THE ABSORPTION OF WATER BY PLANT TISSUE IN RELATION TO EXTERNAL HYDROGEN-ION CONCENTRATION

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(With Three Text-figures.)

In previous papers we have considered in some detail the properties of plant tissues in relation to the hydrogen-ion concentration of the external medium, and our observations have led us to regard some of those properties as being due to the behaviour of the proteins present in the tissues, and in particular to the positions of the isoelectric points of those predominant in the tissues. It is now proposed to consider the water absorption of plant tissues from a similar point of view, and to correlate the results of such a study with the data obtained when the swelling and precipitation of proteins over a pertinent range of hydrogen-ion concentration are investigated.

In order to put our facts on as sound a basis as possible, tissues of various kinds and in different conditions have been examined, our material consisting of potato, turnip and swede tissue, in the living, in the dead, and also in the dried and ground condition. One of the chief factors concerned in water absorption by a living tissue is of course osmosis, and the use of dead and dry ground tissue served to eliminate the osmotic system set up by the presence of living protoplasmic membranes in the cells of the tissue. When, however, the results were tabulated and worked out, it was found possible to explain the behaviour of the living tissue in terms of the action of solutions of various hydrogen-ion concentrations on the protoplasm considered as protein material without reference to the osmotic factor.

The methods used in the cases of the living and dead tissues were very similar. Discs of uniform size and thickness were obtained by slicing cylinders of tissue cut with a large cork borer by means of a coarse hand microtome improvised from a safety-razor blade. Any discs showing peculiarity of structure or texture were immediately rejected. The remainder were washed to get rid of the *débris* incidental on cutting and then thoroughly mixed to distribute as far as possible the discs from any one cylinder of tissue. Sets of discs, each set of a minimum weight of 25 g., were blotted dry with filter paper, weighed, and immersed in a series of solutions of different hydrogen-ion concentration. The sets of discs were re-weighed at intervals after being again dried with filter paper. Any increase in weight was calculated as a percentage gain of the original weight. With potato tissue and white turnip this method appears to give consistent and reliable results, but with swedes considerable fluctuations were observed in successive experiments, so that it was

found necessary to use larger quantities of tissues, *e.g.* 40–50 g. The method of procedure outlined above was also used for dead but undried tissue.

For purposes of comparison and corroboration some experiments were carried out with dried tissue which was ground to a fine powder. Definite amounts (1, 2 or 5 g. in different experiments) were then weighed out and placed in solutions along the required *p*H gradient. For determining swelling, small bore measuring cylinders or flasks with graduated necks were used, and, in the latter case, after the powder had been shaken into suspension, the flasks were inverted and kept in the inverted position so that the amount of swelling could be read off on the graduated neck. In some cases only one quantity of tissue powder was used, the change in volume being noted as the reaction of the supernatant liquid was changed every few hours.

The solutions were brought to the required hydrogen-ion concentration by the addition of hydrochloric acid or sodium hydroxide to distilled water, as this method seemed to have the advantage of introducing only a small quantity of ions, so keeping the system relatively simple, and reducing the effect of ions to a minimum. The tendency towards a change in the hydrogen-ion concentration of the solutions when discs of tissue were used, owing to the rapid absorption of hydrogen-ions by the tissue below *p*H 6, was counteracted by using large volumes of solution (500 c.c.) and by renewing the solutions before the discs were replaced after every weighing. On *a priori* grounds it may seem more difficult for the system to reach equilibrium under this treatment, but the results were in entire agreement with those obtained in the check observations made by using buffered solutions of di-sodium phosphate and citric acid. These were used in dilute form to avoid a tendency to plasmolyse the tissues, and in order to keep constant the salt content—a probable source of error in such determinations—enough citric acid was added to *M*/100 disodium phosphate to bring the solutions to the required reactions. The change produced by the tissues in the hydrogen-ion concentration of these buffered solutions was very slight, and the results for water absorption obtained resembled the data previously got without the use of buffers. Determinations of the hydrogen-ion were made colorimetrically.

ABSORPTION OF WATER BY FRESH POTATO TISSUE.

The data obtained regarding the swelling of fresh potato tissue, already cited in a previous paper (8), are given in Table I, and expressed graphically in Fig. 1 *a*.

It will be noticed that water absorption is least after 10 hours' exposure at *p*H values 6·3, 5·4, 4·5 and 3·5. These points of minimum swelling are not materially altered if exposures are for longer or shorter periods, except that longer exposures increase the tendency to water loss below *p*H 3·5; and this tendency gradually moves towards the alkaline side of the *p*H gradient, until after 4 or 5 days all of the tissues in solutions of *p*H 4·3 and under tend to lose water and die. In solutions still more acid than those given here the water loss is still more pronounced. On the other hand, for alkaline solutions, *i.e.* of *p*H 7 and over, all our observations show a pronounced swelling. The data presented here are in fairly close agreement

with those of Robbins (9) in showing a point of minimum swelling at *p*H 5·6. In graph, however, Robbins has ignored points of minimum swelling other than that which he suggests as the isoelectric point for the tissue, while our own observations point to the conclusion that there is no one tissue isoelectric point. If a curve be made by joining points plotted from his tabulated data, it will be found similar to our own, with three points of minimum swelling.

Table I.

Absorption of water by potato tissue at different pH values.

*p*H	I	II	III	*p*H	I	II	III
2·8	5·6 g.	—	—	4·5	—	12·4 g.	12·4 g.
2·9	—	7·9 g.	—	4·7	13·9 g.	14·2	14·6
3·1	10·2	9·7	—	4·9	—	—	13·1
3·3	11·8	—	—	5·4	—	—	11·5
3·5	—	11·8	—	5·7	13·6	—	—
3·6	—	—	12·8 g.	5·9	—	13·3	—
3·7	12·7	12·4	—	6·0	13·9	—	—
4·0	14·2	—	—	6·1	—	—	12·4
4·1	—	—	13·7	6·3	—	12·2	12·1
4·3	—	—	13·8	7·1	14·7	—	—
4·4	13·7	—	—				

It is of interest to compare with the data of fresh potato tissue the swelling of the powder obtained when potato tissue is dried at 100° C. and ground to a fine powder, for in this case the protoplasm is coagulated and the semi-permeability of the protoplasmic membranes destroyed. Two sets of data are given in Table II, Fig. 1 *b* and *c*.

Table II.

Volume in c.c. of 2 g. of dried potato after different treatments.

I		II		III		IV		V	
*p*H	Vol.	*p*H	Vol.	*p*H	Vol.	*p*H	Vol.	*p*H	Vol.
2·9	18·9	**3·3**	**15·9**	2·8	17·6	4·7	17·4	3·3	16·8
3·1	17·8	3·8	17·3	3·0	16·6	5·1	16·9	3·4	17·3
3·2	**14·9**	4·6	17·5	*3·2*	*14·9*	*5·3*	*15·2*	3·7	17·2
4·6	17·7	5·5	17·3	3·6	16·7	5·4	16·4	4·1	17·6
4·9	16·3	5·9	17·0	4·9	16·5	5·6	17·2	4·6	18·0
5·1	**15·2**	6·0	16·8	**5·2**	**15·3**	5·8	16·8	5·1	16·2
5·4	17·1	6·1	16·7	5·4	17·0	6·2	16·2	6·2	16·5
5·9	17·1	6·2	18·0	5·9	17·0				
6·2	17·4	6·4	18·1	6·0	17·3				
6·4	17·7	6·6	18·2	6·1	17·2				
				6·2	17·3				

I and II—equal weights of potato in solutions of HCl of varying *p*H values for 12 hours.
III, IV and V—2 g. of tissue allowed to swell in water (12 hours), then acidified with successive doses of HCl, the volume being measured 1 hour after treatment.

In series I and II the volumes of 2 g. of the dried potato powder are given after ng left for 12 hours in solutions of different *p*H. In each of the series III, IV and V, however, a single mass of 2 g. was taken and allowed to swell in water for 12 hours. The solution was gradually acidified at intervals, the volume of the

mass being measured an hour after each change in acidity, this having been found a sufficient time for equilibrium to be attained if powder and solution were well shaken. Thus each of the figures given for series III, IV and V represents the volume of the same mass of ground tissue after being subjected to the action of solutions of different *p*H values. The graphical expressions of these results in Fig. 1 *b* and *c*

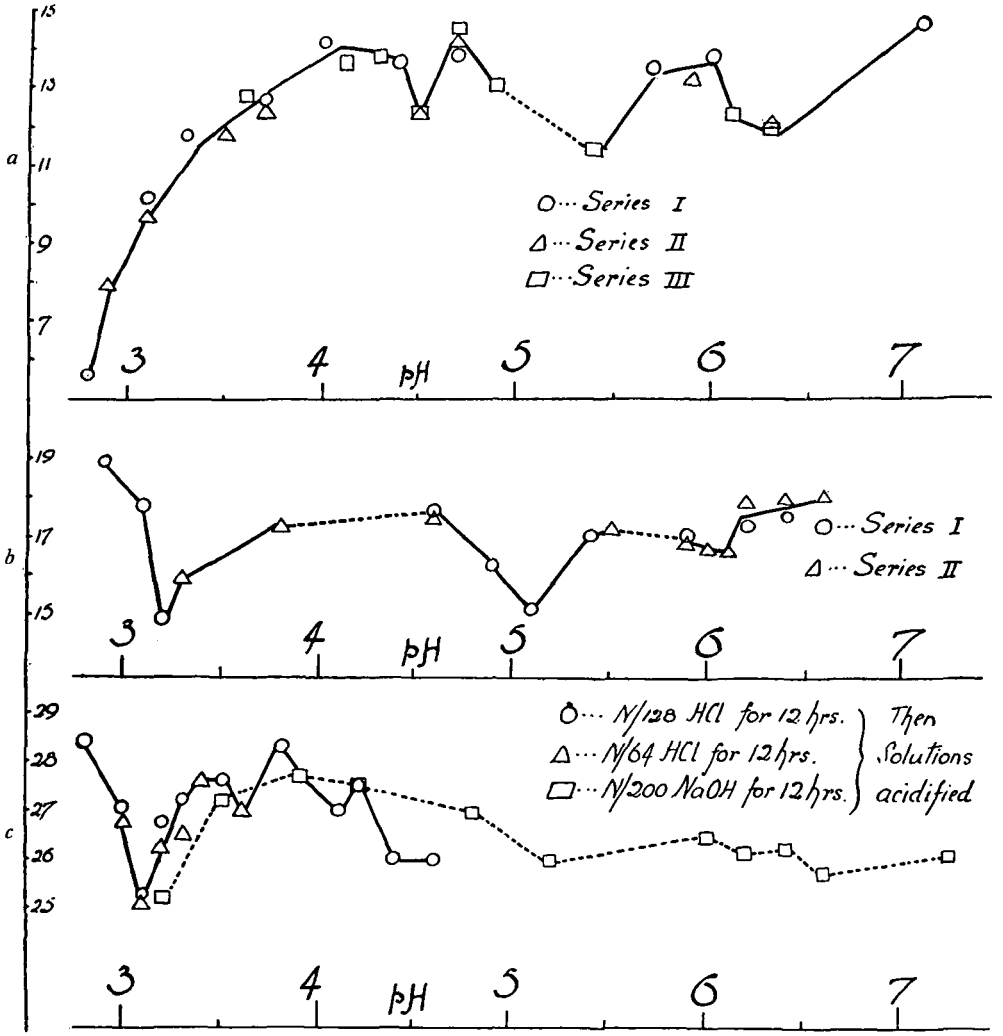

Fig. 1. Swelling of potato tissue at various *p*H values. *a*, percentage increase in weight of fresh tissue, Table I; *b*, volume in c.c. of dried tissue, Table II, series I and II; *c*, volume in c.c. of fresh ground tissue; after standing in acid, continuous line, after standing in alkali, dotted line (Table III).

show that they agree closely. Points of minimum swelling at *p*H 3·2 and [illegible] remain, while those at *p*H 4·5 and 6·3 tend to disappear.

A similar result is obtained after standing the freshly ground tissue in water or very dilute alkali (*i.e.* *p*H 6 or more) for 12 hours, for this treatment also removes

almost all the tuberin from the tissue. On the other hand, if the ground tissue is prepared by standing in dilute acid at *p*H 4·5—thus rendering the tuberin nearly insoluble—a minimum swelling at *p*H 4·4 is obtained. The dried and ground tissue has not been examined in this way, but there can be little doubt that prolonged heating of the moist tissue at 100° C. would be sufficient to degrade the proteins present or at any rate change their properties so profoundly that a change in the proportions of the different depressions is not surprising.

Table III.

Volume in c.c. of fresh ground potato after extraction with dilute acid or alkali and subsequent treatment.

A		*B*		*C*	
*p*H	Vol.	*p*H	Vol.	*p*H	Vol.
4·6	26·0	3·6	27·0	7·4	26·2
4·4	26·0	3·4	27·6	6·6	25·8
4·2	27·5	3·3	26·5	6·4	26·3
4·1	27·0	3·2	26·2	6·2	26·2
3·7	28·3	3·1	25·1	6·0	26·5
3·5	27·6	3·0	26·7	5·2	26·0
3·3	27·2			4·8	27·0
3·2	26·7			4·2	27·5
3·1	25·2			3·9	27·7
3·0	27·0			3·5	27·2
2·8	28·4			3·2	25·2
				3·0	26·7

A = *N*/128 HCl for 12 hours
B = *N*/64 HCl for 12 hours } Then solutions acidified
C = *N*/200 NaOH for 12 hours

WATER ABSORPTION BY SWEDES.

The water absorption of fresh swedes was investigated by similar methods, but owing to the greater variability of the results and the smaller quantity of the water

Table IV.

Percentage increase in weight of swede discs.

Temp.	16° C.				27° C.			16° C.				27° C.	
*p*H	HCl 10 hours	Phos. 10 hours	Phos. 36 hours	HCl 48 hours	Phos. 4 hours	Phos. 18 hours	*p*H	HCl 10 hours	Phos. 10 hours	Phos. 36 hours	HCl 48 hours	Phos. 4 hours	Phos. 18 hours
2·8	7·6	—	—	—	—	—	4·9	—	—	—	19·7	15·4	18·1
3·0	10·1	—	—	1·2	—	—	5·2	16·5	16·4	16·9	—	—	—
3·2	—	—	—	11·2	—	—	5·3	—	—	—	20·0	—	—
3·4	14·0	—	—	—	12·3	−1·0	5·4	—	—	—	—	15·7	18·7
3·6	—	12·0	7·9	20·1	13·7	5·0	5·6	15·5	15·6	17·3	19·0	—	—
3·8	—	15·3	5·1	—	14·6	9·9	5·8	—	17·0	—	—	17·2	19·7
4·0	17·4	16·4	9·8	—	14·7	11·5	6·0	17·3	—	—	20·1	—	—
4·2	—	—	—	19·2	15·3	15·4	6·2	—	15·8	17·0	—	16·2	19·2
4·4	—	14·9	11·0	—	—	—	6·4	16·2	15·7	16·6	18·9	—	—
4·5	15·6	—	—	—	15·6	17·3	6·6	—	—	—	19·0	16·1	19·4
4·6	—	15·4	14·8	—	—	—	6·9	16·2	16·0	17·1	—	—	—

absorbed, it was found necessary to use larger quantities of tissue. Table IV gives the percentage gain in weight due to water absorption at vari hydrogen-ion concentrations for fresh tissue, each batch consisting of a mass of tissue of an initial weight of about 60 g. The data are plotted graphically in Fig. 2,

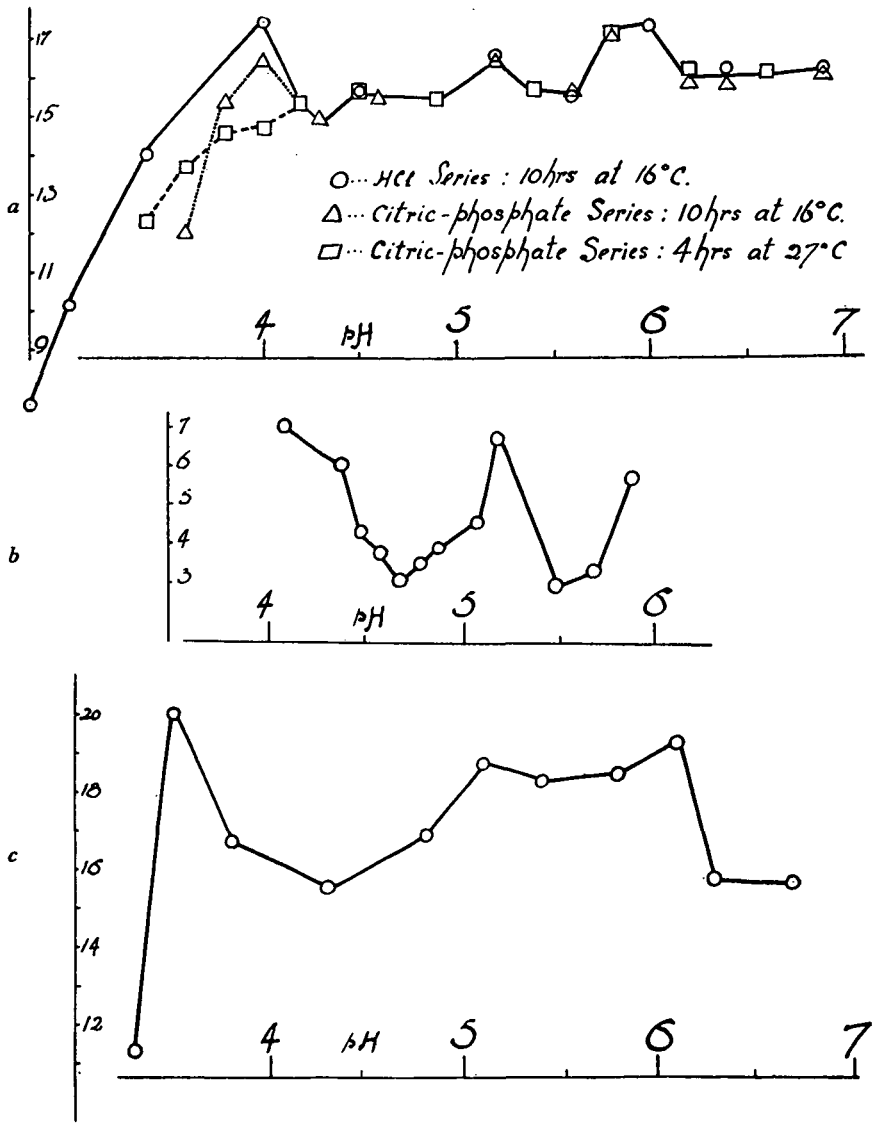

Fig. 2. Percentage increase in weight of tissues placed in solutions of various *p*H values. *a*, fresh swedes; *b*, self-asphyxiated swedes; *c*, fresh turnips.

and give the results of experiments carried on at temperatures of 16 ± 1° C. for 10 hours', and of 27° C. for 4 hours' exposure. On account of the rapid cha in hydrogen-ion concentration of the solution at higher temperatures when hydrochloric acid and sodium hydroxide were used for obtaining the necessary reactions,

the tissues were placed in buffered solutions of disodium phosphate and citric a[illegible] The swelling curves (see Fig. 2) at 16 ± 1° C. (10 hours) and at 27° C. (4 hours) are quite similar whether buffered or unbuffered solutions are used. This agreement is, in fact, so close that we have used the general form of the curve in the graphs for the range from *p*H 4·5 to *p*H 6·9.

The general form of the swelling curve at 16° C. resembles that of potato in having depressions about *p*H 3·2 or less, 4·5–4·9, 5·5 and 6·2–6·9. The tissue, however, swells much more uniformly, and although the high rates of water absorption at *p*H 4 and *p*H 6 are very constant features, that at *p*H 5·2 is variable and would seem to be comparatively unimportant.

ABSORPTION OF WATER BY DEAD SWEDE TISSUE.

Data regarding the swelling of dead but undried swede tissue were obtained in connection with another piece of work. The roots had been sealed up by dipping in melted paraffin wax, and stored at a temperature of 24° C. for four weeks. At the end of that time the tissues were found to be dead, and the protoplasts of the cells plasmolysed. That self-asphyxiation had taken place seemed evident from the interesting fact that the *p*H of the sap (normally about *p*H 5·8) had dropped to *p*H 4·8. Now when the swelling of the tissue was investigated by the methods described for fresh potato and swede tissue, one point of minimum swelling was found at *p*H 4·7, and another at *p*H 5·5–5·6. From the latter point the curve (Fig. 2) rises towards *p*H 6 with a similar rise towards *p*H 3·7, beyond which points data were not available. In this case the graph represents, over the available range of *p*H, the water absorption by the protoplasm, any superimposed osmotic effect being removed. An interpretation of these results will be attempted in the latter part of the paper.

WATER ABSORPTION BY TURNIP TISSUE.

From a limited number of observations on white turnip (*Brassica rapus*), we take for comparison the following data, giving percentage increase in weight of 20–30 g. of turnip tissue:

*p*H	3·3	3·5	3·8	4·3	4·8	5·1	5·4	5·8	6·1	6·3	6·7	11·3
Percentage increase in												
10 hours	11·3	20·0	16·7	15·5	16·8	18·7	18·2	18·4	19·2	15·7	15·6	—
18 hours	8·0	21·3	19·0	17·0	18·5	21·4	21·0	20·5	21·7	17·1	16·9	—

The points of similarity between these results and those for swedes are obvious, and in some of our series there are indications of a slight rise in the curve about *p*H 5·1. It will be noted, however, that the variations in water absorption are much greater than those in swedes.

SWELLING OF THE BROAD BEAN.

[illegible] case of the broad beans (*Vicia faba*), data were obtained by measuring the change in volume of 2 g. of dry roots, which had been grown in Shive's culture solution, washed and then dried in a current of air. The material was finely powdered

and allowed to swell in water acidified to *p*H 4·6 or *p*H 3·3 for 12 hours. The supernatant liquid was then removed and a solution of the required reaction was added. After an interval of 2 hours the volume (in c.c.) of the powdered roots was read off, giving the following results:

*p*H	2·8	2·9	3·0	3·2	3·3	3·4	3·5	3·6	3·8	4·0	4·1
Vol.	20·6	20·5	18·4 19·0	14·4 14·5	17·6	18·6	18·9 18·7	19·0 19·4	19·0	19·0	18·6
*p*H	4·2	4·3	4·4	4·5	4·6	4·8	4·9	5·0	5·1	5·2	5·6
Vol.	18·3	18·0	18·0	16·9	18·0	18·0	18·1 18·6	19·1	19·3	17·4	19·0
*p*H	5·7	6·0	6·3	6·4	6·5	6·6	6·8	7·3	8·0	8·4	8·5
Vol.	18·9	17·7	16·0	16·1	18·2	20·0	21·8	22·5	22·0	22·0	21·6

These data are graphed in Fig. 3, and show depressions at *p*H 3·2, 4·5, 5·2 and 6·3, similar to those previously observed for fresh potato.

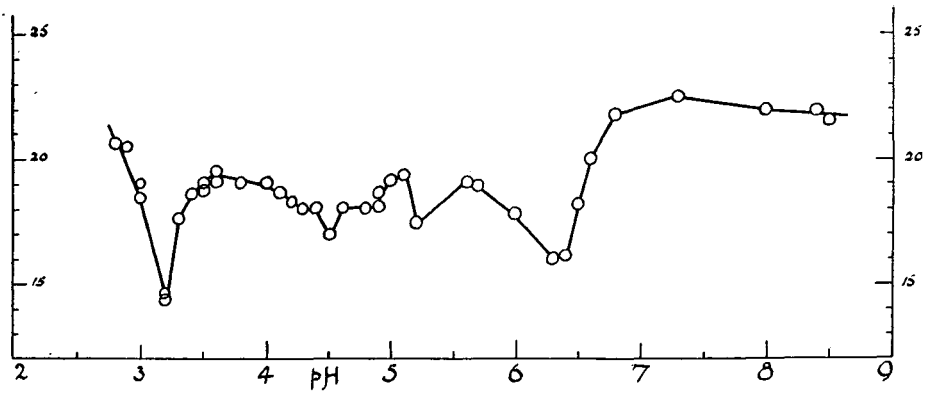

Fig. 3. Volume in c.c. of 2 g. of dried bean roots at various *p*H values.

Some unpublished work* on the swelling in buffer solutions of the air-dry, but living embryos of broad bean seeds shows that these behave in a manner similar to dried root tissue. There is, however, in different experiments, greater variability in the positions where the points of minimum swelling may be found. This may be due in part to the varying salt content of the solutions with which this series of experiments was carried out.

In connection with another investigation a limited number of observations were made upon the change in length of sections of fresh stems of bean seedlings immersed in solutions of various *p*H values. The method employed, that of measuring the change in length by means of a microscope with micrometer eyepiece, does not lend itself to a detailed or exhaustive series of observations at the lower *p*H values. There are, however, undoubted depressions in the swelling curve about *p*H 4·4–4·6, and again at *p*H 6·3–6·4. At *p*H 3·2 the tissue rapidly becomes flaccid.

* We are indebted for these details to Miss R. M. Tupper-Carey.

These three points of minimum swelling also occur in the swelling curve of dried tissue. There can therefore be little doubt that the data for dried tissue closely resemble those for living tissues of embryo and stem.

INTERPRETATION OF RESULTS.

There are two aspects of these results which are worthy of further attention, (1) the varied behaviour of the tissues in the most acid solutions (*p*H 3–4), and (2) the series of depressions on the swelling curves which are common to all the results.

In general, the living tissues in solutions of *p*H 3·2 or less absorb little water at first and later show a marked tendency towards water loss, ultimately becoming flaccid and dying. The extent to which these changes develop varies considerably with the different tissues and the two different types of solution. They are, however, not shown in dead tissue, for in this case there is a marked depression in the swelling curve about *p*H 3·2 (Fig. 3), while the tissues in solutions of still higher hydrogen-ion concentration tend to swell rapidly. It seems clear, therefore, that the tendency towards continued water loss is due to changes in the living protoplasm at low *p*H values, and the most probable explanation of the fact is that the semi-permeability of the protoplasmic membrane breaks down so that the cells are unable any longer to retain water. It has previously been shown (7) that chlorine-ions escape rapidly from potato tissue under these conditions. A more complete explanation of these phenomena seems tentatively possible, if we assume that the changes are due to the precipitation or coagulation of the proteins present in the protoplasm, so that the continuity of the protoplasmic layer is destroyed and its permeability greatly increased. The case can be stated fairly clearly in regard to wheat. Addoms (1) observed that the toxic influence of solutions of *p*H 4·1 or less on the root hairs of wheat was associated with the precipitation of the particles in the protoplasm, and the ultimate coagulation of the latter. This effect is the same as that produced by solutions of this *p*H value upon suspensions of the two chief wheat proteins, which show maximum precipitation about *p*H 4·3–4·5 (6). In the case of tissues, the situation is not quite so straightforward as in solutions of the protein, because the precipitating ions (hydrogen in this case) may not be free to diffuse through the tissue or the protoplasm very rapidly. Thus the *p*H value of the cell sap appears to change very slowly in potato (7), so that, while the protoplasm may be in contact with a solution of high hydrogen-ion concentration outside, yet the internal hydrogen-ion concentration may be low. In general, therefore, it would be anticipated that the hydrogen-ion concentration of the external medium required to cause rapid precipitation of protoplasm, must be higher than the concentration at which the proteins will precipitate most rapidly *in vitro*. Now in potato, water loss ultimately develops in hydrochloric acid solutions of *p*H 3·9 or less after 48 hours, though the value would probably be higher if longer exposures were given. Robbins' figures (9) show that with phosphate buffer solutions, the tendency towards water loss is both more pronounced at 24 hours and also extends to *p*H 4·3. The point of maximum precipitation of tuberin, the chief protein of

potato, lies at *p*H 4·4 (3, 6), so that it is clear that the tuberin would tend to precipitate in the cells when the external *p*H value was 4·3 or less.

The conditions obtaining in swedes are very similar. In this case the tissue contains considerable quantities of two protein-like substances which precipitate most easily *in vitro* at *p*H 3·2 and *p*H 4·3 respectively. Water loss tends to develop below *p*H 3·4 in HCl solutions, but below about *p*H 4·4 in phosphate-citric acid buffer solutions. The greater effect of phosphate on this process was noticed in connection with potato. In that case, however, Robbins' figures show that concentrations of phosphate of ·01 *M* (equal to that in our experiments) produce no enhanced effect, so that the citric acid used in our buffer solutions is probably largely responsible for the increased tendency towards water loss. The effect must clearly be due to the citrate ions as compared with chlorine ions, and it is of interest, therefore, to note that electro-positive proteins are much more readily precipitated by citrate ions (trivalent) than by chlorine ions. Since important proteins in both potato and swede are isoelectric in the region of *p*H 4·4, these proteins would be particularly rapidly precipitated in solutions containing greater concentration of hydrogen ions and also citrate ions. In other words, since the anion effect is only pronounced below *p*H 4·5, then it is only at or below this point that any considerable quantity of protein is electro-positive.

The examination of the figures and graphs for swede tissue shows that temperature also has a well-marked effect on the tendency towards water loss. The water absorption between *p*H 4·5 and *p*H 6·9 is clearly exactly similar for exposures of 10 hours at 16° C. (in either type of solution) and of 4 hours at 27° C. (phosphate citric acid solution). The rate of water absorption is therefore increased by 2·5 times for a rise in temperature of 11° C. A rather similar result was obtained by Stiles and Jorgensen (12) for potato tissue where the temperature coefficient was 2·75 for 10° C. (from 15° C. to 25° C.). The rate of absorption of hydrogen ions is similarly affected by temperature. Our estimations at 16° C. and 27° C. give an average increase in the rate of absorption of 2·45 times for a rise of 11° C., which again is similar to the value of 2·2 for 10° C. obtained by Stiles and Jorgensen (11) for potato. Thus with our material the rate of absorption both of water and of hydrogen ions was affected in a similar way by the higher temperatures employed.

The decrease in the semi-permeability of the protoplasm at 27° C. below *p*H 4·5 cannot, therefore, be attributed to a relatively increased rate of hydrogen-ion absorption as compared with water absorption. It must be due to a greater sensitivity of the protoplasm to the precipitating agent. A similar tendency towards loss of semi-permeability at temperatures of 30° C. or more was observed by Delf (4) for dandelion scapes, and by Stiles and Jorgensen (12) for potato tubers and carrot roots. These effects all seem to be related to the coagulation of protoplasm which is observed at higher temperatures. They also bear a striking similarity to the effects produced when the extracted proteins are precipitated under similar conditions.

For the examination of these effects, the juice was expressed from fr[illegible] ground swedes. Equal quantities of this juice were treated with different proportions of acid (HCl) or alkali (NaOH) so as to bring the juice to a suitable range

of hydrogen-ion concentration between *p*H 2·8 and *p*H 8·0. When these preparations were allowed to stand at 16° C. for not less than 24 hours, considerable quantities of protein were precipitated between *p*H 3 and *p*H 3·5, and also between *p*H 3·8 and *p*H 5, while in the narrow zone between *p*H 3·5 and *p*H 3·8 little or no precipitation occurred. The points of maximum precipitation of the two proteins were respectively *p*H 3·2 and *p*H 4·4. A comparison of the rates of precipitation of these substances under different temperature conditions was then made. Two similar series of test-tube preparations with acidified swede juice were set up, and one series was kept at 27° C. for 4 hours, while the second series was kept at 16° C. for 10 hours. The relative effect of the higher temperature was to cause much more rapid precipitations of the protein precipitating around *p*H 3·2. A considerable quantity of this substance had precipitated after 4 hours at 27° C., while after 10 hours at 16° C. only traces of precipitate were visible. In neither case had the protein showing maximum precipitation at *p*H 4·4 come down. The separate precipitation of two ampholytes from the same solution is unusual, for the usual effect is for both to be precipitated together at some point intermediate between the two points of maximum precipitation. This usual result can be obtained with swede juice by heating the solutions to 40° C. or more when maximum precipitation is about *p*H 3·7 to 3·9 and almost all the protein is removed from solution. The salient features of the evidence are, therefore, that higher temperatures apparently cause an increase in the rate of protein precipitation below *p*H 4·4. This would, on the hypothesis suggested, account for the loss of semi-permeability in this region at 27° C.

The general features of the swelling curves presented in the preceding pages are very consistent in another respect. The various tissues possess minimum volumes at the following four hydrogen-ion concentrations: (1) at or below *p*H 3·2, (2) about 4·5, (3) between *p*H 5·2–5·4 and (4) about 6·2–6·5. These points of contraction may be present whether the cells are alive or dead, and they must be, therefore, attributes of the substances present and not of the vital or structural organisation of the cells. Since the swelling curves are so generally similar, it is probable that the causes of the depressions are of the same general type in the different cases. Carbohydrates are present in considerable quantity in these tissues, and MacDougal (5) has shown that the swelling of these substances may have some relation to the swelling of the tissue. We have not attempted to follow this line of investigation further because examination of potato starch and of cellulose showed no significant change of volume under the conditions of our experiments. It, therefore, seems unlikely that the observed fluctuations of volume are due to carbohydrates. Of fatty materials, the lipins might possibly give rise to changes of volume—but the small quantities which appear to be present in plant tissues are against the supposition that they give rise to such a varied series of swellings and depressions. On the other hand, potatoes, swedes, turnips and beans (in common with other tissues) contain proteins which are isoelectric about *p*H 4·5 (3, 6) (see p. 256), and it seems reasonable to suppose that these tissues would show a point of minimum swelling at this *p*H value. In the case of beans, turnips and swedes

a second ampholyte can be extracted from the expressed juice which is isoelectric about *p*H 3·2, and the point of minimum swelling near this *p*H value may possibly be due to this substance. It is equally possible that all of the four points of minimum swelling may be due to the presence of amphoteric substances isoelectric at these points. In this connection the following figures are of interest. While a list of this sort cannot be regarded either as complete or exempt from revision in the present state of our knowledge, yet it is extremely suggestive:

Isoelectric points of vegetable proteins and their degradation products.

Proteins	*p*H	Proteins	*p*H
Vicilin	3·4	Turnip	4·3
Legumilin	4·5	Beet	4·4
Legumin	4·6	Nucleo protein (typhus bacillus)	4·4
Carrot globulin	4·1–4·4	*Potato (*a*)	3·2
Tomato „	4·6	Glutamic acid	3·3
*Tuberin	4·4	Aspartic acid	3·0
Edestin	5·6	Glycine	6·6
Leucosin (wheat)	4·6	*Leucine	6·5
Glutenin	4·4–4·5	*Lysine	9·0
Orange	4·6	Alanine	6·7
Leaf proteins (Chibnall) ...	4·0–5·0	*Histidine	7·2
Yeast albumin	4·6	*Tyrosine	5·4
„ globulin	4·6	*Asparagine	5·3
Rhubarb	4·5–4·8	Phenylalanine	5·35
Nitella	4·4–4·6	Glycyl-glycine	5·5
Swede (*a*)	3·2	Alanyl-glycine	5·5
„ (*b*)	4·3	Leucyl-glycine	5·7

* Occurring in potato.

An examination of this list shows that, with the exception of the di-amino acids, all the amphoteric substances for which data are available, and which are likely to be found in vegetable tissues, have isoelectric points which group themselves round four distinct *p*H values, viz. 3·2, 4·5, 5·5 and 6·6. It is remarkable that these regions should coincide with those of the points of minimum swelling so closely, and the coincidence seems to permit the tentative suggestion that the unexplained swelling depressions may also be due to amphoteric substances present in the tissue.

In the case of potato tubers also it will be seen that the ampholytes known to occur in the tissue fall into each of the four main groups. For the other tissues used in our experiments the records are too sparse for an adequate tabulation of the amino acids present, but most of the amino acids known have been isolated from leguminous plants, and hence may be present in the broad bean.

The conception that the swelling curve of a plant tissue shows the effect of several isoelectric points is supported by the work of Sakamura and Tsung-Le Loo(10), who investigated the fluidity of *Spirogyra* protoplasm by a viscosity method. They find points of minimum fluidity to occur about *p*H values 5·3, 6·4 and 7·6. Assuming these points to represent zones of low water content, as is indicated in our results, then they should also be points where fluidity of protoplasm is least, as found in *Spirogyra*. Sakamura and Tsung-Le Loo also interpret their results as indicating isoelectric points at the *p*H values given.

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