CXXXII. ON THE APPLICATION OF THE DONNAN EQUILIBRIUM TO THE IONIC RELATIONS OF PLANT TISSUES.

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INTRODUCTION.

It is a matter of general knowledge that, if a plant tissue is placed in a solution of an electrolyte the concentration of which is greater than its concentration in the tissue, the cation and anion are absorbed in different amounts, electrostatic neutrality being preserved by the outward diffusion of other ions from the tissue. The mechanism controlling this unequal intake of ions is one of which we are still in comparative ignorance; but it has been suggested that the interpretation may be associated with the establishment of a Donnan membrane equilibrium between the cells of the tissue and the external solution. It is the purpose of this paper to inquire more closely into the possibility of this suggestion being correct, and into the extent to which other factors may be associated with the ionic equilibria of the living cell.

THE INADEQUACY OF A SIMPLE DONNAN SYSTEM.

If two electrolytes in solution are separated by a membrane which is impermeable to one of the ions of one of the electrolytes, the diffusible ions proceed to an equilibrium at which they are unequally distributed on the two sides of the membrane, but at which the product of the concentrations of each pair of oppositely charged diffusible ions is the same on either side; these products will be equal whether only a single electrolyte is concerned or whether there is a mixture of a number. It is this condition which is known as the Donnan equilibrium.

The distribution of an electrolyte in such a system will be expressed by the following equation¹:

$$\frac{x_i y_i}{v_i^2} = \frac{x_e y_e}{v_e^2} ; \quad \dots\dots(1)$$

where x_i and y_i represent the amounts of cation and anion respectively within the membrane at equilibrium, x_e and y_e represent the equilibrium amounts of the same pair of ions in the external solution, and v_i and v_e denote the

¹ If the valency of the x ions were m and that of the y ions n then we should have $x^m y^n$ instead of xy —the special case for univalent ions. We have limited ourselves to the consideration of univalent ions but our arguments can easily be extended to other cases.

volume of the solvent on either side of the membrane. It is clear that whatever the concentration of indiffusible ions within the membrane the above product equation must hold, since it is derived from the fundamental laws of thermodynamics: and, if there are no indiffusible ions in the system at all, the individual ionic concentrations will all be equal; *i.e.*

$$\frac{x_i}{v_i} = \frac{y_i}{v_i} = \frac{x_e}{v_e} = \frac{y_e}{v_e}.$$

Unfortunately experimental verification of the product law is difficult in the case of ordinary plant tissue¹, owing to the impracticability of estimating x_i, y_i and v_i : while the net amount of ions absorbed from a solution could be calculated from the difference between their concentration before putting in the tissue and after equilibrium had been reached, this would not allow for the amounts of these ions which in many cases would almost certainly be resident in the tissue at the commencement of the experiment.

If we are dealing with a simple Donnan system we shall have the following equation:

$$\frac{(X+x)(Y+y)}{v_i^2} = P, \quad \dots\dots(2)$$

wherein X and Y denote the amounts of the cation and anion respectively present in the tissue, x and y denote the net amounts of the same ions absorbed from the external solution, v_i has the same significance as in equation (1), and P is the product of the concentrations of the two ions in the external solution at equilibrium.

A study has recently been made by Petrie [1927] of the variation with temperature in the uptake of the two ions of single salts by carrot-root tissue². The results of this inquiry provide convenient data for substitution in equation (2); for, with a sufficient number of values for x, y and P , we can solve this equation for the unknowns X, Y and v_i , provided we assume that v_i does not change with temperature. We know that X and Y are the same for each temperature, since a uniform sample of tissue was used throughout any one

¹ *Valonia* and *Nitella*, from which the cell-sap can be extracted and analysed, allow of a more accurate estimate of the internal concentrations; and it was found in the investigations of Osterhout [1926] on the uptake of hydrogen sulphide and of Osterhout and Dorcas [1927] on the uptake of carbon dioxide by *Valonia*, that the ratio $[H^+ \text{ inside}]/[H^+ \text{ outside}]$ varied in the same manner as the ratio $[HS' \text{ outside}]/[HS' \text{ inside}]$ or $[HCO_3' \text{ outside}]/[HCO_3' \text{ inside}]$. The deviations of these ratios from equality were rather great, but according to the writers were "such as might result largely from experimental error." It should be noted with reference to our subsequent remarks that in this case we are dealing with concentration of ions in the cell-sap and not with the average concentration of the ions in the cell as a whole.

² The results of this study could be explained qualitatively on the basis of the Donnan equilibrium in that, while the cation was absorbed in greater amount than the anion, rise of temperature, which caused the cation absorption to decrease, caused the anion intake concomitantly to increase. The opposite drift of cation and anion intake would result in the product being more nearly constant than if the drift had been in one ion alone. Constancy of external product, however, is not implicit in the application of the Donnan equilibrium as was suggested in the paper. Conditions may be such, and in the experiments were such, as to make the drift in the external product inappreciable.

experiment. If X , Y and v_i determined in this way have values which are rendered improbable by other evidence, we shall have some ground for believing that the system is not of the type for which Donnan's equation holds.

Table I. *Equilibria in the absorption of 0.1 M ammonium chloride by carrot tissue at various temperatures*¹.

Temp. ° C.	NH ₄ ⁺			Cl ⁻			Ext. ionic product	Int. ionic product
	Final ext. conc.	Final int. conc.	Absorp- tion ratio	Final ext. conc.	Final int. conc.	Absorp- tion ratio		
	$\left(\frac{x_e}{v_e}\right) \times 10^3 M$	$\left(\frac{x}{v_i}\right) \times 10^3 M$	$\left(\frac{xv_e}{x_e v_i}\right)$	$\left(\frac{y_e}{v_e}\right) \times 10^3 M$	$\left(\frac{y}{v_i}\right) \times 10^3 M$	$\left(\frac{y v_e}{y_e v_i}\right)$	$\left(\frac{x_e y_e}{v_e^2}\right) \times 10^6$	$\left(\frac{xy}{v_i^2}\right) \times 10^6$
4	·0805	·2143	2.662	·0930	·07676	0.8252	·00748	·01644
15	·0825	·1923	2.331	·0916	·09232	1.008	·00756	·01755
23	·0855	·1593	1.863	·0892	·1187	1.330	·00758	·01891

Let us consider for example the results obtained for the intake of ammonium chloride (Table I). It is perhaps reasonable to suppose tentatively that the tissue originally contained no appreciable amount of NH₄⁺, so that we may assume $X = 0$; chlorine, however, is definitely known to be present in considerable quantity in some samples of carrot tissue [Pearsall and Ewing, 1924], and some may probably still have been left in the present instance in spite of the washing to which the tissue was subjected prior to the experiment. For P , x and y we obtain the following values from the experimental results:

P	x	y
7.48×10^{-3}	1.95×10^{-3}	0.70×10^{-3}
7.55×10^{-3}	1.75×10^{-3}	0.84×10^{-3}
7.58×10^{-3}	1.45×10^{-3}	0.08×10^{-3}

We may substitute these values in equation (2) and obtain the most probable values of Y and v_i by the method of least squares; by this procedure we find that

$$Y = 0.417 \times 10^{-3} \text{ g.-mols.},$$

and

$$v_i = 17 \text{ cc.}$$

Let us also determine the values of the unknowns from the results recorded on the intake of potassium chloride. In this case we have to consider that potassium as well as chlorine is previously existent in the cell², so that X will have a finite value. From the experimental results (Table II) we obtain the following values:

P	x	y
8.72×10^{-3}	1.04×10^{-3}	0.27×10^{-3}
8.76×10^{-3}	0.96×10^{-3}	0.31×10^{-3}
8.68×10^{-3}	0.94×10^{-3}	0.42×10^{-3}
8.69×10^{-3}	0.88×10^{-3}	0.47×10^{-3}

¹ Tables I-IV are based on the results of Petrie [1927], Tables V and VI on those of Stiles [1924]. The volume of solution employed in each case was 100 cc. The concentrations are expressed in gram-molecules per litre. The value of v_i is 9.1 cc. in Tables I-IV and 11.3 cc. in Tables V and VI.

² Stiles [1924] found that K⁺ along with Ca⁺⁺ and Mg⁺⁺ diffused out from carrot tissue to replace the excess of Na⁺ absorbed from a solution of NaCl. The previous existence of potassium in the tissue would be a possible interpretation of the small slope of the cation and anion curves for KCl absorption compared with those for NH₄Cl (cf. Petrie [1927], Figs. 2 and 5).

Table II. *Equilibria in the absorption of 0.1 M potassium chloride by carrot tissue at various temperatures.*

Temp. °C.	K ⁺			Cl ⁻			Ext. ionic product $\left(\frac{x_e y_e}{v_e^2}\right) \times 10^6$	Int. ionic product $\left(\frac{xy}{v_i^2}\right) \times 10^8$
	Final ext. conc. $\left(\frac{x_e}{v_e}\right) \times 10^3 M$	Final int. conc. $\left(\frac{x}{v_i}\right) \times 10^3 M$	Absorp- tion ratio $\left(\frac{x v_e}{x_e v_i}\right)$	Final ext. conc. $\left(\frac{y_e}{v_e}\right) \times 10^3 M$	Final int. conc. $\left(\frac{y}{v_i}\right) \times 10^3 M$	Absorp- tion ratio $\left(\frac{y v_e}{y_e v_i}\right)$		
4	·0896	·1143	1.276	·0973	·02968	·3050	·00872	·00339
10	·0904	·1055	1.167	·0969	·03407	·3517	·00876	·00359
21	·0906	·1033	1.140	·0958	·04615	·4817	·00868	·00477
26	·0912	·0967	1.060	·0953	·05165	·5420	·00869	·00500

The number of equations is not sufficient in this case to permit the use of the method of least squares. We may, however, solve for the three unknowns by taking the equations in all possible combinations of three, the average of the results so obtained being as follows:

$$X = -.9523 \times 10^{-3} \text{ g.-mols.};$$

$$Y = -.3754 \times 10^{-3} \text{ g.-mols.};$$

and

$$v_i = +.7243 \text{ cc.}$$

Another series of values might be obtained by taking the alternative roots of X and Y ; they have been neglected, however, since these roots are considerably greater negative quantities than the above values.

Some results of Stiles [1924] also provide data which can be substituted in equation (2). These results were obtained by a similar technique, again using carrot tissue, only in this case the experiments were carried out at the same temperature with different external concentrations. Since the tissue remained turgid throughout the experiments, we may perhaps assume that the volume of the internal solute did not vary appreciably with the external concentration.

Taking for example the experiments with sodium chloride, we have the following data (Table V, p. 1076):

P	x	y
6.860×10^{-3}	2.52×10^{-3}	0.83×10^{-3}
5.380×10^{-5}	3.61×10^{-4}	1.58×10^{-4}
4.778×10^{-7}	3.81×10^{-5}	2.58×10^{-5}

Substituting these values in equation (2) and solving simultaneously the three equations so obtained, we find that

$$X = -.3354 \times 10^{-3} \quad \text{or} \quad -.5758 \times 10^{-3} \text{ g.-mols.};$$

$$Y = +.007979 \times 10^{-3} \quad \text{or} \quad +.1076 \times 10^{-3} \text{ g.-mols.};$$

and

$$v_i = 16.33 \quad \text{or} \quad 16.29 \text{ cc.}$$

Now the volume of the whole tissue employed was, in the case of the ammonium chloride experiment, only 9.1 cc., and in the case of the sodium chloride experiment only 11.3 cc.; so that it is evident that the value of v_i is in these cases impossibly high. It is also unreasonable that either X or Y should be negative as is indicated in the cases of potassium chloride and sodium chloride. We are therefore led to the conclusion that the Donnan equation cannot be applied to these experiments.

The same conclusion can be reached from another aspect. We may take x and y of equation (2) as being the minimum values of x_i and y_i in equation (1), since they do not include any amount of the ions which may have been in the tissue at the commencement of the experiment; we may also take 9.1 or 11.3, as the case may be, and which we may express as v_i , as the maximum value of v_i , since the actual volume of the solute concerned is undoubtedly less than that of the whole tissue. The value of xy/v_i^2 is thus a minimum for the inside product $(X + x)(Y + y)/v_i^2$. Now, if the Donnan equation applies, the outside product should equal the inside product, and hence the quantity xy/v_i^2 should be less than the outside product, the more so the greater the value of X . In the results for potassium chloride (Table II), where one presumes that X is really comparatively high, the relationship actually holds; but in the case of ammonium chloride, where X is probably considerably less, instead of the

Table III. *Equilibria in the absorption of 0.1 M ammonium sulphate by carrot tissue at various temperatures.*

Temp. °C.	NH ₄ ⁺			SO ₄ ^{''}			Ext. ionic product $\left(\frac{x_e^2 y_e}{v_e^3}\right) \times 10^6$	Int. ionic product $\left(\frac{x y^2}{v_i^3}\right) \times 10^6$
	Final ext. conc. $\left(\frac{x_e}{v_e}\right) \times 10^3 M$	Final int. conc. $\left(\frac{x}{v_i}\right) \times 10^3 M$	Absorp- tion ratio $\left(\frac{x v_e}{x_e v_i}\right)$	Final ext. conc. $\left(\frac{y_e}{v_e}\right) \times 10^3 M$	Final int. conc. $\left(\frac{y}{v_i}\right) \times 10^3 M$	Absorp- tion ratio $\left(\frac{y v_e}{y_e v_i}\right)$		
5	.158	.4615	2.920	.0985	.0165	0.1674	.00246	.003515
12	.167	.3626	2.172	.0985	.0165	0.1674	.00275	.002169
17	.170	.2619	1.540	.0953	.0516	0.5420	.00275	.003538
21.5	.192	.0879	0.458	.0897	.1132	1.262	.00331	.000875

Table IV. *Equilibria in the absorption of 0.1 M calcium nitrate by carrot tissue at various temperatures.*

Temp. °C.	Ca ⁺⁺			NO ₃ [']			Ext. ionic product $\left(\frac{x_e y_e^2}{v_e^3}\right) \times 10^6$	Int. ionic product $\left(\frac{x y^2}{v_i^3}\right) \times 10^6$
	Final ext. conc. $\left(\frac{x_e}{v_e}\right) \times 10^3 M$	Final int. conc. $\left(\frac{x}{v_i}\right) \times 10^3 M$	Absorp- tion ratio $\left(\frac{x v_e}{x_e v_i}\right)$	Final ext. conc. $\left(\frac{y_e}{v_e}\right) \times 10^3 M$	Final int. conc. $\left(\frac{y}{v_i}\right) \times 10^3 M$	Absorp- tion ratio $\left(\frac{y v_e}{y_e v_i}\right)$		
4	.0917	.09122	.9947	.199	.01099	.0522	.003633	.0001102
8	.0930	.07693	.8271	.197	.03297	.1673	.003610	.0008362
14	.0950	.05495	.5785	.195	.05495	.2818	.003612	.001660
18	.0959	.04506	.4699	.185	.16480	.8910	.003283	.01225

outside product still exceeding xy/v_i^2 although to a smaller extent, it is actually less. The same phenomenon tends to appear in the results for ammonium sulphate (Table III); and the external product is also considerably less than xy/v_i^2 in Stiles's experiments with sodium and ammonium chlorides (Tables V and VI, p. 1076). Perhaps the same might hold for the experiments on calcium (Table IV) and potassium salts if the probably large values of X and Y were respectively added to x and y .

To make the internal product equal to the external product, either the value of v_i has to be greater than the volume of the tissue (v_t), as resulted in

the calculations for ammonium and sodium chlorides; or the observed values of x and y have to be reduced, as resulted in the calculations for potassium and sodium chlorides, where X and Y in the one case and X in the other case became negative. There thus seems no doubt that the law of equality of products does not hold between the tissue and the external solution: in other words, the tissue as a whole cannot be treated as a simple Donnan system.

Table V. *Equilibria in the absorption of sodium chloride by carrot tissue at 20° (circa) in various concentrations.*

Init. ext. conc. M	Na^+			Cl^-			Ext. ionic product $\left(\frac{x_e y_e}{v_e^2}\right) \times 10^6$	Int. ionic product $\left(\frac{xy}{v_i^2}\right) \times 10^6$
	Final ext. conc. $\left(\frac{x_e}{v_e}\right) \times 10^3 M$	Final int. conc. $\left(\frac{x}{v_i}\right) \times 10^3 M$	Absorp- tion ratio $\left(\frac{xv_e}{x_e v_i}\right)$	Final ext. conc. $\left(\frac{y_e}{v_e}\right) \times 10^3 M$	Final int. conc. $\left(\frac{y}{v_i}\right) \times 10^3 M$	Absorp- tion ratio $\left(\frac{y v_e}{y_e v_i}\right)$		
0.1	0.0748	0.223	2.98	0.0917	0.07345	0.801	6860×10^{-2}	1638×10^{-1}
0.01	0.00639	0.03195	5.00	0.00842	0.0140	1.66	5380×10^{-4}	4474×10^{-3}
0.001	0.000619	0.00337	5.45	0.000772	0.00202	2.62	4778×10^{-6}	6808×10^{-5}

Table VI. *Equilibria in the absorption of ammonium chloride by carrot tissue at 20° (circa) in various concentrations.*

Init. ext. conc. M	NH_4^+			Cl^-			Ext. ionic product $\left(\frac{x_e v_e}{v_e^2}\right) \times 10^6$	Int. ionic product $\left(\frac{xy}{v_i^2}\right) \times 10^6$
	Final ext. conc. $\left(\frac{x_e}{v_e}\right) \times 10^3 M$	Final int. conc. $\left(\frac{x}{v_i}\right) \times 10^3 M$	Absorp- tion ratio $\left(\frac{xv_e}{x_e v_i}\right)$	Final ext. conc. $\left(\frac{y_e}{v_e}\right) \times 10^3 M$	Final int. conc. $\left(\frac{y}{v_i}\right) \times 10^3 M$	Absorp- tion ratio $\left(\frac{y v_e}{y_e v_i}\right)$		
0.1	0.0871	0.1141	1.31	0.0941	0.0520	0.553	8196×10^{-2}	5933×10^{-2}
0.01	0.00428	0.0506	11.8	0.00678	0.0285	4.20	2901×10^{-4}	1442×10^{-3}
0.001	0.0000293	0.00859	293.2	0.0001923	0.00715	37.2	5635×10^{-8}	6142×10^{-4}

THE POLYPHASE SYSTEM OF THE PLANT CELL.

There are various ways in which the tissue may differ from a simple Donnan system so as to explain these experimental results which we have been considering. The Donnan equilibrium applies to free ions only, whereas it is possible that some of the ions in the cell are bound as undissociated salts of protein or other substances, or some of the ions may be present in a state of physical adsorption and so be removed from solution. These ions would thus be present in the cell in addition to those which had entered to satisfy the product equation, and so would result in the internal concentration product being greater than that of the external concentrations. There is evidence that unless in considerable dilution the ionisation of protein salts is incomplete [Manabe and Matula, 1913; Hitchcock, 1923]; but to accept this hypothesis we should require to know the degree of ionisation of the particular salts concerned at their existing concentrations.

Another explanation of the inequality of the products might lie in the possibility, suggested by Butkewitsch and Butkewitsch [1925], that the degree

of permeability of the plasma-membrane is not the same in both directions: if inorganic ions could enter the cell with greater velocity than they could emerge, they would accumulate in greater amount in the cell than the simple Donnan theory would require. While, however, these properties have been shown to characterise some membranes [Wertheimer, 1923, 1924], we have not the same type of evidence that this is so for the material with which we are concerned in this discussion.

Whether either of these factors is responsible for the inequality of the concentration products cannot be determined at present; but, apart from any such possibilities, there are certain characters of the system involved which must tend to result in a higher value for the quantity $(X + x)(Y + y)/v_i^2$ than for the product of the external concentrations. The plant cell is not a simple system, composed of a mere membrane enclosing a homogeneous solution, one of the ions of which is indiffusible: there are at least three phases between which the ions may be distributed—the external medium, the cytoplasm, and the vacuoles. Within the cytoplasm also, and perhaps in the cell-sap, there are micelles, or gel-particles, which can constitute yet other phases. Loeb [1924] has suggested in his work on the viscosity of gelatin and caseinogen solutions that between such particles and the continuous phase a Donnan equilibrium may become established¹. Hence there are a number of phases, all of which are in equilibrium with one another; and it can be shown that the apparent internal ionic product, resulting from the collective effect of all the phases within the tissue under these conditions, must have a higher value than that of the external medium.

Let us consider for example the case where there are two internal systems. Suppose a_1, b_1 and a_2, b_2 represent the amounts of any pair of oppositely charged diffusible ions in the two internal systems respectively; and let v_1 and v_2 be the respective amounts of solvent in which they are dissolved. Finally, let R denote the value of the product of the concentrations of the two ions in the external solution at equilibrium.

When equilibrium has established itself the product of the concentrations of the two ions must be the same in each system; hence

$$R = \frac{a_1 b_1}{v_1^2} = \frac{a_2 b_2}{v_2^2} = \frac{a_1 b_1 + a_2 b_2}{v_1^2 + v_2^2}.$$

Let R' represent the apparent internal ionic product based upon the combined effect of the two internal systems; *i.e.*

$$R' = \frac{(a_1 + a_2)(b_1 + b_2)}{(v_1 + v_2)^2}.$$

We have now to determine the conditions governing the following relationship:

$$R' \geq R,$$

¹ Most investigators assume that a particle of a colloid, large or small, can be treated as a homogeneous solution surrounded by a membrane impermeable to one of the contained ions; we are not concerned here with the validity of this assumption.

$$\text{or} \quad \frac{a_1 b_1 + a_2 b_2 + a_1 b_2 + a_2 b_1}{v_1^2 + v_2^2 + 2v_1 v_2} \geq \frac{a_1 b_1 + a_2 b_2}{v_1^2 + v_2^2},$$

$$\text{or} \quad \frac{a_1 b_2 + a_2 b_1}{2v_1 v_2} \geq \frac{a_1 b_1 + a_2 b_2}{v_1^2 + v_2^2},$$

$$\text{or} \quad \frac{a_1 b_2 + a_2 b_1}{2v_1 v_2} \geq \frac{a_1 b_1}{v_1^2}.$$

Since

$$\frac{a_1}{v_1} \cdot \frac{b_1}{v_1} = \frac{a_2}{v_2} \cdot \frac{b_2}{v_2},$$

or

$$\frac{a_1 v_2}{v_1 a_2} = \frac{b_2 v_1}{v_2 b_1},$$

the expression becomes

$$\frac{1}{2} \left(\frac{a_1^2 b_1 v_2}{v_1^3 a^2} + \frac{a_1 b_1^2 v_2}{v_1^3 b_2} \right) \geq \frac{a_1 b_1}{v_1^2},$$

or

$$\frac{1}{2} \cdot \frac{a_1 b_1}{v_1^2} \left(x + \frac{1}{x} \right) \geq \frac{a_1 b_1}{v_1^2},$$

where

$$x = \frac{a_1}{v_1} \cdot \frac{v_2}{a_2} = \frac{b_2}{v_2} \cdot \frac{v_1}{b_1};$$

whence the expression becomes

$$x + \frac{1}{x} \geq 2.$$

Now

$$x + \frac{1}{x} > 2,$$

except when $x = 1$, *i.e.* except when

$$\frac{a_1}{v_1} = \frac{a_2}{v_2};$$

that is, the apparent internal product, R' , for a system in which there are two internal phases, will be greater than the external product R , except in the one case where the concentration of each of the ions is the same for the two internal phases. A similar conclusion could be drawn for a system of a greater number of internal phases.

The possibility of the concentration of any ion being the same in all the phases of the internal system may be dismissed. For it must be observed that, in plant tissues such as the root of the carrot, the intercellular space system, which from the practical point of view is included with the tissue, represents an internal phase. It is to be expected that this system contains ions in the same concentrations as in the external solution, so that it constitutes a phase which differs from the others within the tissue¹.

THE COMPLICATION OF A MIXED AMPHOLYTE SYSTEM.

If the indiffusible ion in each phase of the internal system has the same type of charge, then the concentration of any diffusible ion of opposite charge in each of these phases, and hence its average concentration in all the internal phases together, should be greater than the concentration of the same ion in

¹ It is evident that a solution of colloid in which some of the colloid is in micellar form, enclosed in a collodion bag, constitutes such a polyphase system as we have been considering. The application of the Donnan equation to such a system is thus unjustifiable if the amount of micellar colloid is appreciable.

the external solution; similarly it follows that this ratio for any diffusible ion of the same charge as the indiffusible ion should be less than unity. Thus, if the indiffusible ion is a negative one, as is generally concluded to be the case in living tissues, we should have

$$\frac{(X+x)v_e}{x_e v_i} > 1,$$

and

$$\frac{(Y+y)v_e}{y_e v_i} < 1;$$

where X and Y are the amounts of the cation and anion respectively initially present in the tissue, x and y are the respective amounts of the same ions absorbed from the external solution, x_e and y_e are the final amounts in the external solution, and v_i is the volume of the internal, and v_e that of the external solution. Actually, taking $xv_e/x_e v_i$ and $yv_e/y_e v_i$ respectively as minimal values of the two above expressions, where v_i is the volume of the tissue employed, we find in certain cases (as will be seen from the accompanying tables) that both ratios may be greater than unity; and, since v_i cannot be greater than v_e it is obvious that the excess over unity may in reality be greater than is apparent.

This anomaly might be accounted for by assuming that some of the anions are bound in the undissociated state; but another possible interpretation lies in the supposition that, while indiffusible anions occur in one of two internal phases, the other contains indiffusible cations. For example, let K be the amount of the indiffusible cation in one phase and A the amount of the indiffusible anion in the other. In the former phase let x_1 and y_1 represent the amounts of the cation and anion respectively of a diffusible salt; let x_2 and y_2 have the same significance in the latter phase; and let x_e and y_e represent the amounts of the same two ions in the external solution. Also let v_1 be the volume of solvent in the first phase, v_2 the volume of solvent in the second phase, and v_e the volume of the external solution. The system may hence be visualised as follows:

$$\begin{array}{ccc|ccc|ccc} K & x_1 & y_1 & & A & x_2 & y_2 & & x_e & y_e \\ & v_1 & & & & v_2 & & & v_e & \end{array}$$

In such a system the following relationships will hold:

$$\frac{y_1}{v_1} > \frac{y_e}{v_e}; \text{ and } \frac{x_2}{v_2} > \frac{x_e}{v_e}.$$

With certain values of K and A , y_1/v_1 may be so much greater than y_e/v_e that the addition of y_2 to the numerator and of v_2 to the denominator still leaves

$$\frac{y_1+y_2}{v_1+v_2} > \frac{y_e}{v_e},$$

while similarly at the same time

$$\frac{x_1+x_2}{v_1+v_2} > \frac{x_e}{v_e},$$

so that in the case of both ions the apparent internal concentration can be greater than the external.

To illustrate the possibility of this we may take the following numerical example, in which we have a system containing only one diffusible electrolyte:

$$K = 9; \quad x_1 = 1; \quad y_1 = 10 \quad \Big| \quad A = 19.5; \quad x_2 = 20; \quad y_2 = 0.5 \quad \Big| \quad x_e = 31.6; \quad y_e = 31.6$$

$$v_1 = 100 \quad \Big| \quad v_2 = 100 \quad \Big| \quad v_e = 1000$$

Here the apparent internal concentration is greater than the external in the case of both cation and anion; that is, for the cation

$$\frac{21}{200} > \frac{31.6}{1000},$$

and, for the anion,

$$\frac{10.5}{200} > \frac{31.6}{1000}.$$

It must be noted that, for this to hold good, K and A must be in different phases; if they were both in the same phase then we should have

$$\frac{x_i y_i}{v_i} = \frac{x_e y_e}{v_e}.$$

Stearn [1927] has recently brought forward evidence from different sources for the belief that protoplasm contains a mixture of ampholytes of different isoelectric points: moreover, in the case of carrot tissue with which we are particularly concerned in this discussion, the work of Cohn, Gross and Johnson [1920] indicates that two proteins are present, one isoelectric at about p_H 4 and the other in the region of p_H 8; and, since the normal reaction of the cell-sap is about p_H 6 [Pearsall and Ewing, 1924] one of these proteins must be dissociated as an acid and the other as a base. If these proteins occur in the cytoplasm as separate colloidal particles, they would behave as separate phases of the internal system; so that there is a possibility that the cells of this tissue contain such an ampholyte system as we have been considering. In terms of this system of two proteins dissociated, the one giving rise to cations and the other to anions¹, and occurring in different phases of a poly-phase system, we have therefore a hypothesis whereby we may interpret some of the phenomena of the ionic equilibria of the living cell; but to what extent such a hypothesis is a true explanation of the facts it remains for further data to show.

THE EFFECT OF TEMPERATURE ON THE UNEQUAL INTAKE OF IONS.

It is of interest now to consider how the effect of temperature on the unequal intake of the two ions of an electrolyte, observed by Petrie [1927], can be correlated with this new conception; for temperature may be expected to produce various changes in the system we have been considering.

¹ The effect of the protein existing as a negatively charged ion will be supplemented by indiffusible organic acids, particularly the amino-acids, and the feebly dissociated carbohydrates such as the pentosans. We must not forget also the phosphatides, which like the proteins are amphoteric: Feinschmidt [1912] found the isoelectric point of lecithin (which was, however, probably impure) to lie between p_H 2 and p_H 4; he found also that lecithin could combine with proteins to give a compound with an isoelectric point different from that of either of the constituents.

Thus, since the solubility of proteins increases with temperature, the disperse phase in the protoplasm may be reduced by the passing of some of the micellar proteins into crystalloidal solution; one of the phases in the heterogeneous internal system would in this way tend to pass into another. On the other hand, the effect of temperature might be regarded as increasing the swelling of the micelles of protein: Jordan-Lloyd and Pleass [1927] have shown that the swelling of gelatin rises up to about 18° and it is only after this that increased solubility conspicuously manifests itself. Increased swelling would lead to an increase rather than a decrease in the volume of the micellar protein phase.

Such changes as these, however, could not explain the observed decrease in cation intake and increase in anion intake associated with rising temperature. It is true that they would alter the internal product; but, if electrostatic neutrality is to be preserved, such alterations could be achieved only by equal transference of ions of both charges from one phase to another.

The decrease of the difference between cation and anion intake as the temperature rises necessitates a decrease of the difference between indiffusible anions and cations in the tissue. If the tissue consisted of a single phase with an indiffusible anion, then the effect of temperature would be explained if the acidic dissociation constant of the ampholyte in the cells was decreased with rise of temperature. If, however, the system consists of two phases, one with colloidal anion and the other with colloidal cation, then the possibilities are considerably increased. If the dissociation of both decreases as the temperature is increased, the conditions of the whole system may be such that the dominating effect of the anion also decreases. For example, if the two internal phases were of equal volume, but the anion in one greater than the cation in the other, then halving their concentrations would still leave the anion dominant but to a decreased extent. The effect of temperature on ion uptake could, however, be reconciled with an increase of both indiffusible anions and cations in the tissue, the increase of the latter more than counterbalancing the increase of the former, and the more so the higher the temperature. The fraction of the one ampholyte which is in the form of anions will be $\frac{k_a}{[H^+] + k_a}$, where k_a is the acidic dissociation constant. Similarly, the fraction of the other ampholyte in the form of cations will be

$$\frac{k_b}{\frac{k_w}{[H^+]} + k_b},$$

where k_b is the basic dissociation constant and k_w is the dissociation constant of water. If k_a and k_b are increased by rising temperature the absolute increase in the fraction dissociated will be at a maximum in the case of the acidic dissociation when $[H^+] = k_a$, and in the case of the basic dissociation when $[H^+] = k_w/k_b$. Hence, if the hydrogen ion concentration is a long way removed from k_a and nearer to k_w/k_b , then the effect of the increase in cations may more than counterbalance the effect of the increase in anions. At present, however,

we have no knowledge of the dissociation constants of the substances in the system or of the way in which temperature affects them.

We do not suggest that our interpretation of the phenomena of ionic absorption by tissues in terms of the Donnan equilibrium is the true one; it does, however, seem possible that this equilibrium plays a part, even though the system is not a "simple" one as has been suggested in some papers.

SUMMARY.

The conception of a simple Donnan membrane equilibrium operating between two homogeneous phases is inadequate to explain the phenomena of ionic intake by plants. Experimental evidence indicates that the product of the apparent internal concentrations of cation and anion, estimated on the basis of the total volume of the tissue, may be greater than that of the concentrations of the two ions in the external medium, instead of equal to it, as the above conception would require.

It is pointed out that the interior of the tissue comprises a number of phases each of which may be in Donnan equilibrium with the external solution. The product of the apparent internal ionic concentrations resulting from the total effect of all these internal phases is shown under such circumstances to be greater than the external product.

Even in such a system as this, however, if the ratio of the apparent internal to the external concentration of a cation is greater than unity, the same ratio of the corresponding anion should be less than unity; whereas the experimental evidence indicates that both may at times be greater than unity. It is shown that this may be accounted for if we suppose that one internal phase contains indiffusible cations and that another contains indiffusible anions.

The effect of temperature on the relative uptake of the cation and anion of a salt can be interpreted in terms of its possible effect on the dissociation of the tissue colloids.

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