Studies in Permeability.

V. The Swelling of Plant Tissue in Water and its Relation to Temperature and various Dissolved Substances.¹

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With ten Tables and nine Figures in the Text.

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INTRODUCTION.

THE passage of substances between plants and their surroundings, and between cell and cell, is a subject of fundamental importance. Thus among the problems to which such phenomena have application may be mentioned those of carbon assimilation, translocation, transpiration, and root absorption, with their bearing on agriculture; the extraction of substances from living or dead plant tissue; and even such problems as those involved in the preservation of vegetables and fruits. Nevertheless, in spite of the wide importance of the subject, the known facts are few and often not very definite, so that the information so far available does not enable us to attempt an analysis of the processes involved. For although the single term 'permeability' is used in relation to these phenomena, it is to be expected that the processes are numerous and not all of the same nature.

Obviously a detailed analysis of the processes of permeability is

¹ The terms swelling and shrinkage are used in a general sense and refer to the total gain or loss of water by the cell under various conditions. Pfeffer used the term swelling in a restricted sense to indicate the water imbibed by the protoplasm, as distinct from that absorbed by osmosis.

required, similar to that which has been attempted in the case of carbon assimilation, in which, even if our knowledge is still very imperfect, we have a considerable amount of information regarding the complexity of the processes involved and the factors influencing them. Without such an analysis the putting forward of theories is clearly of little help to the advancement of the subject, and undoubtedly, as with carbon assimilation, the development of the subject has been hampered by undue speculation.

As to the water relation in permeability, after the extensive pioneer investigations of de Vries and Pfeffer, little work of any value was done, and no advance in the subject was brought about until the recent introduction by A. J. Brown (3, 4, 5) and by F. F. Blackman (6) of fresh methods of attack in which changes are measured quantitatively and are followed from time to time, thus rendering possible an investigation of the kinetics of the changes taking place.

METHOD OF EXPERIMENT.

The observations on the passage of water between the cell and surrounding medium recorded in this paper have been made on potato and carrot. The changes with time have been followed quantitatively by weighing the tissue experimented upon at intervals, as was done in the experiments of A. J. Brown (3, 4, 5) on the intake of water by seeds of barley.

The method of experimentation is as follows: Cylinders of tissue having a diameter of 1.8 cm. are cut from the potato or carrot by means of a cork-borer. These cylinders are then cut up by means of a hand microtome into a number of discs having a definite thickness. In all the experiments dealt with here the thickness was 2 mm. After they are cut, the discs are rapidly dried between blotting-paper and then weighed out into lots of 10 or 20 gm. It is not difficult to obtain such lots which contain the same number of discs and differ in weight by not more than 2 per cent. The weight of each such set is taken to the nearest centigramme; more accurate weighing is not justified on account of errors arising from other causes, namely drying of the discs and variability of the samples. Each lot of discs is transferred to a bottle or flask containing a comparatively large volume of liquid (100 c.c.—700 c.c.) which is kept at constant temperature in a thermostat. At intervals the discs are removed from the liquid, their surfaces are dried as rapidly as possible with blotting-paper, and they are weighed. They are immediately replaced in a fresh quantity of the experimental liquid.

Alterations in the weight of the discs are almost entirely due to the passage of water into or out of the tissue. The weight of the small quantity of dissolved substances which may pass into or out of the cells is negligible compared with the weight of water which passes across the tissue boundaries.

From the data so obtained curves are constructed, showing the relation between time and the absorption by, or exosmosis from, the tissues under different conditions.

THE VARIABILITY OF THE TISSUE USED AND THE
PROBABLE ERROR OF EXPERIMENT.

Although it might be expected that potato and carrot would yield very uniform tissue, so that individual variations would be largely eliminated in experiments with 20 gm. of tissue (40 discs), yet actual measurements show this is far from being the case. The variability of such tissue in regard to water absorption and excretion is indeed surprisingly large. Probably the previous history of the tissue is in a great measure responsible for this variability, although inherent differences may possibly play a part. In the experiments with potato the same variety was used throughout so that inherited differences should be reduced to a minimum. It is a matter of common knowledge that organs such as potato and carrot gradually lose water during storage, and evidently different degrees of desiccation of different samples will give rise to notable differences in regard to absorption or exudation of water.

In Table I are shown the amounts of water absorbed by nine separate lots of potato discs cut at the same time and subjected to the same experimental conditions. In these experiments, as they were cut, the discs were dried between blotting-paper and weighed out into lots of 20 gm. It will be observed how wide are the variations between individual samples, and that these are indeed due to differences in the tissue itself is indicated by the fact that the differences are maintained throughout the experiment.

TABLE I.

Water absorbed by 20 gm. of Potato from Distilled Water.

Sample.	Gain in Weight of 20 gm. of Potato after various times.				
	0.8 hr.	3.8 hrs.	18.15 hrs.	47.3 hrs.	70.8 hrs.
	gm.	gm.	gm.	gm.	gm.
1	0.52	1.08	2.20	2.77	3.04
2	0.53	1.37	2.49	3.05	3.22
3	0.59	1.43	2.63	3.31	3.60
4	0.36	1.08	2.31	2.60	3.40
5	0.70	1.49	2.93	3.66	4.12
6	0.61	1.63	2.63	3.28	3.60
7	0.56	1.36	2.33	2.70	3.24
8	0.50	1.39	2.33	3.00	3.67
9	0.57	1.53	2.47	3.17	3.57
Mean	0.55	1.37	2.48	3.05	3.50
P _M	±0.02	±0.04	±0.07	±0.075	±0.07

It is possible to form an idea of the degree of accuracy of the mean values by calculating their probable error. From Table I it will be observed

that the probable errors (P_M) of the mean results there recorded lie between 2 and 4 per cent.

With carrot the variability of the tissue is somewhat greater, as the following table shows:

TABLE II.

Water absorbed by 20 gm. of Carrot from Running Tap-water.

Sample.	Increase in Weight of 20 gm. of Carrot after various times.			
	21.03 hrs.	44.4 hrs.	68.8 hrs.	123.5 hrs.
	gm.	gm.	gm.	gm.
1	2.53	2.41	2.60	2.48
2	2.52	2.20	2.54	2.57
3	2.24	2.37	2.74	2.69
4	2.14	2.26	2.56	2.46
5	3.09	2.83	3.00	3.00
6	3.21	3.36	3.66	3.66
7	2.79	3.00	3.57	3.61
8	3.10	3.34	3.25	3.22
9	2.66	3.00	2.74	2.87
Mean	2.70	2.75	2.96	2.95
P_M	± 0.08	± 0.10	± 0.07	± 0.10

It is thus possible when working with nine samples to obtain results in which the probable error of the mean result of the nine samples is about 3 per cent. Thus differences of about 9 per cent. have a reasonably certain significance in relation to different experimental conditions. This is not a particularly high order of accuracy having regard to the labour entailed when sets of nine different samples are used. We therefore adopted the following method in order to reduce the sampling error:

All the discs required for a particular experiment in which the results were to be compared were cut, dried with blotting-paper, and then thoroughly mixed before they were weighed out into lots of 10 or 20 gm. This preliminary mixing has the result of reducing the sampling error considerably, as the following table shows. Thus when carrot is used with only four different samples the probable error of the mean does not generally rise much above 2 per cent. With potato it is possible to work with sets of three samples and obtain this same degree of accuracy.

TABLE III.

Water absorbed by 20 gm. of Carrot from Distilled Water.

Sample.	Increase in Weight of 20 gm. of Carrot after various times.					
	0.5 hr.	1.0 hr.	2.0 hrs.	5.0 hrs.	22.5 hrs.	49.5 hrs.
	gm.	gm.	gm.	gm.	gm.	gm.
1	1.43	1.95	2.75	3.17	3.48	3.65
2	1.50	2.11	3.10	3.45	3.87	4.00
3	1.47	2.02	2.85	3.10	3.40	3.75
4	1.47	1.94	2.74	2.92	3.19	3.62
Mean	1.47	2.00	2.86	3.16	3.47	3.75
P_M	± 0.01	± 0.03	± 0.06	± 0.07	± 0.10	± 0.06

In the majority of cases the differences recorded are many times larger than any that could be accounted for by sampling error, and they may therefore be regarded as having a real significance.

By this method it is only those experiments conducted at the same time with material from the same general sample of mixed discs that can be directly compared, as otherwise differences due to previous history and inherent causes may become evident. An example of this we shall have an occasion to quote in the section dealing with the influence of temperature.

We may take this opportunity of calling attention to the necessity of determining the error of experiment in plant physiological work. Probably little plant tissue is so uniform as potato and carrot, and it is shown here how necessary it is, even with this tissue, to determine the probable error of experiment before drawing conclusions from observed differences in behaviour in relation to different conditions. In very few plant physiological researches has this very necessary calculation been made, with the result that conclusions are constantly drawn for which there is really no experimental evidence.

EXPERIMENTAL RESULTS.

(a) *The Swelling of Potato and Carrot in Water.*

When living tissue of potato or carrot is immersed in distilled water there is a passage of water into the cell. The rate of this process, rapid at first, gradually slows down until a condition of equilibrium is reached.

The curves marked P_D and C_D in Fig. 1 illustrate the general course of this swelling in distilled water at 13°C . It will be observed that, in the case of carrot, 90 per cent. of the total swelling takes place during the first two days. The maximum swelling is reached after about six days; little alteration takes place during the next six days, at the expiration of which time the experiments were discontinued.

FIG. 1. Swelling of potato and carrot in distilled and in tap water. C_D , carrot in distilled water; C_T , carrot in tap-water; P_D , potato in distilled water; P_T , potato in tap-water.

The course of swelling with the potato is similar, but the initial rate of swelling is less rapid (see Fig. 2). This fact we have noticed throughout our experiments. Equilibrium is, however, reached in about the same time as with carrot, and is maintained for a considerable time just as with that tissue.

The behaviour of the two tissues with tap-water is different. With carrot there is the same rapid initial rate of swelling, but the maximum value of this is less and is reached somewhat sooner, after which there is a slight, though distinct, gradual shrinkage of the tissue for the next eleven days. Having regard to the course of events observed with potato to be immediately described, there is little doubt that this shrinkage would have continued until death of the tissue finally resulted.

The line in Fig. 1 marked P_T illustrates the course of swelling of potato in running tap-water. The tissue swells at first in the same way as in distilled water. As with carrot a lower maximum swelling is reached than with distilled water, and in this case after about 2.5 days, and then immediately a shrinkage of the tissues sets in, which was continued very uniformly until the end of the experiment. Thus, after 17 days in running tap-water, the potato tissue had shrunk to its original weight.

FIG. 2. Swelling of potato and carrot in distilled water. C_D , carrot in distilled water; P_D , potato in distilled water.

De Vries and Pfeffer explained the plasmolysis of plant tissue in more concentrated solutions of various substances on the theory that the limiting layer of the protoplasm of the cell acts as a semi-permeable membrane, allowing the passage of water, but not permitting the passage of the particular solutes. On this view such a swelling as we have recorded for potato and carrot would have to take place, if it is assumed that the various crystalloidal substances of the cell cannot pass through the membrane, or can pass only with great difficulty and slowness. If the cell wall were absolutely elastic the intake of water should continue indefinitely, as equilibrium could only be reached when there was the same osmotic concentration inside and out. On the other hand, if the cell wall were perfectly inelastic and quite incapable of stretching, water would pass in as before on account of the difference in osmotic concentration on the two sides of the cell boundary. This will result in an increase in pressure inside the cell which tends to force water out of the cell. Under these conditions water will pass in by osmosis until the pressure forcing water into the cell by osmosis is equal to the pressure forcing water out of the cell owing to its compression inside. In this condition the cell will be in equilibrium with the external medium in regard to the exchange of water.

In reality the condition of affairs is intermediate between these two extremes. Although the rigidity of the cell wall prevents indefinite increase in volume of the protoplast, yet the wall is capable of a certain amount of stretching so that a certain increase in volume takes place. Hence the actual course of events may be considered as follows: Water passes into the cell in consequence of the difference in osmotic pressure on

the two sides of the plasma membrane. This produces an increase in pressure inside the cell and in consequence the cell wall stretches. As the water passes in, the pressure inside the cell increases, and continued stretching of the cell wall takes place until ultimately the pressure of the water inside is equal to the osmotic pressure forcing water into the cell.

That such high turgor pressures can exist in the cell as are indicated by these considerations has been generally admitted by physiologists, and they are indicated also by the very turgid condition of discs of tissues so swollen in distilled water.

We have considered the swelling of plant tissue in water in terms of the original theory of de Vries and Pfeffer. However, we may perhaps indicate that we use this theory only as a working hypothesis which explains the facts; we do not wish to urge that it is the only hypothesis which will explain the facts or that it is either correct or complete.

The depression of swelling observed when tap-water is used instead of distilled water may be explained on this hypothesis by the supposition that certain salts in the tap-water are absorbed and bring about an increase in permeability of the cell. The subsequent diffusion out of dissolved substances from the cell as a result of this lowers the osmotic pressure of the cell, and so water passes out into the external liquid.

(b) *The Influence of Temperature on the Swelling of Potato and Carrot in Water.*

Our earlier experiments on the influence of temperature on the swelling of plant tissue were made on a number of different days. The results obtained showed no correlation between the swelling and the temperature, as the curves exhibited in Fig. 3 indicate.

Such a result can only be due to variations in the samples used in the experiments conducted on different days. The sampling error is indeed reduced as regards the comparison of results obtained from experiments started at the same time, by the thorough mixing of the discs as already described; but the error is not eliminated as regards experiments done at different times, and necessarily on different samples of potatoes or carrots.

In order therefore to avoid this difficulty the measurements of swelling at different temperatures were made at the same time on discs all taken from the same mixed sample. The swelling at four temperatures was measured, the vessels containing the discs being kept during the experi-

FIG. 3. Swelling of different samples of carrot at different temperatures.

ment in four different thermostats. The swelling of potato at 10° C., 20° C., 30° C., and 40° C. measured in this way is shown in Table IV.

TABLE IV.
Swelling of Potato in Distilled Water at Different Temperatures.

10° C.		20° C.		30° C.		40° C.	
Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.
		0.65	5.6	0.53	9.1	0.60	19.6
1.23	4.4	1.27	8.7	1.28	12.6	1.30	14.4
1.95	5.8	2.00	10.2	2.00	15.1	2.02	10.3
2.85	7.2	2.87	11.8	2.87	17.7	2.87	5.8
4.98	8.9	5.03	14.5	5.05	20.6	5.08	-5.8
6.57	10.3	6.65	16.2	6.67	22.5	6.08	-8.2
18.80	19.8	19.23	20.6			19.68	-14.6

These results are shown graphically in Fig. 4. They clearly illustrate the fact that the temperature exerts a marked influence on the rate of

FIG. 4. Curves illustrating the influence of temperature on the swelling of potato in distilled water.

water absorption by potato tissue—the higher the temperature the more rapid the swelling. With higher temperatures, however, secondary actions occur which transform the swelling into a rapid shrinkage. Measurements were not taken at temperatures higher than 40° C., but the results obtained leave little doubt that the initial period of swelling would be less at higher temperatures, and the shrinkage still more rapid. The results are analogous to those obtained by F. F. Blackman for carbon assimilation, where assimilation falls off with time at higher temperatures owing to secondary actions.

The influence of temperature on the swelling of carrot is similar to its influence on potato, although it is not so marked. The numbers obtained by experiments are given in Table V.

TABLE V.
Swelling of Carrot in Distilled Water at Different Temperatures.

10° C.		20° C.		30° C.		40° C.	
Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.
0.63	9.4	0.52	10.8	0.52	12.6	0.58	12.0
2.27	15.2	2.28	16.0	2.28	16.5	2.35	10.9
3.13	16.6	3.15	17.2	3.13	17.3	3.22	8.8
14.90	20.1	15.13	18.8	15.08	16.6	15.23	-1.7

There will again be observed the increase in the rate of swelling with

increased temperature and the subsequent shrinkage at higher temperatures. It would appear that even after half an hour the shrinkage at 40° C. has already commenced. In Fig. 5, where these results are shown graphically, the first part of the curve of swelling at this temperature is assumed.

From the values obtained from fifteen to twenty hours, in both the cases of carrot and potato, it seems reasonable to conclude that temperature exerts no marked influence on the equilibrium value of maximum swelling, apart from the secondary action at higher temperatures to which reference has already been made.¹

The rate of swelling at any particular moment is given by the tangent of the angle made with the time axis by the tangent drawn to the curve at that particular point.

In order to measure the effect of temperature on swelling it is necessary to compare the rates of swelling at the same stage of swelling at the different temperatures.

The numbers given in Table VI are obtained in this way. The table shows the comparative rates of swelling at different temperatures when the tissue has increased 10 per cent. in weight, i.e. when it has swollen to about half the maximum possible swelling.

It does not follow, however, that carrot and potato are in the same absolute stage of swelling, as we cannot conclude that the initial conditions of the two tissues are the same in regard to water-content and turgor pressure. Nevertheless, although the comparison of the temperature coefficients of potato and carrot obtained here must be made with a certain amount of reservation, yet the recorded differences are striking and indicate considerable differences in constitution of the two tissues.

TABLE VI.

Rate of Swelling of Potato and Carrot at Different Temperatures.

Temperature.	Potato.	Carrot.
10° C.	0.15	1.3
20° C.	0.45	1.7
30° C.	1.2	2.5

These numbers give the following values for the temperature coefficient of swelling:

TABLE VII.

Temperature Coefficients of Swelling of Potato and Carrot.

Temperature.	Potato.	Carrot.
10°–20° C.	3.0	1.3
20°–30° C.	2.7	1.6

¹ Cf. Pfeffer (14), p. 138: 'Since by a rise of temperature of 15° C. the (osmotic) pressure is only raised from 100 to 105.5, it is evident that temperature can never exercise any marked direct effect turgor in plants.'

FIG. 5. Curves illustrating the influence of temperature on the swelling of carrot in distilled water.

It is not advisable to enter into a discussion of the meaning of these temperature coefficients while our analysis of the processes involved is so incomplete. As the rate of swelling is dependent not only on the permeability of the protoplasm to water, but also on the elasticity of the cell wall and probably on other factors also, as for instance permeability to substances dissolved in the cell sap, it is clear that at present we can do no more than record the fact that temperature increases the rate of swelling in the manner indicated. Moreover, it does not follow that tissue in which the inherited factors are different or which has previously been subjected to different external conditions will give the same values for the temperature coefficient of swelling as recorded here.

(c) *The Influence of Dissolved Substances on the Swelling of Potato.*

I. *Sodium chloride.*

It was shown by de Vries that above a certain concentration of solute, solutions of various substances brought about a contraction of the protoplasm of the cell away from the wall. This was explained by de Vries and Pfeffer as due to the outer layer of the protoplast acting as a semi-permeable membrane which allowed water to pass through, but which prevented the passage of the dissolved substance. A solution of the same osmotic concentration as the cell sap should produce no effect whatever, but above that concentration it should bring about plasmolysis. Thus, the terms hypotonic, isotonic, and hypertonic were introduced to describe solutions respectively lower, equal, or higher in osmotic concentration than the cell sap.

If in the case of hypertonic solutions the cell wall should shrink to some extent along with the protoplast, water should be exuded and the tissue should lose weight. As in distilled water the cell wall stretches, so it is reasonable to expect it to be capable also of shrinkage, and the experiments of Miss Delf (6), in which the rate of shrinkage in length of tissues in such solutions is measured, show that this is the case.

The following table shows the swelling and shrinkage of potato tissue in various strengths of sodium chloride solution. The values are given in terms of percentages of the original weight of the tissue :

TABLE VIII.

Swelling and Shrinkage of Potato in Sodium Chloride Solutions.

<i>Distilled Water.</i>		$\frac{N}{12}$ NaCl.		$\frac{N}{10}$ NaCl.		$\frac{N}{8}$ NaCl.		$\frac{N}{7}$ NaCl.		$\frac{N}{6}$ NaCl.	
Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.
0.57	6.4	1.15	4.0	0.75	1.8	0.92	0.6	1.72	-1.6	1.62	-4.6
3.12	13.6	3.28	3.6	3.18	1.8	3.25	1.2	2.42	-3.0	2.42	-5.6
6.80	17.2	7.12	2.6	6.92	1.2	5.83	0.4	3.25	-3.0	3.20	-6.8
21.80	22.4	22.03	2.8			7.00	0.2				

These results are shown graphically in Fig. 6. They show clearly the effect of increasing quantities of sodium chloride in the external solution in producing a progressive decrease in swelling until above a certain concentration shrinkage is produced.

It will be observed how, after a certain time, the swelling or shrinkage reaches a position of approximate equilibrium which is maintained for at least sixteen hours. On the de Vriesian view this indicates that the sodium chloride does not enter the tissue in any quantity. For if it did enter the cell it would either alter the permeability or it would not. In the latter case, since the substances dissolved inside the cell cannot pass out, the osmotic pressure inside would be raised, and, when the concentration of sodium chloride inside and outside was the same, the tissue should ulti-

FIG. 6. Curves illustrating the absorption and excretion of water from potato in sodium chloride solutions of various concentrations.

FIG. 7. Curves illustrating the absorption and excretion of water from potato in sucrose solutions of various concentrations.

mately swell to the same extent as in distilled water. Similarly, if the permeability to the substances inside the cell were decreased, there would still be swelling as in distilled water. On the other hand, if the permeability were increased so that dissolved substances inside the cell diffused out, one would expect increased swelling, at first due to the increase in salt concentration inside the tissue, followed by a shrinkage as the permeability increased and the internal solutes diffused out. This is not indicated by the curves obtained with sodium chloride, although such is indeed the course of events observed with some substances which for other reasons we know bring about an increase of permeability.

It will be noted that with these samples of potato a solution of $\frac{N}{8}$ sodium chloride was approximately isotonic on the osmotic hypothesis with the cell sap.

A similar series of experiments with carrots showed, however, that a solution of sodium chloride of a concentration of about $\frac{N}{3}$ was required to reduce the swelling to zero.

2. *Sucrose.*

In Fig. 7 are shown graphically the results obtained for the swelling of potato in solutions of cane sugar of various concentrations. It will be observed that the results are almost identical with those obtained with sodium chloride. A solution of concentration $\frac{M}{4}$ is approximately isotonic, the shrinkage in this solution being almost negligible. Thus, an isotonic solution of sucrose has a concentration of almost double that of an isotonic solution of sodium chloride. This is exactly the result to be expected as sucrose is completely undissociated in solution, while sodium chloride is almost completely dissociated into its constituent ions.

3. *Ethyl alcohol.*

In the solutions of ethyl alcohol employed the general course of swelling is quite different from that obtained with sodium chloride or sucrose.

FIG. 8. Absorption and excretion of water by potato in solutions of ethyl alcohol of various concentrations.

If ethyl alcohol behaved similarly to these substances we should expect a concentration of ethyl alcohol of about $\frac{M}{4}$ to be isotonic, and solutions of higher concentration to bring about shrinkage and those of lower concentration swelling. As a matter of fact swelling takes place at first in much higher concentrations than this. Thus, potato swells at first in a solution eight times as strong ($2M$). In this strength, however, the swelling phase is of short duration and reaches a low maximum after about half an hour.

Shrinkage then commences and continues for at least sixteen hours, after which time the tissue may have lost in weight by about 20 per cent. (Fig. 8). In higher strengths than 2 *M* the preliminary swelling phase is reduced, and in solutions of 5 *M* and 10 *M* shrinkage is considerable after half an hour. It is clear that the higher the concentration of alcohol the more rapid is the rate of shrinkage.

With concentrations less than 2 *M* the preliminary swelling period is more marked. Thus, in a gram-molecular solution maximum swelling is reached after about ten hours and is a higher value than the maximum swelling in the case of a 2 *M* solution. With $\frac{M}{2}$ ethyl alcohol the maximum is not reached even after sixteen hours, nevertheless the swelling is lower than in distilled water.

The explanation of these phenomena on the membrane hypothesis is simple. Ethyl alcohol is supposed to penetrate the membrane, and in so doing to alter its constitution in such a way that it becomes permeable to the dissolved substances inside the cell. In the first stage of the process, therefore, the rise in osmotic pressure inside the cell owing to the addition of the alcohol produces swelling in what would have been hypertonic solutions if the alcohol had not passed through. If the membrane remained unaltered the cell should ultimately swell as much as in distilled water, provided no reactions took place between the alcohol and cell contents. But if the membrane is rendered more permeable to the solutes dissolved in the cell sap, these will diffuse out of the cell, slowly at first, then more rapidly as more of the membrane is changed, and finally more slowly again as the difference in concentration inside and outside of the cell becomes small. The exosmosis of the dissolved substances reduces the osmotic pressure inside the cell, which therefore loses water to the external medium. As the destruction of the membrane will take place more rapidly the higher the concentration of the alcohol, the exosmosis from the cell, and consequently the shrinkage, will be more rapid the higher the concentration. The curves given in Fig. 8 show that this is exactly what actually occurs.

Moreover, that the exosmosis of dissolved substances takes place as this explanation supposes, has already been shown in regard to electrolytes in the preceding paper in this series (16). Reference may be made to the series of curves obtained for ethyl alcohol given in Fig. 6 of that paper, in which the exosmosis of electrolytes from potato tissue immersed in various strengths of ethyl alcohol is shown. It will be observed how exactly the exosmosis curves and swelling curves correspond.

It is thus necessary to distinguish between the shrinkage due to plasmolysis and that due to injury. So Osterhout (11) distinguishes between true and false plasmolysis, the latter being the shrinkage which results from injury.

4. *Octyl alcohol.*

In the preceding paper of this series it was shown how the complexity of the molecule in the series of monohydric alcohols influenced the exosmosis of electrolytes. The higher the alcohol the lower is the concentration which produces equal exosmosis. This relation ought also to appear in regard to the swelling of tissue in solutions of different alcohols; that is, the higher the alcohol the lower should be the concentration required to produce the shrinkage which is correlated with exosmosis.

This is found to be so, and the case of octyl alcohol, the most complex alcohol used, may be cited. It was previously found (17) that the exosmosis produced by a 1.4 *M* solution of ethyl alcohol was produced by a concentration of a secondary octyl alcohol

of 0.003 *M*. The swelling in three strengths of this alcohol are indicated in the following table. In 0.001 *M* the swelling is approximately the same as in distilled water. In 0.002 *M* the swelling is the same as in 0.001 *M* after one or two hours, but after sixteen hours the swelling is depressed just as in the case of 0.5 *M* ethyl alcohol. In a solution of concentration 0.003 *M* the swelling is the same after 1.3 hours, but by the end of sixteen hours there is a great shrinkage.

TABLE IX.

Swelling of Potato in Solutions of a Secondary Octyl Alcohol.

<i>Time in hours.</i>	<i>Swelling in percentages of original weight.</i>			
	0.000	0.001 <i>M</i> .	0.002 <i>M</i> .	0.003 <i>M</i> .
1.33	7.2	7.2	7.3	7.4
16.50	16.9	16.9	13.9	-19.2

The form of the swelling curves given for ethyl alcohol may be regarded therefore as the typical form of such curves when the substance in solution outside the tissue enters the cell and produces changes which render the cell permeable to the solutes inside.

5. *Sulphuric acid.*

That alcohols enter the cell is not easy to show by direct measurement; it is, on the other hand, comparatively easy to show by direct measurement that acids, or at any rate the hydrogen ions of their molecules, are readily absorbed by plant cells. This has been done in the first three papers of this series (15, 16, 8), and it has also been shown (14, 8) that the absorption of the hydrogen ion is accompanied by the exosmosis of electrolytes from the cell. It would be expected then that the swelling of potato in acids would follow a similar course to the swelling in alcohols, that is, there would be a preliminary swelling, the magnitude of which

would depend on the dilution of the acid, followed by a shrinkage which would appear sooner the stronger the solution.

Fig. 9 shows that this is the case. With $\frac{N}{50}$ sulphuric acid the maximum swelling is reached in an hour, and with acid as dilute as $\frac{N}{1,000}$ the tissue begins to shrink after four hours' immersion. Shrinkage was observed in $\frac{N}{2,000}$ acid by the sixth hour of immersion, and even $\frac{N}{5,000}$ acid brought about shrinkage before the elapse of twenty-four hours.

It will be observed that in the lower strengths of sulphuric acid the swelling is at first actually greater than in distilled water. It is possible that this may be referred to the well-known phenomenon of increased swelling of proteins in acid solutions as compared with pure water, a phenomenon on which much stress has been laid by Fischer (7), who successfully shows the similarity between the swelling of such proteins as gelatine and blood-fibrin with certain animal tissues, frog's muscle and sheep's eyes.

The observed increased swelling of potato in dilute acid solutions appears to be a definite increase and not merely due to variations in the samples, as the differences are for the most part considerably greater than the probable error. These are given in Table X.

FIG. 9. Curves illustrating influence of different concentrations of sulphuric acid on the swelling of potato.

TABLE X.

Swelling of Potato in Various Concentrations of Sulphuric Acid.

Distilled Water.		$\frac{N}{5,000} H_2SO_4$		$\frac{N}{2,000} H_2SO_4$		$\frac{N}{1,000} H_2SO_4$		$\frac{N}{50} H_2SO_4$	
Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.	Time. hrs.	Swelling. per cent.
0.50	4.0 ± 0.05	0.58	5.7 ± 0.2	0.50	4.5 ± 0.0	0.50	4.3 ± 0.0	0.25	0.37
2.00	9.4 ± 0.3	2.23	10.7 ± 0.25	2.17	9.9 ± 0.2	2.00	9.5 ± 0.25	1.02	0.53
3.60	11.2 ± 0.4	3.67	12.8 ± 0.3	3.63	12.1 ± 0.15	3.58	10.0 ± 0.3	2.52	-0.40
5.85	12.7 ± 0.35	5.88	14.0 ± 0.35	5.87	11.8 ± 0.25	5.88	7.3 ± 0.25	3.32	-1.16
7.00	13.5 ± 0.3	7.05	14.8 ± 0.25	7.03	11.4 ± 0.25	7.00	5.1 ± 0.4	4.37	-1.59
21.90	17.4 ± 0.15	24.18	13.0 ± 0.1	24.78	2.6 ± 0.4	24.20	-7.5 ± 1.3		

However, as we hope to deal on a later occasion both with the question of the action of dilute solutions and with the general behaviour of plant tissue towards acids, we do not propose to enter into a detailed discussion of these results here.

CONCLUDING REMARKS.

In the preceding pages we have recorded the results of some preliminary experiments on the exchange of water between the plant cell and the surrounding medium. The chief difference between these experiments and most of the earlier ones on the same subject lies in the fact that the method employed is quantitative and the reaction is followed with time. In work with such complex systems as the plant physiologist has to deal with, it is extremely easy to draw incorrect conclusions and to refer observed effects to wrong causes, and this is especially the case where the course of the reaction with time is not followed. It is only necessary for us to refer to the conclusions drawn by van Rysselberghe (18) from his experiments on our subject, as an example of this. Thus, Miss Delf (6) has recently shown that van Rysselberghe's conclusions are false, and that it would have been impossible for him to have drawn them if he had made measurements in his experiments from time to time. Again, it is only by quantitative work that an analysis of complex processes will ever be made and the laws underlying the processes discovered. The whole history of physics and chemistry exemplifies this.

By far the most important work on the subject here dealt with which has appeared since Pfeffer's fundamental researches, is that of Miss Delf (6) on the effect of temperature on the passage of water out of the cell when slightly plasmolysed, in which the method employed was quantitative and sensitive and in which the change with time was followed. Her conclusion that the rate of passage of water out of the cell is greatly increased by rise of temperature agrees well with the results of the authors' experiments, which show a similar effect of temperature on the rate of intake of water by the cell surrounded by distilled water.

As regards the magnitude of the coefficient, it is impossible to make any definite statement. The actual rate of swelling depends on the previous history (environmental and genetic) of the tissue employed, and it is at least not improbable that the temperature coefficient of swelling may show similar variations with the previous history. For there are at least four different processes concerned, namely, the absorption of water by the cell wall, the absorption of water by the protoplasm, the passage of water into the vacuole, and the stretching of the cell wall, and if these are differently affected by temperature and also by the factors to which they are subjected by previous history, the temperature coefficient of swelling will depend on the previous history.

This complexity of the system, as we have already said, makes it difficult to draw conclusions as to the causes of the observed phenomena. With quantitative data and proper methods, however, mathematical treatment becomes possible and will in the future no doubt be a great aid in the analysis required for an understanding of the processes of plant physiology. However, although mathematical treatment is possible in regard to the subject dealt with in this paper, we have avoided this, as it seems to us considerably more data are required before this can be done profitably in this connexion.

The explanations so far put forward to account for swelling and plasmolysis probably do not take enough account of the complexity of the system. Of the two chief theories, one, that of de Vries and Pfeffer, explains the absorption of water on the basis of semi-permeable membranes and osmosis.¹ The protoplast is supposed to be surrounded by membranes which are permeable to water and impermeable to certain substances.

The argument advanced against this theory, that salts get into the plant, and therefore the plasma membrane cannot be impermeable to salts, is frivolous, for in the first place the plasma membrane may undergo reversible changes in regard to its permeability,² and, secondly, plasmolysis would still take place if the membrane were not absolutely semi-permeable (as no membrane is) but let through the salt more or less slowly.

Although we do not think the theory of de Vries is likely to be a complete expression of the facts, yet we may point out that there is not a fact recorded in this paper which cannot be explained on its basis. The swelling of tissue in distilled water, the plasmolysis in sodium chloride and sucrose, the behaviour in solutions of substances with poisonous properties and which are known to enter the cell, such as acids and the primary alcohols, are all phenomena which follow directly from the semi-permeable membrane theory. On the other hand, unpublished work of Stiles and Kidd indicates that sodium chloride passes rapidly into the cells of potato and carrot, while Brooks (2), using de Vries's method of tissue tension, concludes that sucrose as well as sodium chloride passes readily into the scapes of the dandelion.³

Such difficulties as this have caused some workers on animal cells, e.g. Moore, Roaf, and Webster (9, 10) to reject the osmotic theory altogether and to explain all permeability phenomena as due to the presence of

¹ Pfeffer was well aware of the capacity of protoplasm to absorb water on account of its colloidal nature, but apparently did not regard it of importance in such observations as are recorded here. Cf. Pfeffer (14), p. 136.

² 'Apparently it is by temporary or permanent alterations in the specific nature of the plasmatic membrane that an absorption (or excretion) of a particular substance may be temporarily or permanently permitted or prevented' (Pfeffer, 14, p. 102).

³ Pfeffer points out (14) that the formation of starch in the plastids, or the accumulation of other carbohydrates, when plants are supplied externally with sugar, indicates that sugar can be absorbed under certain circumstances.

a colloid in contact with a crystalloidal solution. The difficulties of this theory (see e. g. Bayliss, 1) are so great, and it has explained so little, that it has found practically no support. Martin Fischer (7), as a result of an extensive series of experiments with gelatine, blood fibrin, frog's muscle, and sheep's eyes, has also rejected the osmotic theory and attempted to explain swelling of animal and plant tissue and related phenomena as due to the colloidal nature of the tissue. In view of the fact that the causes of the swelling of colloids are not by any means settled, and especially when it is considered that the swelling may be an osmotic phenomenon, this explanation of the swelling of living tissue is not so far very helpful. Probably the most striking result of Fischer is the exact similarity in the effect on acids in increasing swelling of gelatine and blood fibrin on the one hand, and muscle and sheep's eyes on the other. This may very well be the same phenomenon, and the indication of an increased swelling in dilute acid in our experiments may also be due to the colloidal nature of the protoplast, but it scarcely justifies Fischer's conclusion that in all cases the swelling in water is due to the production of acid as a result of the conditions of experiment. In our experiments a very low hydrogen ion concentration is sufficient to kill the cell, which would appear to dispose completely of the view that the total swelling of plant tissue in water is due to the formation of acid in the protoplast.

In such a complex organization as the cell it is unlikely that any one single explanation will explain all the phenomena of permeability. We know that there are at least three structures of which account must be taken: the cell wall, the protoplast, and the vacuole. We also know that at the junction of two immiscible phases the properties of the material may be different from those in the body of either phase, and indeed we find that de Vries postulated two limiting membranes to the protoplast, an outer one surrounding the protoplast (the plasma membrane or ectoplast), and an inner one surrounding the vacuole (the vacuole wall or tonoplast).¹ It would for instance be reasonable to suppose that the protoplast behaved towards external solutions in much the same way as gelatine or blood fibrin, while at the same time it formed a semi-permeable membrane between the vacuole and external solution.² Such a theory might be elaborated to explain the permeability of the cell, but we feel, as we have already emphasized in previous papers in this series, that we know so little

¹ The arguments for the existence of plasmatic membranes with diosmotic properties distinct from those of the body of the protoplast have been stated by Pfeffer (13). They cannot be regarded as very convincing.

² 'The general principles previously laid down as governing the exchanges of substance taking place between the protoplast and the external world would still hold good, even though no differences existed between the diosmotic properties of the general mass of the protoplast and the limiting membranes which enclose it; if, for example, the entire thickness of the primordial utricle behaved like a single very thick plasmatic membrane' (Pfeffer, 14, p. 107).

of the facts of permeability, that a great amount of experimental work is needed before the presentation of fresh theories is justified.

SUMMARY.

1. A method for investigating the passage of water between the cell and its surroundings is described, which is quantitative and allows the investigation of the kinetics of the changes taking place.

2. The probable error of experiment is determined, and the means of reducing it indicated.

3. The tubers of potato and roots of carrot in distilled water absorb water (swell) for some days before equilibrium is attained. Equilibrium is maintained for a considerable time.

4. The rate of swelling of carrot is much greater than that of potato.

5. The previous history of the tissue influences greatly the magnitude of the swelling.

6. In tap-water, i. e. a weak solution of a number of salts, the magnitude of swelling is less than in distilled water and equilibrium is not maintained for so long (especially in the case of potato), a shrinkage of the tissue supervening. This is presumably due to the absorption of substances which cause an increase in permeability.

7. Increase of temperature increases the rate of swelling. The temperature coefficients (Q_{10}) determined were, for carrot, about 1.5, and for potato, about 3.0. These are probably not definite values characteristic of the tissue, but probably vary with the previous history of the tissue.

8. In solutions of sodium chloride and sucrose the swelling of potato is reduced with increase of concentration of the solution. With the sample of tissue used sodium chloride produced no swelling in a concentration of about $\frac{N}{8}$ and sucrose in a concentration of about $\frac{M}{4}$. No swelling of the

carrot used took place in a concentration of about $\frac{N}{3}$ sodium chloride. With concentrations above these values swelling took place; with lower concentrations, shrinkage. The shrinkage obtained in these cases is identical with the shrinkage usually called plasmolysis.

9. In the case of certain substances which enter the cell, such as the primary alcohols, preliminary swelling takes place in solutions of much higher concentration than isotonic solutions as determined by experiments with sodium chloride and sucrose. Subsequently shrinkage or depressed swelling occurs in all concentrations, which is correlated with the alteration of the permeability of the protoplasm and the death of the tissue. This shrinkage is due to toxic action and not plasmolysis.

10. Acids behave similarly to the primary alcohols, and produce the preliminary swelling and the subsequent shrinkage correlated with toxic action.

11. The bearing of the facts described on some theories of permeability is discussed.

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