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ELECTRIFICATION OF WATER AND OSMOTIC FLOW¹

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THE exchange of water and solutes between the cell and the surrounding fluid is one of the important factors in the mechanism of life, and a complete theory of the osmotic flow is therefore a postulate of biology. It was a marked advance when the experiments of Pfeffer and de Vries led van't Hoff to the formulation of the modern theory of osmotic pressure. According to this theory the molecules of the solute behave like the molecules of a gas in the same volume and at the same temperature, and the gas pressure of the solute measures the "attraction" of a watery solution for pure water through a strictly semipermeable membrane. Yet it is obvious to-day that in a liquid the electrical forces between solvent and solute must play a rôle and no adequate provision is made for these forces in van't Hoff's law. Traube rejected van't Hoff's theory altogether, suggesting instead that the osmotic flow was from the liquid with lower to the liquid with higher surface tension (and higher intrinsic pressure).

Tinker has shown that van't Hoff's theory for osmosis holds strictly only in the case of *ideal* solutions, *i.e.*, when the process of solution occurs without heat of dilution and change in volume, but that in the case of *non-ideal* solutions Traube's ideas explain the deviations from the gas law which are bound to occur. When two different *ideal* solutions containing equal numbers of particles of solute in equal volume are separated by a strictly semipermeable membrane, equal numbers of molecules of water will diffuse simul-

¹ Presidential address prepared for the Chicago meeting of the American Society of Naturalists, December 30, 1920.

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taneously in opposite directions through the membrane and no change in volume will occur. When, however, the same experiment is made with two non-ideal solutions containing equal numbers of molecules in equal volume, the result is different. As Tinker has demonstrated mathematically, in this case the flow of water must be from the solution having the lower intrinsic pressure and lower surface tension to the solution with higher intrinsic pressure and higher surface tension. This is what Traube claims, and his theory explains therefore, as Tinker points out, the deviations from the gas law in the case of non-ideal solutions, but it does not prove that the gas law of osmotic flow does not hold in the case of *ideal* solutions and Traube's theory can not therefore replace van't Hoff's theory.

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There is a second group of forces not taken into consideration in van't Hoff's law, namely the influence of the chemical nature of the membrane on the solvent. These forces become noticeable when the membrane separating the solution from the pure solvent is not strictly semipermeable. When water is in contact with a membrane it undergoes as a rule an electrification and this electrification of the particles of water plays a great rôle in the rate of the osmotic flow when the solution into which the water diffuses is an electrolyte.

diffusing The assumption that water through a membrane is as a rule, electrified, is justified by a large number of observations. Quincke demonstrated that when water is pressed through capillary tubes it is found to be electrically charged (the sign of charge being more frequently positive); while the tube has the opposite sign of charge, e.g., negative, when the water is positively charged. When two solutions of weak electrolytes are separated by a membrane (which may be considered as a system of irregular capillary tubes) an electric current causes water to migrate to one of the two poles, according to the sign of its charge. By this method of so-called electrical endosmose it can be shown

that water diffuses through collodion membranes in the form of positively charged particles. Collodion bags, cast in the shape of Erlenmeyer flasks, are filled with a weak and neutral solution of an electrolyte, e.g., M/256 Na_oSO_o and dipped into a beaker filled with the same solution of M/256 $Na_{a}SO_{4}$. The opening of the collodion bag is closed with a rubber stopper perforated by a glass tube serving as a manometer. When a platinum wire, forming the negative electrode of a constant current, is put through the glass tube into the collodion bag while the other pole of the battery dips into the outside solution, the liquid in the glass tube rises rapidly with the potential gradient between the two electrodes. The water therefore migrates through the collodion membrane in the form of positively charged particles. The writer has made a number of experiments² concerning the osmotic flow through collodion membranes, and it is the purpose of this address to give a brief survey of the results.

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When a collodion bag is filled with a solution of a crystalloid, e.g., sugar or salt, and dipped into a beaker containing pure water. the pure water will diffuse into the solution and the level of liquid in the capillary glass tube serving as a manometer will rise. At the same time particles of the solute will diffuse out of the bag (except when the solute is a protein solution or a solution of some other colloid). The concentration of a crystalloid solute inside the collodion bag will therefore become constantly smaller until finally the solution is identical on both sides of the membrane. Nevertheless the relative force with which a given solution inside the collodion bag "attracts" the pure water into which the bag is dipped can be measured by the initial rise in the level of water in the manometer, before the concentration of the solution has had time to diminish to any great extent through diffusion. Since in the

² Loeb, J., J. Gen. Physiol., 1918–19, I., 717; 1919–20, II., 87, 173, 273, 387, 563, 659, 673. first minutes accidental irregularities are liable to interfere with the result, we measure the rise in the level of liquid in the manometer during the first 20 minutes.

If the initial rise of level of liquid in the solution is thus measured it is noticed that it occurs approximately in proportion with the concentration of the solution when the solute is a non-electrolyte. The rate of diffusion of pure water into a solution of cane sugar through a collodion membrane is therefore approximately a linear function of the concentration of the solute within the limits of O and 1 M. This is what we should expect on the basis of van't Hoff's theory of osmotic pressure.

If, however, a watery solution of an electrolyte is separated from pure water by a collodion membrane, water diffuses into these solutions as if its particles were positively charged, and as if they were attracted by the anion of the electrolyte in solution and repelled by the cation with a force increasing with the valency of the ion (and another property of the ion to be discussed later).

Pure water diffuses into a M/128 solution of NaCl through a collodion membrane more rapidly than it diffuses into a M/64 solution of cane sugar; water diffuses into a M/192solution of Na₂SO₄ or Na₂ oxalate still more rapidly than into a M/128 solution of NaCl; and into a M/256 solution of Na₂ citrate water diffuses more rapidly than into a M/192solution of Na_2SO_4 , and into a M/320 solution of $Na_4Fe(CN)_6$ still more rapidly than into a M/256 solution of Na₂ citrate. Assuming complete electrolytic dissociation of the electrolytes in these cases, the influence of the five solutions mentioned should be identical according to van't Hoff's theory. We notice, instead, that the "attraction" of the solutions for water increases with the valency of the anion. This is true for all neutral solutions of salts contained in a collodion bag, regardless of the nature of the cation.

If a collodion bag containing a neutral solution of a salt with bivalent cation, e.g., M/192 CaCl₂ or MgCl₂, or with a trivalent

cation, eg., M/256 LaCl₃, is dipped into a beaker with pure water we notice no rise in the level of water in the manometer. In solutions with bivalent or trivalent cations the repulsion of the cation equals or exceeds therefore the attraction of the anion for the positively charged particles of water diffusing through the pores of the collodion membrane. Hence we conclude from these (and numerous similar) experiments that the particles of water diffuse through a collodion membrane as if they were positively charged and as if they were attracted by the anion of an electrolyte and repelled by the cation with a force increasing with the valency of the ion.

It seemed of interest to find that concentration of a cane sugar solution which just suffices to prevent the diffusion of water into a given solution of an electrolyte. Into each of a series of beakers, all containing the same neutral salt solution, e.g., M/192 Na₂SO₄, was dipped a collodion bag containing a cane sugar solution of different concentration, from M/128 to 1 M, and it was observed in which of these sugar solutions the level in the manometer rose during the first 10 minutes, in which it fell, and in which it remained constant. It was found that the cane sugar solution which was just able to balance the

TABLE I

Approximate Concentration of a Solution of Cane Sugar Required to Balance the Osmotic Attraction of the Following Solutions of Electrolytes for Water

Molecular Concen- tration	Electrolyte Used	Approximat tration of of	e Molecular Balancing S Cane Sugai	Concen- Soluti on
M/128	.KCl	•		M /8
M/192	.K ₂ SO ₄	. Between	M/4 and	M/2
M/192	.K ₂ oxalate	•		M/2
M/192	.K ₂ tartrate	. Slightly	above	M/2
M/256	.K ₃ citrate	. Slightly	above	3M/4
M/128	.RbCl			M/4
M/128	.KCl	•		M/8
M/128	.NaCl	•		M/8
M /128	.LiCl	. Slightly	above	M/32
M/192	.MgCl ₂			M/64
M/192	.CaCl ₂	. Below		M/64
M/192	.SrCl ₂	. Below		M/64
M/192	.BaCl ₂	•	· •••••••••	M /64
M/192	. CoCl ₂	. Below		M/64
M/192	.MnOl,	. Below		M/64

"attraction" of the M/192 solution of Na₂SO₄ for water had to have a concentration of about or over M/4. If the gas pressure effect alone determined the relative attraction of the two solutions for water the concentration of the sugar solutions required to osmotically balance the M/192 solution of Na_2SO_4 should have been M/64 (or slightly less). Hence the sugar solution balancing osmotically a M/192 Na₂SO₄ solution was found to be 16 times more concentrated than the theory of van't Hoff demands. This high concentration of cane sugar was needed to overcome the powerful "attractive" influence of the anions of a M/192 solution of Na₂SO₄ for the positively charged particles of water. Table I. shows the results of a few such ex-The solution of the electrolyte periments. was in these experiments always theoretically isosmotic with a M/64 cane sugar solution (on the assumption of complete electrolytic dissociation). The data contained in Table I. have only a qualitative value since no attempt at an exact determination of the concentration of the balancing sugar solutions was made. The data show, however, that the "attraction" of M/128 KCl for positively charged particles of water is eight times as great, that of K₂SO₄ sixteen times as great, and that of M/256 K_s citrate almost forty-eight times as great as that of M/64 cane sugar; while the "attraction" of a M/192 solution of a salt with a bivalent cation and monovalent anion, like MgCl_o, for water is not greater than that of a M/64 solution of cane sugar.

These experiments then prove that the rate of diffusion of water from the side of pure water through a collodion membrane into a solution of an electrolyte increases with the valency of the anion and diminishes with the valency of the cation. They give also a rough idea of the relative influence of these ions upon the rate of diffusion of positively charged water through the pores of the collodion membrane from the side of pure water to the side of the solution.

A second fact brought out in these experiments was that the relative influence of the oppositely charged ions of an electrolyte in solution upon the rate of diffusion of positively charged water from the side of pure water to the side of the solution is not the same in all concentrations. Beginning with the lowest concentrations the "attractive" effect of the anion for positively charged water increases more rapidly with increasing concentration than the "repulsive" effect of the cation until the concentration of the electrolyte is about M/256; from then on the "repulsion" of the cation upon positively charged water increases more rapidly than the "attractive" effect of the anion. As a consequence we can say that in concentrations of neutral salts between M/256 and M/8 the "attraction" of the solution for water diminishes with increasing concentration. This is the reverse of what we should expect if the gas law alone determined the attraction of water by solutions of electrolytes. When the concentration of the solution is M/8, the apparent electrostatic effects of the ions upon the positively charged particles of water disappear and for concentrations above M/8 the curves for the attraction of water by electrolytes and by sugar solutions show less difference.

We have already mentioned the fact that the valency of the ion is not the only quantity which determines its influence on the rate of diffusion of water through a collodion membrane. In addition to the valency (or the number of electrical charges) a second quantity of the ion enters which may be designated provisionally as the influence of the radius of the ion. In the case of monovalent and monatomic cations the retarding influence on the rate of diffusion of positively charged particles of water through the collodion membrane from the side of pure water into a solution increases inversely with the radius of the ion, namely in the order Li > Na > K > Rb, where the retarding effect is greatest in the case of Li and least in the case of Rb; while in the case of monatomic monovalent anions the accelerating effect upon the rate of diffusion of positively charged particles of water from the side of pure water through the membrane into the solution increases directly with the radius of the ion I > Br > Cl; where I has the greatest and Cl the smallest attractive action.

This might be intelligible if the action of the ions on the particles of water were electrostatic, since in this case the action of the anion depends on the negative charge in its outermost shell of electrons and the electrostatic effect should be the greater the farther the shell is removed from the positive nucleus of the ion; while the electrostatic effect of the cation is due to the positive charge of the nucleus and this should be the greater the smaller the distance between nucleus and the outermost layer of electrons, *i.e.*, the closer the positive nucleus can approach the water particles or the membrane particles on which the ion is to act.

IV

We have alluded to the fact that collodion membranes are not strictly semipermeable and that crystalline solutes diffuse out from the collodion flasks in our experiments. It might be argued that the differences in the flow of water measured in the preceding chapter are due to differences in the rate of diffusion of electrolytes from the side of the solution to the side of pure water through the collodion membrane. This assumption is, however, not tenable since it can be shown that the diffusion of the solutes into the pure water through the collodion membrane seems to follow Fick's diffusion law according to which the rate of diffusion of a solute is directly proportional to its concentration and this seems to hold equally in the case of electrolytes and non-electrolytes. The specific influence of solutions of *electrolytes* on the rate of diffusion of water from pure water through collodion membranes into solutions can therefore not be due to any difference in the rate of diffusion of electrolytes and nonelectrolytes through the membrane into the pure water, but must be ascribed to a difference in the behavior of water towards these two types of solutes.

v

We have thus far mentioned only the influence of electrolytes on the rate of diffusion of positively charged particles of water. Perrin found in his experiments on electrical endosmose that in certain cases the water migrated to the positive electrode, namely when the solution had an acid reaction, while it migrated to the negative electrode when the solution had an alkaline reaction. No such reversal in the sign of electrification of water can be produced in the case of pure collodion membranes, since in this case the water is always positively charged no matter whether the solution is acid, neutral, or When, however, we deposit a film alkaline. of a protein on the inside (or on both sides) of the collodion membrane the latter becomes amphoteric. When the solution is sufficiently acid, the water migrates through the membrane as if its particles were negatively charged, while when the hydrogen ion concentration is lower, *i.e.*, when the solution is only very faintly acid or neutral or alkaline, the water particles move through the protein film of the membrane as if they were positively charged.

When we separate an acid solution of a salt by a collodion membrane possessing a protein film, from a solution of a pure acid of the same hydrogen ion concentration as that of the salt solution, the hydrogen ion concentration being equal to or above 10^{-4} N, the water migrates through the pores of the membrane as if its particles were negatively charged and as if they were "attracted" by the cation and "repelled" by the anion of the electrolyte in solution with a force increasing with the valency of the ion. In this case, water is "attracted" more powerfully by salts with trivalent cation, e.g., AlCl₃ or LaCl₃, than by salts with bivalent cation e.g., MgCl₂ or CaCl₂; and it is "attracted" more powerfully by the latter than by salts with monovalent cation, e.g., NaCl or KCl; while negatively charged water is not "attracted" by salts with bivalent or trivalent anions, e.g., Na_2SO_4 or Na^4 oxalate or $Na_4Fe(CN)_6$, etc.

In the case of salts with monatomic and monovalent cations the "attraction of" the salt for negatively charged water seems to increase inversely with the radius of the cation in the order Li > Na > K > Rb, where Li with the smallest radius "attracts" the negatively charged water most and Rb with the largest radius "attracts" the water least. The monatomic monovalent anions "repel" the negatively charged particles of water directly in proportion with the radius of the ion in the order I > Br > Cl, where I with the greatest radius "repels" the negatively charged water most, and Cl least.

The relative "attractive" and "repelling" action of the two oppositely charged ions of an electrolyte for negatively charged water is not the same in all concentrations. In the lowest concentrations the attractive influence of the cation for negatively charged water increases more rapidly with increasing concentration than does the repelling action of the anion; while beyond a certain concentration the repelling action of the anion on the negatively charged water increases more rapidly than the attractive action of the cation. Finally a concentration is reached where the electrical effects of the two oppositely charged ions balance each other more or less and from then on the solution behaves more like that of a non-electrolyte.

VI

In the course of these experiments facts were observed which indicate a chemical source for the electrification of water when in contact with a collodion membrane. We have mentioned the fact that when a membrane has been treated with a protein, the sign of the electrification of water in contact with the membrane can be reversed by acid. The protein forms a fine film on the surface and probably inside the pores of the collodion membrane. In an alkaline or neutral, and often even a very faintly acid concentration the water in contact with the protein film is positively charged, but when the hydrogen ion concentration exceeds a certain limit the water assumes a negative charge. The writer has measured the hydrogen ion concentration at which this reversal occurs and has found that it changes in a characteristic way with a certain chemical constant of the protein which constitutes the film, namely its isoelectric point. Proteins are amphoteric electrolytes which behave differently on the two sides of a hydrogen ion concentration which is termed the isoelectric point. On the alkaline side from the isoelectric point proteins behave like a fatty acid, e.g., CH, COOH, forming metal proteinates with alkalies, e.g., Na proteinate. On the acid side of the isoelectric point the proteins behave like NH₃, forming protein-acid salts, e.g., protein We may imagine that proteins chloride. exist in the form of two isomers, one on the alkaline side of the isoelectric point possessing COOH as the active chemical group; the other on the acid side of the isoelectric point possessing NH₂ as the chemically active group. The isoelectric point, i.e., the hydrogen ion concentration at which the reversal of one type of protein salt to the other occurs, is a characteristic constitutional property of each protein. Its value is, according to L. Michaelis, a hydrogen ion concentration of $10^{-4.7}N$ for gelatin and for casein, $10^{-4.8}N$ for crystalline egg albumin, and $10^{-6.8}N$ for oxyhemoglobin.

The writer has been able to show that the reversal of the sign of charge of water when in contact with a collodion membrane possessing a protein film practically coincides with the isoelectric point of the protein used. lying slightly on the acid side of this point. The method of determining the hydrogen ion concentration at which the reversal in the sign of electrification of water occurs is as follows: We have shown that M/64 CaCl, or M/256 LaCl_a "attract" negatively charged water powerfully, while these two salts do not "attract" positively charged water. On the other hand, Na_2SO_4 "attracts" positively charged water powerfully while it does not "attract" negatively charged water. We fill a series of collodion bags previously treated with a protein each with a M/64 CaCl, solution, and dip each collodion bag into a beaker with distilled water. The M/64 CaCl, solution in each bag is brought to a different hydrogen ion concentration by adding suitable quantities of HNO_3 or NaOH to the

solution; and the distilled water in the beaker is always brought to the same hydrogen ion concentration as that of the M/64 CaCl₂ solution inside the collodion bag dipped into the beaker. Similar experiments are made with Na₂SO₄ brought to a different hydrogen ion concentration. The result of these experiments is striking. There is always one definite hydrogen ion concentration at which the "attraction" of both M/64 CaCl₂ (or $LaCl_2$) as well as that of M/256 Na_2SO_4 for water is almost zero. As soon as the hydrogen ion concentration rises, the attraction of M/64 CaCl₂ for water becomes noticeable and increases with a further increase in the hydrogen ion concentration until it reaches a maximum (at a hydrogen ion concentration of about 10⁻³N). The attraction of M/256 $Na_{a}SO_{4}$ for water rises when the hydrogen ion concentration falls below the point where the attraction is zero. M/256 Na₂SO₄ attracts water when it is positively charged and M/64 CaCl_o does so when water is negatively charged. Where neither solution "attracts" water the latter is not electrified. (It should be mentioned that the attraction of a cane sugar solution of M/64 or below for water is very slight and scarcely noticeable, and that this is the reason that when water is not electrified it is not noticeably attracted by M/64 $CaCl_2$ or M/256 Na₂SO₄.) Table II. shows the close relation of this hydrogen ion concentration and that of the isoelectric point for different proteins. Water begins to become negatively charged in contact with a collodion membrane as soon as the hydrogen ion concentration is slightly on the acid side of the

TABLE II

Nature of Protein Film on the Membrane	Hydrogen Ion Concentration where Water is Un- charged	Isoelectric Point of Protein
Gelatin	Between $10^{-4.0}$ and $10^{-4.6}N$	10 ^{-4.7} N
Casein		$10^{-4.7}N$
Egg albumin	** ** ** **	$10^{-4.8}N$
Oxyhemoglobin	About 10 ^{-6.0} and 10 ^{-7.0} N	$10^{-6.8}N$

isoelectric point of the protein forming a film on the membrane.

The quantitative agreement between the isoelectric point of the protein forming the

film on a collodion membrane and the point of reversal of the sign of electrification of water is such that it is difficult to question the connection between the chemical constitution of the protein and the sign of electrification of water. It is also obvious that the density of the charge varies with the hydrogen ion concentration.

When the collodion membrane is not treated with a protein the water is always positively charged and no reversal in the sign of the charge can be obtained by an increase in the hydrogen ion concentration. This harmonizes with the fact that collodion is not an amphoteric electrolyte.

It is to be expected that in addition to the chemical nature of the membrane the chemical nature of the liquid in contact with the water also influences the sign (and density) of the electrical charge at the boundary of the two phases. Indications supporting this view exist but they can not be discussed in this connection.

VII

van't Hoff's theory of osmotic pressure confronted the physiologists with the puzzling fact that in the phenomena of secretion water diffused often from places of higher to those of lower osmotic pressure. In 1908 Girard suggested that such cases of abnormal osmosis as occur in organisms might be explained on the assumption that the opposite sides of a membrane separating pure water from an acid or alkaline solution are oppositely charged, and that therefore Perrin's experiments on electrical endosmose furnish the explanation of these phenomena. According to Girard, only H or OH ions should produce such a difference in charge and neutral solutions of electrolytes should behave like solutions of non-electrolytes which is, however, not correct. Bernstein, in 1910, also reached the conclusion that electrical endosmose might be utilized for the explanation of abnormal osmosis as manifested in secretion and in his book on "Electro-Biology" many speculations in this direction are offered but unfortunately very few experiments. He also assumes that the opposite sides of the membrane of a gland are oppositely charged. Under such circumstances positively charged water particles will be driven in the direction from the positive to the negative side of the membrane. As soon as the positively charged water particle reaches the negative side of the membrane it gives off its charge. This enables other positively charged water particles to follow.

Ideas similar to those offered by Girard and by Bernstein have been expressed by way of explanation of other cases of abnormal osmosis by Bartell and his collaborators, and by Freundlich.

Whatever the ultimate theory of the driving force in these cases may be, we have a right to state that the electrification of the particles of water migrating through a membrane is a fact; that the sign of this electrification seems to depend on the chemical nature of the membrane in contact with water; that the rate of migration of these charged particles of water through the membrane from the side of pure water to the side of the solution is accelerated by the ions of the opposite sign of charge and retarded by the ions with the same sign of charge as that of the water with a force increasing with the valency of the ion; and that the relative acceleration and retarding effects of the two oppositely charged ions on the rate of diffusion of electrified water are not the same for all concentrations, that in lower concentrations of electrolytes the accelerating action of the oppositely charged ion increases at first more rapidly than the retarding effect of the other ion; while for higher concentrations the reverse is the case, until finally a concentration of the electrolyte is reached where the effects of the oppositely charged ions more nearly balance each other.

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HENRY ANDREWS BUMSTEAD

My personal acquaintance with Henry A. Bumstead dates from a meeting of the British

Association in Winnipeg in the summer of 1909. He had studied in Cambridge, England, where his engaging personality, keen intelligence, and unusual *savoir faire* had made him a place in the hearts and homes of English scientists which has been held by few Americans. I was then almost unknown both to him and to them, but I soon learned that if Bumstead was in any gathering I should at once feel at home.

I was walking with him one day through one of the busy streets of Winnipeg when he asked if I would not step into a shop with him while he bought a little memento for Mrs. Bumstead, a "bad habit" which he said he had formed on trips away from home.

I mention these two trivial incidents because they reveal the soul and heart of the man; and what, after all, is either science or art in comparison?

When in 1917 the important and difficult post of scientific attaché in London was created, Bumstead was the only man considered, for no scientist in this country had his tact, his judgment, his knowledge of England, and his ability to assist in bringing about what was then, and what is now, the most important need of the modern world, namely, the cooperation and mutual understanding of the two great branches of the Anglo-Saxon race.

Bumstead's success in London was extraordinary. The British liked and trusted him. Admiral Sims and our own War Department placed large responsibilities upon him, and his office became the center of a very active and Young American very important service. officers who went abroad on scientific missions found him the center of their contacts and the prime source of their usefulness. They all became his devoted admirers. Not one or two but a dozen or more of both British and American officers who came to Washington during the war told me that they owed their success in their work in England and the continent primarily to Bumstead, and counted it the most valuable part of their experience that they had had an opportunity to become acquainted with him. One of these officers de-