A CONTRIBUTION TO THE PHYSICAL ANALYSIS OF THE PHENOMENA OF ABSORPTION OF LIQUIDS BY ANIMAL TISSUES, VOL. X, PP. 105-134

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RALPH W. WEBSTER

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A CONTRIBUTION TO THE PHYSICAL ANALYSIS OF THE PHENOMENA OF ABSORPTION OF LIQUIDS BY ANIMAL TISSUES

BY

RALPH W. WEBSTER

ASSISTANT IN PHYSIOLOGICAL CHEMISTRY

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A CONTRIBUTION TO THE PHYSICAL ANALYSIS OF THE PHENOMENA OF ABSORPTION OF LIQUIDS BY ANIMAL TISSUES

RALPH W. WEBSTER

I. INTRODUCTION

THE following paper is, as the title indicates, intended to be a contribution to the physical analysis of phenomena of absorption by living tissues. That the older experiments on absorption could not lead to any satisfactory explanation of the processes involved seems evident from the fact that only recently has there been discovered one of the most fundamental theories concerning the exchange of liquids separated by membranes, more or less semi-permeable. Only such papers can be expected to throw a light on this subject as take cognizance of this theory of osmotic pressure.

Van't Hoff, applying certain facts brought out by Traube and Pfeffer regarding the influence of semi-permeable membranes upon processes of osmosis, showed that substances in solution obey the ordinary laws of gases, as brought forth by Boyle, Henry, Gay-Lussac, and Avogadro. In consequence of this similarity between gases and substances in solution, the latter will exert a pressure upon the walls of a containing vessel equal to the pressure which the dissolved substance would exert were it present in the gaseous form under the same conditions of temperature and molecular aggregation. Whether this pressure, which van't Hoff calls "osmotic pressure," be due to the impacts of the dissolved particles against the walls of the containing vessel, as the kinetic theory of gases would demand, or whether it be an expression of the attraction of the dissolved particles for water, concerns us, in these experiments, only in so far as our work has to do with the dynamics of the process of absorption.

From these facts it is evident, as van't Hoff shows, that the pressure of a substance in solution depends both upon the concentration of the substance and upon the temperature at which the observation is made. By the concentration we mean, not the number of molecules, as such, contained in a definite amount of the solvent, but, rather the total number of "active" particles contained in a definite (usually 1 liter) amount of solvent. This fact was made clear by the endeavor to collate the pressures of various salt solutions with those of organic substances, or, in other words, of electrolytes with those of non-electrolytes. Clausius had shown that the molecules of substances conducting electricity, viz., of electrolytes, are dissociated into ions, which have a movement independent of one another. Arrhenius, in his work on *Dissociation of Substances Dissolved in Water*, advanced the hypothesis that the molecules of substances in solution suffer a dissociation into their electrically-charged ions (an ion being considered as an atom, or group of atoms, carrying an electric charge + or −, according

to the electric nature of the element). This conception of Arrhenius gave the new idea that the dissociation was due to the mere dissolving of the electrolyte in the water, and not, as Clausius had claimed, to the action of the electric current. This electrolytic dissociation of substances in watery solution is by no means complete at every concentration, but increases with the dilution until, at infinity dilution, a complete dissociation of the molecules into their respective ions takes place. We have, therefore, in a watery solution of an electrolyte two kinds of molecules — the active (electrically dissociated), and the inactive (non-dissociated). Inasmuch as the ions of the dissociated molecules each exert pressure, we may readily understand how the osmotic pressure of a substance in watery solution will show a much higher value as the degree of dissociation becomes greater.

The bearing of the theory of osmotic pressure upon phenomena of absorption is easily seen to be of the first magnitude. If a substance in watery solution be separated from the pure solvent by a membrane permeable to the solvent, but not to the solute, we have the conditions necessary for the solving of problems of osmosis. In such a case, as van't Hoff showed, water will pass into the solution, and, after a time, will establish a condition of equilibrium due to the pressure of the water which enters in minimal quantities. Of course the water, under such circumstances, does not give rise to the osmotic pressure measurable by a manometer, inasmuch as it is present on both sides of a membrane permeable to it. The pressure is, in this case, due solely to the dissolved particles, and may be explained by the kinetic theory, or the water-attraction of the parts dissolved.

If, instead of a membrane strictly impermeable to the dissolved particles, we have one which allows the passage of some, at least, of the dissolved particles we have a slightly different condition of affairs. In such a case the osmotic pressure will be a minimum and the process will resolve itself into one of diffusion, as the membrane being permeable to the solvent and solute, will not in any way hinder the diffusion process. However, if we add to such a condition the further complexity of two solutions separated by a membrane, freely permeable to the solvent and difficultly so for the solute, we have the conditions as they exist in the various cells of the body.

Experiments on absorption of liquids, or, in other words, upon processes involved when the conditions stated above exist, have been made on organized as well as unorganized material. The tissues chiefly involved in such work have been red-blood corpuscles, muscles, and intestines; while the unorganized material has been that known under the general name of colloid matter, including, here, gelatine, albumin, sodium-oleate, silicon-dioxide, etc. In the present work I have confined myself chiefly to the effects of solutions of various electrolytes and non-electrolytes upon absorption by muscular tissue. However, a few introductory experiments upon red blood-corpuscles were made, as it had been shown by various workers that absorption by these cells obeys the laws of osmotic pressure to a large extent, while my results on muscular tissue seem to show that variations in osmotic pressure cannot account, entirely, for phenomena noted in the latter case.

II. EXPERIMENTS ON RED BLOOD-CORPUSCLES

IT seems to be generally agreed that these cells may be regarded as systems surrounded by semi-permeable membranes. If two solutions of different osmotic pressure be separated by a more or less elastic, semi-permeable membrane, an attempt is made to equalize the pressure on each side of this membrane, with the result that phenomena occur of swelling or of shrinking of the cell in question.

Donders, Hamburger, Koeppe, Gryns, Hedin, Overton, Roth, and others have concluded, from their work on these corpuscles, that the phenomena mentioned above depend on the difference of osmotic pressure between the solution outside and that inside the corpuscular membrane, which membrane may be regarded as a thickening of the protoplasm of the corpuscle. So convinced is Koeppe of the rôle of osmotic pressure in life phenomena that he gives expression to the generality: "We cannot imagine a single phenomenon in the living organism in which osmotic pressure may not have a share."

One of the most important points to be considered in this discussion of effects of difference in osmotic pressure is that of the permeability of the corpuscle to the molecules, or ions, of the substances investigated. If it can be shown that the corpuscle be permeable to these particles, then, of course, the laws of diffusion replace those of osmotic pressure. The process, in this latter case, would then resolve itself simply into a discussion of the relative rate of diffusion of the various particles in question.

Certain facts and experiments show, rather conclusively, that the corpuscular membrane is to be regarded as a semi-permeable membrane, permeable to the molecules of water (and to ions, or molecules, of certain inorganic and organic substances) but impermeable, to a large extent, to substances in solution both as regards the solution inside and that outside the membrane. As Koeppe states, the red corpuscles contain no sodium chloride, while the serum contains 5.546 gms. in 1,000 gms. On the other hand, the corpuscles contain 3.679 gms. of potassium chloride, while the serum contains only .39 gms. in 1,000 gms. These facts, in themselves, show that the corpuscle is impermeable to the ions of the salts in question, else an equilibrium would be established on both sides of a permeable membrane. Gryns, Eykman, Hedin, and Oker-Blom have carried out extensive series of experiments to show the relative permeability of the corpuscle to various substances. Gryns, in his work on *Influence of Dissolved Substances upon Red Blood-Corpuscles in Connection with Phenomena of Osmosis and Diffusion*, gives a tabular list of substances to which the corpuscle is permeable and impermeable. Among these we find salts of metals, certain NH_4 salts, such as sulphate, nitrate, phosphate, and tartrate, glycocoll, sugar, etc., to be incapable of penetrating the corpuscle; while other NH_4 salts, such as chloride, bromide, and oxalate, alcohol, glycerine, urea, etc., easily penetrate this cell. Hedin and Oker-Blom, working with methods different from those of Gryns, confirm his results.

A fact of the utmost importance in this discussion of the permeability of the corpuscular membrane, as well as of any other membrane, to different substances is the following: A removal of free ions from a solution is possible only when ions of oppo-

site electric charges are removed together, because the electric charges of the ions hinder the free movement from the solution. Therefore, if a semi-permeable membrane is impermeable to one ion of a molecule it is impermeable to the other, for, if this were not true, a separation of positive or negative electricity would take place.

In solutions, therefore, of any of these substances to which the corpuscular membrane is impermeable, phenomena of swelling or of shrinking occur if the osmotic pressure of the outside solution be less, or more, than that of the solution within this membrane. This osmotic value of the solution within the corpuscular membrane varies, according to Hamburger, between that of a .75 per cent. NaCl solution and that of a .9 per cent. NaCl solution.

If, now, we place the corpuscles in solutions of substances to which they are permeable, osmotic pressure effects play no rôle whatever, even though the concentration of the outside solution be of higher, equal, or lower value than that inside the membrane. We find that, in these latter cases, phenomena of swelling or shrinking do not occur. As Hedin points out, the blood corpuscles do not, under such circumstances, obey the laws of osmotic pressure, and, as a result, they give up their haemoglobin to the outside solution.

Hamburger, in his earlier experiments, had attempted to apply a method based upon that of De Vries's classic work on plasmolysis in plants. He used, in these experiments, the point at which the corpuscle began to lose its haemoglobin as his isosmotic point. He maintained that the corpuscles were, to a high degree, permeable to the salts in question. As a result of this, he says, an equilibrium is established between the osmotic pressure on each side of the membrane. These ideas are, as shown above, totally at variance with the theory of osmotic pressure as advanced by van't Hoff.

In his work Oker-Blom advanced the idea that entirely erroneous conceptions of the osmotic equivalents of the corpuscles must creep in by use of solutions of salts in water. He advocates the use of solutions in serum, inasmuch as the variation introduced in osmotic pressure is, then, referable to the salt added, without considering the consequent lowering of the concentration of the blood on addition of a watery solution of the salt. He shows that, in previous work along these lines, there has always been a question of reciprocal action between the substances present in the animal cells and those with which the cells are brought into contact. In view of such conditions, he says, only the total osmotic pressure of the fluids under observation has been considered, while, from his work, it seems quite evident that the entrance of a substance into a red blood-corpuscle is, clearly, under the influence of the partial osmotic pressure of the substance added, inasmuch as no great increase in total osmotic pressure has been caused by this addition.

My own experiments, few in number, were planned simply with the idea of showing, if possible, that we are dealing, in the case of the red corpuscle, with osmotic pressure effects to a large extent. These experiments have no claim to originality, but are confirmatory of the work previously mentioned. The method followed in this work is a slight modification of Hedin's and Koeppe's hæmatokrit method. Mixtures

were made of defibrinated blood and of isosmotic salt solutions in various dilutions. In some cases equal parts of each (blood and salt solution isosmotic with blood) were taken. In other cases, 2, 3, 5, and 10 parts of solution to 1 of blood were used. These mixtures were placed in dishes containing, approximately, 25 c.c., and allowed to stand for intervals of 1, 3, 6, and 24 hours. At the end of each interval readings were taken by the hæmatokrit method, using one revolution per second for 3 minutes. By this method the percentage relation of corpuscles to serum was obtained. These experiments can have no further value than a comparative one, inasmuch as the osmotic pressure of the mixtures was not determined, and thus the concentration of the solution acting on the corpuscle was unknown. However, as solutions of salts isosmotic with the blood serum and, therefore, with one another were used, we may readily show whether osmotic pressure differences account for phenomena noted or whether we are dealing with specific ionic effects. Below will be found a table embodying the results of these experiments:

TABLE I

Substance	Concentration	Blood Dilution		Per Cent. Vol. of Corpuscles in 3 Hours	Substance	Concentration	Blood Dilution		Per Cent. Vol. of Corpuscles in 3 Hours
		Solution	Blood				Solution	Blood	
NaCl	½ m	10	5	10	KCl....	½ m	10	5	11
NaCl	½ m	10	10	19	KCl....	½ m	10	10	22
NaCl	m	10	5	8	KCl....	½ m	5	5	17
NaCl	m	10	10	15	KCl....	¼ m	10	5	10
NaCl	½ m	10	5	9	KCl....	¼ m	15	5	7
NaCl	½ m	10	10	20	KCl....	¼ m	10	10	25
NaCl	¼ m	10	5	12	KCl....	⅛ m	60	20	21
NaCl	¼ m	10	10	22	KCl....	1/16 m	10	5	dissolution
NaCl	⅛ m	5	5	17	KCl....	1/16 m	10	10	32
NaCl	⅛ m	10	5	11	KCl....	1/32 m	10	5	dissolution
NaCl	⅛ m	15	5	9	KCl....	1/32 m	10	10	7
NaCl	⅛ m	10	10	28	$CaCl_2$..	⅛ m	30	20	33
NaCl	⅛ m	20	20	42	$CaCl_2$..	⅛ m	60	20	12
NaCl	⅛ m	60	10	10	$CaCl_2$..	⅛ m	60	10	8
NaCl	⅛ m	60	20	19	$CaCl_2$..	¼ m	5	5	15
NaCl	1/16 m	10	5	dissolution	$CaCl_2$..	¼ m	10	5	12
NaCl	1/16 m	10	10	30	$CaCl_2$..	¼ m	15	5	8
NaCl	1/32 m	10	5	dissolution	NH_4Cl.	¼ m	5	5	dissolution
NaCl	1/32 m	10	10	8	NH_4Cl.	¼ m	10	5	dissolution
KCl	½ m	10	5	6	NH_4Cl.	½ m	15	5	dissolution
KCl	½ m	10	10	16	NH_4Cl .	½ m	10	10	dissolution
KCl	m	10	5	5	Urea ...	.2286 m	5	5	dissolution
KCl	m	10	10	12	Urea ...	.2286 m	10	5	dissolution
KCl	½ m	10	5	9	Urea ...	.2286 m	15	5	dissolution
KCl	½ m	10	10	18	Urea ...	.2286 m	10	10	dissolution

A study of this table shows two facts quite clearly. The first is, that we have two classes of substances represented here. The one class, to which the corpuscular membrane is permeable, embraces NH_4Cl and urea; the other class, to which the