. Bartell in 1923 suggested that an entire local circuit might be included within a single pore with current flowing in one direction through the center and in the other through the electrical double layer; but, as emphasized by Söllner, this would mean that regions at different potentials would be in electrical contact. Finally, in 1930, Söllner offered a solution for the difficulty based upon the results of Michaelis and his associates. A finding of these workers in addition to the one previously discussed was that with graded colloidion membranes the effect of the membrane on the boundary potential increased with diminution of pore size. Söllner pointed out that in any membrane there must exist pores of a great variety of sizes and that, since the potential resulting from diffusion through any pore varies with the size of the pore, local circuits may be assumed in which the current flows in one direction through one pore, say a large one, and in the return direction through another, say a small one. With Grollman, Söllner went on to a second demonstration of importance. Prior to their work, in spite
of the excellent evidence that many cases of anomalous flow were in response to a membrane potential, no one had actually demonstrated electroosmosis experimentally with an impressed potential of the order of magnitude available in anomalous osmosis; Söllner and Grollman accomplished this.

At present, after the work of Söllner and of Söllner and Grollman, there remains little doubt but that in many cases anomalous osmotic effects are due to spontaneous electroosmosis of the Helmholtz type. In so far as the volume changes in any case of electroosmosis are not due to the Helmholtz mechanism, there is no alternative at present but to consider that they result from ordinary conductance phenomena as in certain of the cases previously mentioned as having been studied by Remy; and, without departing from the view that anomalous osmosis is spontaneous electroosmosis, it is entirely reasonable to postulate that the Remy mechanism may be of importance. The fact that in the literature to date there has been not even a suggestion of the potential importance of the Remy mechanism in anomalous osmosis, indicates than an experimental investigation of this important possibility should be made.

In all the years of the present century only one theory in addition to the spontaneous electroosmotic theory has received serious consideration, the imbibition or swelling (32) theory. This theory is due to Flusin, who in 1908 observed the rates and degrees of swelling of membrane
materials in various solutions and pure liquids, and the
direction and rates of the osmosis which occurred when a
membrane of one of the materials separated a pair of these.
In every case the flow was in the direction of the liquid
for which the membrane material showed the lower affinity;
not only this, but the rate of flow in the particular direc-
tion varied with the difference between the affinity of the
material for the two liquids. Flusin's conclusion was that,
in the cases studied by him, the relative affinities for
the two liquids of the membrane material employed played the
major role in determining the direction and rate of the os-
motic flow; and, at least by implication, that imbibition
can per se be a causal factor in anomalous osmosis. In dis-
cussing Flusin's view Freundlich believed that imbibi-
tion need not necessarily be considered as a mechanism apart
from spontaneous electromosmosis; the parallelism noted by
Flusin might well be due to the fact that anomalous osmosis
and swelling result from the same fundamental causes rather
than that swelling per se brings about anomalous osmosis.
Freundlich went on to say that at the time of writing appro-
priate experimental evidence for deciding the point was not
available.

The present writer goes further than Freundlich, and,
in agreement with Nugent and Coffer, believes that no
mechanism, such as a swelling mechanism, which results in
the attraction of liquid into a membrane can of itself be
responsible for the passage of liquid through the membrane as in osmosis. On the other hand Söllner is most careful to differentiate between anomalous osmosis with "non-swelling" membranes and those with "swelling" membranes; he limits his considerations to the former and states that Flusin has offered a satisfactory explanation for anomalous osmosis with the latter; he classifies the gelatin-treated membranes of Loeb as "swelling" membranes. It appears that the decision as to whether or not imbibition must be considered apart from spontaneous electroosmosis as a causal factor in anomalous osmosis remains to be settled; it is indeed one of the major problems facing workers in the field of membrane phenomena.
THE PROBLEMS AND THE PLAN FOR SOLVING THEM

The present study came as the result of a wish to aid in the solution of the two major problems which came to light in the preceding discussion, the first being whether or not the Remy mechanism is important in anomalous osmosis, and the second whether with membranes of the swelling type there is operative an imbibition mechanism distinct from spontaneous electroosmosis.

The first step in the plan of procedure was to select the type of osmosis which appeared to be most favorable. A number of cases were then to be studied to determine how completely the flows obtained could be described in terms of spontaneous electroosmosis of the Helmholtz type; the effort spent upon any case which was found to be completely describable in these terms would be well spent because there is a definite need for a knowledge of a greater number of completely described cases. When complete description in terms of the Helmholtz mechanism were found not to be possible, that in terms of some combination of the Helmholtz and Remy mechanisms, or in terms of the Remy mechanism alone would be attempted; in such cases important clues might well be obtained from the degree of departure of the experimental results from those predicted on the basis of the Helmholtz mechanism. If complete description were still not possible,
it was hoped that the data accumulated would provide a means of determining the nature of the other causal factor or other causal factors, in particular whether imbibition were important per se.

The type of osmosis selected obviously had to involve a swelling membrane and be one not included among those for which Loeb had demonstrated a quantitative explanation in terms of the Helmholtz theory; since the search was to be for unusual causal factors, a type exhibiting unusual results was desirable; and, finally, for convenience in experimentation and as an aid in securing comparable results, the necessary membranes should be easily obtainable and one of them should be as much like another as possible. With these conditions in mind, the selection was not difficult; Loeb's negative osmoses with gelatin-treated collodion membranes (35) and solutions of acids and bases were clearly indicated; thus, gelatin-treated collodion membranes are easily prepared with results which are sufficiently reproducible; Loeb had not investigated the quantitative application of the Helmholtz theory to them, and, in fact, no case of negative osmosis had been investigated from this point of view; and the nature of the osmotic flow in these cases is distinctly unusual, as will be explained in the next paragraph.

Gelatin-treated collodion membranes and animal membranes have at least one important characteristic in common, in that both have protein surfaces; that the treatment of
collodion with a gelatin solution results in a protein surface is indicated by the fact that under these conditions the particles are isoelectric at the isoelectric point of gelatin; electroosmosis through animal membranes indicates that the surfaces of these are at least largely composed of protein. Anomalous osmosis as observed with each type, is unusual as compared with the more familiar examples observed with untreated collodion membranes and membranes composed of inorganic materials. In the vast majority of cases observed with these latter types the anomalous osmosis is obtained with solutions of salts, is positive at all concentrations, and exhibits a maximum value at a concentration in the range from thousandth to two hundred fiftieth molar; curves II and III in Fig. 1 illustrate cases in point. In marked contrast, anomalous osmosis as observed with membranes with protein surfaces is strong with solutions of acids and bases rather than with those of salts, is characteristically negative, and exhibits a maximum value at a concentration in the range from sixty-fourth to one molar; curves IV and V in Fig. 1, which illustrate cases in point here, clearly differ in a striking manner from curves II and III.

The statements concerning anomalous osmosis with membranes with protein surfaces cover all cases studied, but it should be noted that the number of such cases is small. Loeb's general result has been confirmed by Coffer with
gelatin-treated collodion membranes and solutions of oxallic acid. It is significant that Graham's observations of negative osmosis were made with animal membranes and acid solutions. Quantitative work with such membranes was carried out by Flusin using pig's bladder and solutions of organic acids; Flusin's work is very important because maximum flow was found at concentrations in the range from sixty-fourth to one molar, exactly the range in which Loeb's maxima occurred. Additional quantitative results were obtained by Bartell and Sims with goldbeater's skin and solutions of acids and bases with maximum flow also in this concentration range.

It is apparent from the plan of procedure and from the nature of the osmosis chosen, that, while the problems which gave rise to the investigation are of importance in the general study of anomalous osmosis, the specific results concern the mechanism of negative osmosis with protein membranes. This aspect was chosen as the basis for the title.

The General Plan for the Experimental Work

Originally chosen for study were three of Loeb's cases, those of gelatin-treated collodion membranes respectively with solutions of oxalic acid, hydrochloric acid, and sodium hydroxide; and for comparison one case in which negative osmosis has been reported with a non-protein membrane, that of an untreated collodion membrane with solutions of
calcium chloride. In each of the four cases it was planned first to run a curve of the type shown in Fig. 1 for direct comparison with those previously reported. Next in each case over the same concentration range there was to be determined the variation with concentration of the electro-kinetic potential, the potential across the membrane during osmotic flow, and the electroosmotic flow; during the determinations of the variation of electroosmotic flow, which were to be carried out with constant voltage, there was to be observed the variation with concentration of the electric current. Actually, the investigation of the electrokinetic potentials was carried out first, and, because of lack of time, the remaining investigations have been limited to the cases of oxalic and hydrochloric acids with gelatin-treated collodion membranes.

It was believed that, in a manner which will be explained in detail in connection with the discussion of the experimental results, the data indicated would definitely serve to determine whether the flows in the cases studied resulted completely, partially, or not at all from spontaneous electroosmosis of the Helmholtz type; and if these flows did not take place completely in accord with such electroosmosis, any role of the Remy mechanism or of an imbibition mechanism would be indicated.
EXPERIMENTAL METHODS AND RESULTS

Electrokinetic Potentials

The determination of the electrokinetic potentials could obviously not be based upon electroosmosis because this method assumes that the electroosmotic flow is due solely to the Helmholtz mechanism. The best alternative was to base them upon measurements of electrophoresis, there being ample evidence that the values obtained in this way would be those in effect in the osmotic experiments if the flow in these latter is due to the Helmholtz mechanism.

The electrophoretic measurements were made with a Northrop-Kunitz micro-electrophoresis cell with arrangement of accessory apparatus according to Mudd. In accord with the results of others, observations made at the "stationary levels" respectively at 0.21 and 0.79 of the depth of the cell below the upper surface agreed with those calculated from observations made at several levels. Results at the "stationary levels" were therefore used throughout.

The recorded value in each case is the mean of eight or ten taken in equal numbers at the upper and lower levels. Each single observation involved noting with a stopwatch the time for a particle in good focus to move between two lines of the micrometer eyepiece and back to the first line on reversing the polarity. The details of the use of the
cell with accessory apparatus according to Mudd has been
fully described by others.

After a suitable particle was found, the stopwatch was
started as the particle crossed one of the large unit lines.
When the particle reached the next line, the polarity was
reversed quickly; then the watch was stopped as the particle
crossed its initial line. This method eliminated any error
due to slight eddy currents in the cell. The temperature
remained practically constant, and rarely was there any dif-
ference in the rates which was dependent on the direction of
flow. When the particles moved so slowly that they would
drift out of focus before reaching the unit line, they were
reversed as they crossed the half-line, or the smaller lines
between the units if necessary. The time was then multi-
plied by the appropriate factor to make the distance trav-
eled the same as for the faster particles. Immediately be-
fore or after each day's readings, the pH's of the solutions
were taken, using a Beckmann pH-meter, laboratory model.
The reciprocal of the time required for a particle to trav-
el an arbitrary unit distance is directly proportional to
the electrokinetic potential, and since only relative values
were needed such reciprocals were recorded as measures of
the potentials. The points for any one curve were obtained
consecutively, starting with the most dilute solution. Be-
cause they are to be used in comparison with molarities,
they were plotted that way, but better curves are obtained
using pH.

Measurements were made with collodion particles in the series of solutions indicated in Table I. The results are listed in that table and are shown graphically in Fig. 2. Similarly, measurements were made with gelatin-treated collodion particles, the results being listed in Table II and shown graphically in Fig. 3.

The method of preparing the collodion particles was essentially that of Loeb; one change from his procedure was employed in that the acetone was evaporated by bubbling compressed air through the solution rather than by vacuum distillation. The precipitated particles were washed three times by suspension in distilled water followed by centrifuging. Thereafter, if they were not to be treated with gelatin, they were stored in a few ml. of distilled water to which a crystal of thymol had been added to make conditions comparable to those under which the gelatin-treated particles were stored; the use of thymol with the treated particles was to prevent deterioration of the gelatin. The gelatin treatment consisted of mixing freshly washed particles with two ml. of a one percent solution of the Eastman Kodak Company's de-ashed gelatin and allowing the mixture to stand for about twelve hours at 35 degrees with a crystal of thymol added; the treated particles were stored in the same gelatin solution with the crystal of thymol present, but at room temperature. In making the electro-
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(The negative sign indicates the sign of charge on the particles.)
**FIGURE 2.**

Charge on Particles:
- HCl
- $\text{H}_2\text{C}_2\text{O}_4$
- NaOH
- CaCl$_2$

Relative Electrophoretic Velocity

Log Molar Concentration
## TABLE II

**RELATIVE ELECTROPHORETIC VELOCITY OF GELATIN-TREATED COLLODION PARTICLES IN DIFFERENT ELECTROLYTES**

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<td>5.71</td>
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<td>5.00</td>
<td>-0.021</td>
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<td>$10^{-4.75}$</td>
<td>4.73</td>
<td>+0.0324</td>
</tr>
<tr>
<td>$10^{-4.5}$</td>
<td>4.56</td>
<td>+0.040</td>
<td>$10^{-4.5}$</td>
<td>4.21</td>
<td>+0.044</td>
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<tr>
<td>$10^{-4.0}$</td>
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</tr>
<tr>
<td>$10^{-3}$</td>
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</tr>
<tr>
<td>$10^{-2}$</td>
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<td>3.30</td>
<td>+0.086</td>
</tr>
<tr>
<td>$10^{-1}$</td>
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<td>+0.049</td>
<td>$10^{-3}$</td>
<td>3.05</td>
<td>+0.098</td>
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<td></td>
<td></td>
<td></td>
<td>$10^{-2}$</td>
<td>2.09</td>
<td>+0.086</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>$10^{-1}$</td>
<td>1.30</td>
<td>+0.049</td>
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<table>
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<th>Molarity</th>
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<th>Velocity</th>
<th>Molarity</th>
<th>pH</th>
<th>Velocity</th>
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<tr>
<td>$10^{-6}$</td>
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<td>-0.058</td>
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<td>6.07</td>
<td>-0.067</td>
</tr>
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<td>$10^{-5}$</td>
<td>5.61</td>
<td>-0.050</td>
<td>$10^{-5}$</td>
<td>6.13</td>
<td>-0.082</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>5.64</td>
<td>-0.036</td>
<td>$10^{-4}$</td>
<td>7.05</td>
<td>-0.094</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>6.30</td>
<td>-0.025</td>
<td>$10^{-3}$</td>
<td>10.69</td>
<td>-0.084</td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>8.18</td>
<td>-0.010</td>
<td>$10^{-2}$</td>
<td>11.81</td>
<td>-0.082</td>
</tr>
<tr>
<td>$10^{-1}$</td>
<td>10.74</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
phoretic observations, a few drops of a suspension of the
collodion or gelatin-treated collodion particles were added
to twenty ml. of the appropriate solution.

Osmosis

Collodion membranes treated with gelatin were used in
all electrosmotic and osmotic experiments, and, while it was
found impossible to make all the observations with one mem-
brane, the different ones used gave similar results, never
varying more than the results with one membrane. The elec-
troosmosis was all done with one membrane, and the negative
osmosis with another. Values obtained with two other mem-
branes were used in the membrane potential curves in addition
to those obtained with the membranes used for osmosis.

To make the collodion solution for the membranes, twelve
grams of J. T. Baker's collodion cotton was soaked in one
hundred ml. of absolute alcohol for fifteen minutes; then it
was dispersed by shaking with three hundred ml. ether, and
filtered through glass wool. A membrane was prepared by
pouring 25 ml. of this collodion solution on a clean mercury
surface in a fourteen cm. crystallizing dish, and allowing
the ether to evaporate slowly in still air. When the sur-
face took on the appearance of hammered metal and just began
to dry at the edges, two hundred ml. of water was poured on
it, and one minute later the membrane was turned over after
having been loosened at the edges. The membrane was rinsed
thoroughly with distilled water and either stored in it directly or treated with a half percent solution of the Eastman Kodak Company's de-ashed gelatin. Gelatin treatment was continued for ninety-six hours in an oven set at 35 to 38 degrees C., and was followed by a twenty-four hour period in distilled water at the same temperature. Treated membranes were also stored in distilled water. Small membranes, about four cm. in diameter, were cut from stored membranes as needed. A good membrane could be used for two or three weeks at least if it was returned each night to distilled water to which a crystal of thymol had been added to prevent deterioration of the gelatin. A crystal of thymol was also kept present during the treatment of the membranes with gelatin solution and with water. Bartell and others have used the general method of preparation described. The method of treatment with gelatin solutions is that of Loeb as modified by Coffer. (51)

(52)

The membrane was held between the clean paraffined surfaces of two large rubber stoppers that extended about a millimeter beyond the smooth edges of the cells, of the osmometer shown in Fig. 4. To prevent leaking, the stoppers were paraffined at both junctions with the glass. The two half-cells were held firmly together by screws connecting brass plates which pressed against the stoppers. The membrane surface touched only paraffin; there was no leakage around it, and the exposed surface was defined by the
opening of the cells. As is apparent from Fig. 4, the apparatus consisted of two half-cells each connected to a capillary tube; the capillary tubes were for measuring flow. It is essentially a modification of a well-known type of (54) electroosmotic apparatus. Calibration of the measuring tubes was accomplished by gluing a strip of millimeter graph paper behind the tube, the glass and liquid serving to magnify the markings and to make a reading of the position of the meniscus very easy. Osmosis was registered by a movement in the same direction of the meniscus in each of the tubes, showing a loss of volume on one side and a gain on the other.

The cells were rinsed and filled through the vertical tubes; the osmometer was then clamped in position, and the measuring tubes were attached. The most difficult part of setting up the apparatus was leveling the measuring tubes, because a barely perceptible slant might cause considerable difference in the rates. The whole apparatus, with the exception of the measuring tubes, was placed in a water bath thermostat at 25.00 ± 0.02 C. A notched cork stopper fitting in each vertical tube held a thermometer and the end of the salt bridge (agar jelly saturated with potassium chloride) used in connection with the measurement of the membrane potentials. When a run was to be made, the measuring tubes were detached from their rubber connections and cleaned thoroughly with hot cleaning solution; the left cell was
rinsed several times with the acid solution it was to contain; and finally the acid and water were placed in their compartments and a stopwatch started immediately. As soon as the cell was clamped in position, a reading of the membrane potential was taken by the method to be described, and the tubes were leveled. Liquid was added or taken out through the vertical tubes until each meniscus remained at about the middle of the measuring tube; the movement of each meniscus was then recorded at intervals. As soon as the rate became constant the apparatus was taken out and refilled, and a check run was made. Overnight, the cells were left with water and a crystal of thymol on each side of the membrane.

In most cases an entire range of concentrations was run in one day. The pH value of each solution was taken for later reference, a Beckmann pH-meter being employed. Before and after each series of runs a check run was made using sixteenth molar oxalic acid to ascertain whether the membrane had remained in its original condition. The result tabulated for each concentration is the mean of all observations of the flow in both tubes, the values indicating the rate of movement of the meniscus expressed in cm. per minute. An analysis of the deviations showed that it was entirely legitimate from the point of view of the purposes involved to employ such mean values. The results are listed in Table III and are shown graphically in Fig. 5.
TABLE III

RATE OF OSMOSIS OF SOLUTIONS OF HCl AND H₂C₂O₄ IN CM./MIN.
THROUGH A GELATIN-TREATED COLLODION MEMBRANE

<table>
<thead>
<tr>
<th>Molarity</th>
<th>H₂C₂O₄</th>
<th>Rate</th>
<th>Molarity</th>
<th>HCl</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/4096</td>
<td>3.57</td>
<td>.0015</td>
<td>M/4096</td>
<td>3.80</td>
<td>.030</td>
</tr>
<tr>
<td>M/1024</td>
<td>3.01</td>
<td>.012</td>
<td>M/1024</td>
<td>3.32</td>
<td>.030</td>
</tr>
<tr>
<td>M/256</td>
<td>2.47</td>
<td>-.020</td>
<td>M/256</td>
<td>2.78</td>
<td>.022</td>
</tr>
<tr>
<td>M/64</td>
<td>1.93</td>
<td>-.086</td>
<td>M/64</td>
<td>2.20</td>
<td>.039</td>
</tr>
<tr>
<td>M/16</td>
<td>1.47</td>
<td>-.207</td>
<td>M/16</td>
<td>1.30</td>
<td>-.073</td>
</tr>
<tr>
<td>M/4</td>
<td>1.07</td>
<td>-.089</td>
<td>M/4</td>
<td>1.04</td>
<td>-.156</td>
</tr>
<tr>
<td>M/1</td>
<td>0.64</td>
<td>-.080</td>
<td>M/1</td>
<td>0.60</td>
<td>-.100</td>
</tr>
</tbody>
</table>
FIGURE 5.

FIGURE 8.
Membrane Potentials

In measuring membrane potentials tubes containing agar jelly saturated with potassium chloride connected two saturated calomel electrodes respectively with the solutions on the two sides of the membrane in the osmometer, the calomel electrodes being also immersed in the water bath thermostat. Contact with the solutions was made through the vertical tubes of the osmometer shown in Fig. 4. The differences in potential were measured with a potentiometer; the difference in potential between the two electrodes when dipping in the same solution with no membrane present was negligible. The general method of measuring membrane potentials with two calomel electrodes has been employed by Bartell and Michaelis and their associates.

The values upon which theoretical conclusions of the paper are based were obtained actually during the osmotic experiments, these experiments having started with a membrane which had been immersed for some time in water. Each chamber of the osmometer was rinsed with the liquid which it was to contain and then filled with that liquid; an initial potential measurement was made as soon as possible after the filling, and others were made at regular time intervals during the run. The first reading was invariably high and was followed by a rapid decrease during the first five or six minutes; thereafter there was a slow, regular decrease, due presumably to the gradual diffusion of solute to the
water side; the decrease during a period of one hour was of
the order of 0.02 volts. The recorded value in each case
was the mean of those observed after the steady decrease had
set in. Bartell and Carpenter noted variations in membrane
potentials with time somewhat similar to those described
(57)
here.

A second series of measurements of membrane potentials
was made using a different procedure carried out indepen-
dently of the osmotic experiments. The solution to be em-
ployed was placed on both sides of the membrane in the os-
mometer for a period of five minutes; the solution on one
side was then removed and replaced with distilled water
after rinsing; a series of observations of the potential
were then made at regular time intervals as in the previous
procedure, with the exception that observations of osmosis
were not carried out simultaneously. The essential dif-
fERENCE BETWEEN THIS method and the first one appears to be
that at the start of the experiment the membrane is satura-
ated with solution rather than with distilled water; in any
case it led to somewhat different results: the initial
values were the same as those observed with the first method,
but were not followed by the rapid drop during the first few
minutes; the slow, steady decrease set in practically from
the beginning. The curve for the variation of the
value with time was symbatic with, but somewhat above the
corresponding curve obtained with the first method.
Although the second method gave somewhat steadier and more reproducible results, these results are recorded for future reference only; the results of the first method are used as the basis for discussion, because, as previously indicated, the measurement of these actually accompanied the observations of osmosis. The results obtained by both methods are recorded in Tables IV and V, and those obtained by the first method only are shown graphically in Figs. 6 and 7.

**Electroosmosis**

Electroosmosis was carried out with the same apparatus as used in the osmotic experiments with the substitution of platinum wire electrodes for the calomel electrodes. As has been pointed out, the arrangement is actually essentially an adaptation of a well-known electroosmotic apparatus. The platinum electrodes were connected through a milliammeter to storage batteries supplying twelve volts. Although the voltage dropped slightly at the highest concentrations, being about 11.75 with molar solutions, it may be considered to have been constant so far as the purposes of this study are concerned. Also with solutions eighth molar and above there was a rise in temperature of the solution on each side of the membrane of from 0.1 to 0.3 degrees; since this rise was equal on the two sides, it is presumed not to have affected the results.
**TABLE IV**

**POTENTIAL MEASURED IN VOLTS ACROSS A GELATIN-TREATED COLLOIDION MEMBRANE SEPARATING HCl SOLUTIONS OF DIFFERENT CONCENTRATIONS FROM WATER IN OSMOSIS EXPERIMENTS**

<table>
<thead>
<tr>
<th>Molarity</th>
<th>pH</th>
<th>$E_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/4096</td>
<td>3.32</td>
<td>.0174</td>
</tr>
<tr>
<td></td>
<td>3.66</td>
<td>.0171</td>
</tr>
<tr>
<td></td>
<td>3.80</td>
<td>.0112</td>
</tr>
<tr>
<td>1/1024</td>
<td>3.02</td>
<td>.0502</td>
</tr>
<tr>
<td></td>
<td>3.03*</td>
<td>.0437</td>
</tr>
<tr>
<td>1/256</td>
<td>2.78</td>
<td>.0398</td>
</tr>
<tr>
<td></td>
<td>2.46</td>
<td>.0585</td>
</tr>
<tr>
<td>1/64</td>
<td>2.20</td>
<td>.0680</td>
</tr>
<tr>
<td></td>
<td>1.88</td>
<td>.0593</td>
</tr>
<tr>
<td></td>
<td>1.85*</td>
<td>.0798</td>
</tr>
<tr>
<td>1/16</td>
<td>1.61</td>
<td>.1290</td>
</tr>
<tr>
<td></td>
<td>1.30</td>
<td>.0799</td>
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<td>1/8</td>
<td>0.99*</td>
<td>.1000</td>
</tr>
<tr>
<td>1/4</td>
<td>1.04</td>
<td>.0791</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
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<td>0.53</td>
<td>.1014</td>
</tr>
<tr>
<td>1</td>
<td>0.60</td>
<td>.0832</td>
</tr>
</tbody>
</table>

*Values obtained by special method*
### Table V

Potential measured in volts across a gelatin-treated colloidion membrane separating oxalic acid solutions of different concentrations from water in osmosis experiments.

<table>
<thead>
<tr>
<th>Molarity</th>
<th>pH</th>
<th>E_m</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/4096</td>
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<tr>
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<td>3.60</td>
<td>.0148</td>
</tr>
<tr>
<td>1/1024</td>
<td>3.01</td>
<td>.0446</td>
</tr>
<tr>
<td></td>
<td>3.01</td>
<td>.0374</td>
</tr>
<tr>
<td>1/256</td>
<td>2.47</td>
<td>.0532</td>
</tr>
<tr>
<td></td>
<td>2.46</td>
<td>.0522</td>
</tr>
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<td>1/64</td>
<td>1.93</td>
<td>.0668</td>
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<td>1.93</td>
<td>.0796</td>
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<tr>
<td>1/32</td>
<td>1.79</td>
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<td>1/16</td>
<td>1.47</td>
<td>.0831</td>
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<td>.0955</td>
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<td>.0925</td>
</tr>
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<td>1.07</td>
<td>.0590</td>
</tr>
<tr>
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<td>1.04</td>
<td>.0760</td>
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<tr>
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<td>.1048</td>
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<td>.0726</td>
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<tr>
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<td>.0922</td>
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<tr>
<td></td>
<td>0.63*</td>
<td>.1160</td>
</tr>
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</table>
Figure 6.

Log Molar Concentration of HCl

Figure 7.

Log Molar Concentration of H₂C₂O₄
As soon as the flow became constant in one direction and remained so the polarity was reversed, and observations made with it proceeding in the opposite direction. The rate tabulated for any solution in cm. per minute is the arithmetical mean of all observations in both tubes with both initial and reversed polarity; about ten observations were made with each solution. An analysis of the deviations clearly justified the use of the mean values which are listed in Table VI and shown graphically in Fig. 8.

For each solution, except in one case omitted due to an oversight, the electric current flowing was noted by means of the milliammeter, the value remaining essentially constant throughout any run with one solution. The results, which in a few cases were checked with a copper coulometer, are listed in Table VII and shown graphically in Fig. 9.
TABLE VI
RATE OF ELECTROOSMOSIS IN CM./MIN. SOLUTIONS
OF HCl AND H₂C₂O₄

<table>
<thead>
<tr>
<th>Molarity</th>
<th>H₂C₂O₄</th>
<th>Rate</th>
<th>Molarity</th>
<th>HCl</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/4096</td>
<td>3.56</td>
<td>-.010</td>
<td>M/4096</td>
<td>3.64</td>
<td>-.003</td>
</tr>
<tr>
<td>M/1024</td>
<td>3.01</td>
<td>-.010</td>
<td>M/1024</td>
<td>3.03</td>
<td>-.048</td>
</tr>
<tr>
<td>M/256</td>
<td>2.45</td>
<td>-.014</td>
<td>M/512</td>
<td>2.73</td>
<td>-.087</td>
</tr>
<tr>
<td>M/64</td>
<td>1.92</td>
<td>-.057</td>
<td>M/256</td>
<td>2.42</td>
<td>-.223</td>
</tr>
<tr>
<td>M/32</td>
<td>1.79</td>
<td>-.130</td>
<td>M/64</td>
<td>1.85</td>
<td>-.196</td>
</tr>
<tr>
<td>M/16</td>
<td>1.47</td>
<td>-.145</td>
<td>M/16</td>
<td>1.27</td>
<td>-.104</td>
</tr>
<tr>
<td>M/8</td>
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<td>-.100</td>
<td>M/8</td>
<td>0.99</td>
<td>-.116</td>
</tr>
<tr>
<td>M/4</td>
<td>1.04</td>
<td>-.105</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 M</td>
<td>0.63</td>
<td>-.023</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(The negative sign indicates the flow of liquid toward the positive electrode.)
### Table VII

Variation of Electric Current in Electroosmosis

With Concentration of Acid,

With Applied Potential and Temperature Constant

<table>
<thead>
<tr>
<th>Molarity</th>
<th>HCl</th>
<th>PH</th>
<th>Current in milliamps</th>
<th>H2C2O4</th>
<th>PH</th>
<th>Current in milliamps</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/4096</td>
<td></td>
<td>3.64</td>
<td>1.5</td>
<td></td>
<td>1/4096</td>
<td></td>
</tr>
<tr>
<td>1/1024</td>
<td></td>
<td>3.03</td>
<td>2.0</td>
<td></td>
<td>1/1024</td>
<td></td>
</tr>
<tr>
<td>1/512</td>
<td></td>
<td>2.73</td>
<td>3.0</td>
<td></td>
<td>1/512</td>
<td></td>
</tr>
<tr>
<td>1/256</td>
<td></td>
<td>2.42</td>
<td>4.0</td>
<td></td>
<td>1/256</td>
<td></td>
</tr>
<tr>
<td>1/64</td>
<td></td>
<td>1.85</td>
<td>13.0</td>
<td></td>
<td>1/64</td>
<td></td>
</tr>
<tr>
<td>1/32</td>
<td></td>
<td>1.27</td>
<td>52.0</td>
<td></td>
<td>1/32</td>
<td></td>
</tr>
<tr>
<td>1/16</td>
<td></td>
<td>1.27</td>
<td>52.0</td>
<td></td>
<td>1/16</td>
<td></td>
</tr>
<tr>
<td>1/8</td>
<td></td>
<td>0.99</td>
<td>112.0</td>
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<td>1/8</td>
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</tr>
<tr>
<td>1/4</td>
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<td>0.53</td>
<td>250.</td>
<td></td>
<td>1/4</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>0.63</td>
<td>190.</td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
FIGURE 9.
DISCUSSION OF THE EXPERIMENTAL RESULTS

Two theoretical expressions are of particular importance in the discussion of the experimental results, the equations respectively for the volume of flow in electro-osmosis and for an electrolytic solution boundary potential. The rate of electroosmotic flow is given by the expression

\[ V = \frac{qSXD}{4\pi\eta} \]  

(1)

in which \( V \) is the volume of flow in unit time; \( q \) is the sum of the cross sections of the pores of the membrane; \( D \) is the dielectric constant of the solution; \( S \) is the electrophoretic potential; \( X \) is the potential gradient across the membrane which is determined by the membrane potential \( (E_m) \); and \( \eta \) is the viscosity of the solution. In the experiments under discussion, \( q \) is assumed to be constant, because in successive experiments either the same membrane was employed or there was employed another membrane prepared in the same manner as the first; and \( D \) and \( \eta \) are assumed to be constant on the basis that, from the point of view of the present study, the variation of dielectric constant and viscosity of solutions of the electrolytes employed is negligible in the concentration range up to one molar.

Examination of the two dissociation constants of oxalic acid indicates that the concentration of the divalent anion
in solution is very small as compared with that of the monovalent anion. This being so, the expression for the liquid-liquid boundary potential between solutions of either oxalic or hydrochloric acids is

\[ p = \frac{(u-v)}{(u+v)} \times 0.058 \log \left( \frac{C_2}{C_1} \right) \]  

where \( p \) is the potential in volts; \( u \) and \( v \) are respectively the transference numbers of the cation and the anion, and \( C_2 \) and \( C_1 \) are the concentrations respectively of the more and less concentrated solutions.

Several of the experiments performed were for the primary purpose of checking results obtained previously by Loeb, and excellent agreement was obtained in six of the seven cases in which direct comparison was possible. Loeb's curves for the osmosis of oxalic and hydrochloric acids gave slight positive results in the range close to two hundred fifty-sixth molar; his curve for oxalic acid was not carried through its minimum negative value, but the one for hydrochloric acid, shown as curve IV in Fig. 1, gave a clearcut maximum with a fourth molar solution. In these experiments the initial level of the liquid in the measuring tube was at -20; hence the rise above this value shown by curve IV in Fig. 1 indicates positive osmosis. In the present work positive osmosis was also obtained with both acids in the concentration range close to two hundred fifty-sixth molar, and the position of the maximum negative flow with
hydrochloric acid agreed exactly with that obtained by Loeb. This latter agreement is brought out clearly by a comparison of Fig. 5 and curve IV of Fig. 1.

Loeb's curves showing the variation of the electrokinetic potential with concentration for gelatin-treated colloidion particles and solutions of hydrochloric acid and sodium hydroxide have maxima respectively in the regions close to five hundredth and ten thousandth molar; Fig. 3 shows that good agreement with these values was obtained. Loeb's concentration corresponding to the maximum electrokinetic potentials for untreated colloidion particles and solutions of sodium hydroxide, at close to one thousandth molar was also checked as is evident from Fig. 2; his corresponding value for solutions of hydrochloric acid, also at about thousandth molar, was not checked, the value obtained in the present work, as shown in Fig. 2, being closer to ten thousandth molar.

It is apparent that Loeb's negative osmosis with gelatin-treated colloidion membranes is in qualitative accord with the theory that the flow is spontaneous electroosmosis of the Helmholtz type; the solution side of the electrical double layer is in all cases negatively charged, and flow is to the water side of the membrane which is positively charged due to the high mobility of the hydrogen ion. Although not immediately connected with the problems under consideration, the fact is noted for future reference that
the positive flow in the concentration range close to five hundredth molar is not in accord with the theory and, at present, can only be explained on the basis of the assumption that normal positive osmosis is dominant in this range.

If the negative flow shown in Fig. 5 were completely in accord with the Helmholtz mechanism, and if the membrane potential did not vary with concentration, equation (1) indicates that maximum flow should occur at the concentration of maximum electrokinetic potential; a comparison of Fig. 5 with Fig. 3, however, shows clearly that the maximum negative flow in both cases studied occurred at a much higher concentration than that of maximum electrokinetic potential, at about sixteenth molar with solutions of oxalic acid and at about fourth molar with solutions of hydrochloric acid, in comparison with the value of about thousandth molar at which maximum electrokinetic potential was observed with solutions of both acids. Prior to the measurement of the membrane potentials, it had seemed possible that they would not vary with concentration. As indicated by equation (2) the potentials would be expected to depend upon the ratio of the concentrations on the two sides of the membrane; Loeb's finding that flow of solute to the water side was directly proportional to the initial concentration in the solution side indicates that the ratio might well not vary with concentration. Fig. 6 shows, however, that instead of remaining constant, the membrane potential increases steadily
with concentration up to about sixteenth molar, with some indication of approaching a constant maximum value at higher concentrations.

If, when the faster moving ion bears the same sign of charge as the fixed side of the electrical double layer, a membrane is placed at the junction of solution and solvent, the results of Michaelis and his associates (68) indicate that the boundary potential is lowered. These conditions are met in both of the cases studied. The reason for the increase in membrane potential with concentration is not known, and, so far as can be determined, has not been investigated. It may be that it is due to the fact that with increase in concentration of the acids in the pores of the membrane the boundary potential approaches more and more closely to the value which would obtain with no membrane present.

The fact that the increase in membrane potential with concentration continues well past the point of maximum electrokinetic potential pointed clearly to the fact that, with increasing concentration, the product of the electrokinetic potential and the membrane potential must pass through a maximum value, and to the possibility that this maximum value would correspond to the concentration of maximum negative osmotic flow; such a correspondence would indicate that the flow was entirely in accord with the Helmholtz mechanism. It would be, in fact, entirely analogous to the correspondence previously described upon which Loeb
based his demonstration of the quantitative correspondence
between the Helmholtz mechanism and anomalous osmosis with
untreated collodion membranes and salt solutions. The
values for the products of the relative electrophoretic mo-
ibilities and the membrane potentials were accordingly cal-
culated with results which are listed in Table VIII and
which are plotted against the respective concentrations in
Fig. 10. The values for the relative electrophoretic mo-
ibilities used in calculating these products were those for
solutions the concentrations of which were one half those of
the solutions employed; because the concentration of the so-
lution in the pores of a membrane is not definitely known,
and is best assumed to be the mean of those on the two sides
-- that is, initially half the concentration of the solution
employed.

The curves in Fig. 10 are strikingly similar to the
osmotic curves shown in Fig. 5, but differ from them in two
particulars: they are less steep, and the minima occur at
somewhat lower concentrations. It seems possible that the
greater steepness of the osmotic curves may be due to the
positive osmosis which accompanies the anomalous flow. The
minima in the osmotic curves, which correspond to maximum
negative osmotic flow, occur with solutions of oxalic and
hydrochloric acids respectively at sixteenth and fourth
molar, while the corresponding curves in Fig. 10 have minima
respectively at concentrations of thirty-second and
FIGURE 10.

Product of $E_{M}$

Log Molar Concentration

HCl
H$_2$C$_2$O$_4$
TABLE VIII
THE PRODUCT OF MEMBRANE POTENTIAL AND RELATIVE ELECTRO-
PHORETIC VELOCITY OF GELATIN-TREATED COLLODION
IN SOLUTIONS OF HCl AND H₂C₂O₄ *

<table>
<thead>
<tr>
<th>Molarity</th>
<th>-log M</th>
<th>Eₘ M</th>
<th>Rate</th>
<th>Product</th>
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<tr>
<td>1/4096</td>
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<td>.015</td>
<td>.069</td>
<td>.00103</td>
</tr>
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<td>1/1024</td>
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<td>.032</td>
<td>.087</td>
<td>.00282</td>
</tr>
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<td>1/256</td>
<td>2.408</td>
<td>.049</td>
<td>.090</td>
<td>.00441</td>
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<tr>
<td>1/64</td>
<td>1.806</td>
<td>.064</td>
<td>.074</td>
<td>.00475</td>
</tr>
<tr>
<td>1/16</td>
<td>1.204</td>
<td>.076</td>
<td>.051</td>
<td>.00388</td>
</tr>
<tr>
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<td>.082</td>
<td>.030</td>
<td>.00246</td>
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<tr>
<td>1</td>
<td>0.000</td>
<td>.083</td>
<td>.014</td>
<td>.00116</td>
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</table>

<table>
<thead>
<tr>
<th>H₂C₂O₄</th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
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<td>.014</td>
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</tr>
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<td>0.000</td>
<td>.081</td>
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<td>.00148</td>
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</table>

*Assuming the concentration in the pores to be half the molecular concentration. (Values are taken from the curves of Figs. 3, 6, and 7.)
sixty-fourth molar. The fact that equation (1) predicts that maximum anomalous flow should occur at the concentration at which the product of the electrokinetic potential and the membrane potential is a maximum, and that experimentally the concentrations of maximum product and maximum flow are no farther apart than sixteenth and thirty-second molar, definitely points to the conclusion that the flow in this case is primarily spontaneous electroosmosis of the Helmholtz type.

The difference between the concentrations at which the two minima occur in osmotic and product curves for solutions of hydrochloric acid, respectively fourth molar and sixty-fourth molar, is sufficient to indicate that some important influence in addition to spontaneous electroosmosis of the Helmholtz type may be operative in the anomalous osmosis of these solutions. Nevertheless, the general similarity in the two curves is taken to indicate that spontaneous electroosmosis of the Helmholtz type is of fundamental importance. The indication of the fundamental importance of the Helmholtz mechanism in the anomalous osmosis with gelatin-treated collodion membranes and solutions of oxalic and hydrochloric acids provides the added indication that for the explanation of anomalous osmosis with this type of membrane, and, in fact, with protein membranes in general, no special imbibition mechanism need be invoked.

The next step in the experimental procedure will be to
examine the considerable discrepancy between the locations of the minima of the two curves with hydrochloric acid and the smaller discrepancy between the locations of the minima of the two curves with oxalic acid, to see whether they can be explained in terms of volume changes resulting from ordinary electrolytic transport of ions which occurs in response to the membrane potential; in other words, to the Remy effect as applied to spontaneous electroosmosis. Time available prior to writing this report did not permit the inclusion of experiments pointed toward the solution of this phase of the problem.

Direct comparison between maxima and minima obtained with the same membrane and the same series of solutions in anomalous osmosis and electroosmosis appears to be unsafe; there is considerable uncertainty in the matter of evaluating the actual potentials which are effective in the electroosmosis, due to the possibility of polarization phenomena and variation with concentration of the resistance of the membrane. Thus it can not be assumed that the effective difference in potential acting between the sides of the membrane is constant merely because the potential applied at the platinum electrodes was constant. It would appear on the basis of the Helmholtz mechanism that, if it had been constant, maximum electroosmotic flow would have occurred with solutions of each acid at a concentration of about thousandth molar, this being the concentration of maximum
electrokinetic potential in each case. It therefore appears that either the effective potential varies or that some other mechanism is active in the electroosmosis, presumably the Remy mechanism.

The minimum in the electroosmotic curve for oxalic acid requires further consideration, because it not only resembles the osmotic curve in shape, but occurs at closely the same concentration. The immediate impulse is to conclude that the identical factors are operative; but on the basis of the theoretical considerations which have been advanced the point of view must be taken that the coincidence of these minima is very possibly fortuitous.

The curves in Fig. 9, each showing a sharp upswing of current at a concentration of about thirty-second molar, afford a strong indication that Remy effects are important in the observed electroosmosis; because the greater the current the greater the rate of transport of ions through the membrane.
SUMMARY AND CONCLUSIONS

A plan has been proposed for the experimental investigation of the mechanism of the flow in negative osmosis with protein membranes, and considerable progress has been made in the experimental work. Although the determination of the mechanism in this special type of anomalous osmosis is important in itself, the more general purposes of the study were to determine whether the volume changes manifested in this type include effects due to the ordinary electrolytic transport of ions through the membrane, as shown to enter in certain cases of electroosmosis by Romy (70) and his associates; and whether any role is played by imbibition per se, as was originally suggested by Flusin to be the case with protein membranes and later supported by Sölner (71).

The experimental plan was to choose certain cases of negative osmosis with gelatin-treated collodion membranes; and after checking the osmotic results obtained by Loeb with (73) these cases, to study the electrokinetic and membrane potentials involved, and the electroosmosis with the same membranes and solutions as employed in the osmotic experiments. The data would then be in hand for determining the extent to which spontaneous electroosmosis of the Helmholtz type was operative. If the flows in the cases chosen were
found not to be completely describable in terms of the Helmholtz mechanism, the divergence from the behavior predicted by the Helmholtz theory would be examined to determine whether it, in turn, could be explained in terms of the Remy mechanism, or of imbibition, or of some combination of these two.

The electrophoretic determinations and the determinations of the membrane potentials and of electroosmosis have been completed with solutions of hydrochloric acid and oxalic acid. The results indicate that the Helmholtz mechanism plays a dominant role in both cases. There is also the indication that there is some residual effect; and it is believed that this will be accounted for in a continuation of the present work in terms of the Remy mechanism. The apparent dominant role of the Helmholtz mechanism appears to render invalid the point of view supported by Söllner that the mechanism of anomalous osmosis with gelatin-treated collodion membranes, and with protein membranes in general, must be considered apart from that with "non-swelling" membranes.

At the time of writing, the work reported is considered to lead to indications rather than to the establishment of facts; with complicated phenomena like those under consideration incomplete explanations are usually best considered to be tentative. Progress has been made in the matter obtaining
experimental results, and techniques have been developed with which it is planned to carry the study to conclusion.
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(47) The cell with accessory apparatus may be obtained from the Arthur H. Thomas Co., Philadelphia.


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