Studies in Permeability.

III. The Absorption of Acids by Plant Tissue.

BY

MILDRED HIND.

With eleven Figures in the Text.

IN the first of these studies it has been shown that cells of potato tuber absorb hydrogen ions from aqueous solutions of hydrochloric acid with considerable rapidity. In this paper an account is given of more detailed experiments made to determine to what extent this rapid absorption is a characteristic of acids in general.

Earlier work (4) appears to indicate that with strong acids it is the concentration of the hydrogen ion which is the determining factor of the action of acid on plants, while with organic acids the anion is stated to influence the action (6). So far, the methods which have been used have given little information as to the extent to which acids are actually absorbed by plant tissue, nor have these methods yielded results of any great accuracy. In the experiments recorded in this paper the methods of physical chemistry advocated and described in previous papers of this series have been used.

Again, so far there has not been produced much evidence as to the substances which are responsible for the absorption of acids. For instance, Czapek (1, 2, 3) seems to be of the opinion that the action of acids on the fat emulsions of the plasma is responsible for the effects produced; on the other hand, Loeb (5, 7), Pauli (11), and Osterhout (9) think that protein substances are active in many of the phenomena of permeability. Some experiments have accordingly been made in the course of this work in regard to the action of acids on various plant substances, but however suggestive the results of these experiments may be, it is of course impossible to draw from a few isolated experiments any definite conclusions in regard to the structure and function of cell membranes.

This work was undertaken at the suggestion of Mr. Stiles and Mr. Jørgensen, whom I would here thank for their advice during its progress.

METHODS.

1. *Electrical Conductivity Methods*.—By measuring the change in electrical conductivity of a solution surrounding plant tissue, some idea of the effect of the solution on plant cells can be obtained. If the substance in solution enters without harming the cell and causing an exosmosis of the cell-contents, a decrease in conductivity will be the result; whereas if the plasma-membrane is affected in such a way that the substances within the cell diffuse out, the conductivity of the external solution will be increased on account of this. The change in conductivity in such cases will therefore be the resultant of the diffusion of the external solute into the cell and of exosmosis of electrolytes from the cell.

These experiments were carried out in the same way as the electrical conductivity experiments described in the first of these studies. Twenty discs of potato tuber were put in a stoppered bottle containing 100 c.c. acid, the conductivity of which had been measured. The conductivity of the acid solution was then taken at intervals. Dilute solutions of each acid in various concentrations were used. The experiments were all done in duplicate; they were carried out at constant temperature—18° C.—as it has already been shown that temperature exerts a considerable influence on the rate of absorption (13).

The curves were obtained by plotting the increase or decrease in electrical conductivity against the time.

2. *Volumetric Analytical Methods*.—In order to measure the acidity of the external solution after it had been in contact with the potato discs for different times, the solutions were poured off and titrated with standard alkali. No reliable results, however, were obtained, owing to the great dilution of the solutions, and possibly to the diffusion out from the cells of substances which interfered with the indicators.

3. *Electrometric Method*.—The method of experimentation involving the use of electrical conductivity can only give a rough approximation to the actual course of absorption of electrolytes, owing to the diffusion out of electrolytes from the cell, which takes place with most substances and even to some extent in distilled water. By measuring the concentration of the hydrogen ions in acid solutions before and after they have been in contact with potato discs, the actual rate of absorption of the hydrogen ion can be measured. Experiments were carried out in the way described in the second paper of this series (13).

The hydrogen-ion concentration was calculated in terms of the original pure acid solution. The curves were obtained by plotting the relative hydrogen-ion concentration against the time. The bottles containing the discs and acid were kept at a temperature of 18° C. throughout the experiments.

This method gives no definite information as to the absorption of the anion. Whether the acid is absorbed as such, or whether the kation and anion enter the cell at different rates, is a question at present unsolved. The work of Pantanelli and Sella (10) indicates the possibility of this latter alternative with its resulting complications.

THE RESULTS.

I. The Absorption of Acids by Potato Tissue.

Hydrochloric Acid.

Series 1.—The rapid absorption by plant cells of the hydrogen ion of hydrochloric acid in a concentration of $\frac{N}{1000}$ has been indicated in previous

FIG. 1. Potato in Hydrochloric Acid.

papers (12, 13). In the present investigation a wide range of acid concentrations was employed, varying from $\frac{N}{1000}$ to $\frac{3N}{50000}$. The curves shown in Fig. 1 indicate that a marked decrease in the electrical conductivity of the acid solution occurs with all strengths of acid except the very dilute ones. In all cases examined, the conductivity rose after a time. These curves indicate that in all cases a rapid absorption of acid by the potato

cells takes place, but in the case of the lowest concentrations of acid the increase of conductivity due to the electrolytic exudate from the cells more than counterbalances the decrease, owing to removal of hydrogen ions by the potato tissue. This influence of the exudate on the conductivity curves makes itself obvious after a time in the case of the higher strengths of acid.

Series 2.—More definite information as to the absorption of hydro-

FIG. 2. Potato in Nitric Acid.

chloric acid was obtained by the electrometric measurement of the change in hydrogen-ion concentration of the solution. Experiments were carried out with acid of concentrations $\frac{N}{1000}$, $\frac{N}{2000}$, $\frac{N}{5000}$. In all cases there was a rapid absorption of hydrogen ions, as already indicated for the case of $\frac{N}{1000}$ acid in previous papers of this series.

Nitric Acid.

Series 3.—Conductivity measurements were made of the external solution, as in Series 1, when potato discs were immersed in nitric acid solutions of concentrations $\frac{N}{1000}$, $\frac{N}{2000}$, $\frac{N}{3000}$, $\frac{N}{10000}$.

The accompanying figure (Fig. 2) shows that the result with nitric acid is similar to that obtained with hydrochloric acid.

Series 4.—Experiments made with the electrometric method of measurement, to determine the rate of absorption of the hydrogen ion from

FIG. 3. Potato in $\frac{N}{1000}$ Nitric Acid.

nitric acid solutions, show that the kation is absorbed rapidly, as in the case of hydrochloric acid. The curve shown in Fig. 3 indicates the rate of absorption of the hydrogen ion when $\frac{N}{1000}$ nitric acid was used.

Sulphuric Acid.

Series 5.—Similar experiments were made with sulphuric acid as with hydrochloric and nitric acids. The change in conductivity of the external solution, when solutions of strengths $\frac{N}{500}$, $\frac{N}{1000}$, $\frac{N}{2000}$, $\frac{N}{5000}$, and $\frac{N}{10000}$

FIG. 4. Potato in Sulphuric Acid.

were used, are shown graphically in Fig. 4. It will be observed that the general results are similar to those given with hydrochloric and nitric acids.

Oxalic Acid.

Series 6.—The absorption of oxalic acid by potato tissue was investigated in the cases of solutions of concentrations $\frac{N}{500}$, $\frac{N}{1000}$, $\frac{N}{5000}$. The change which takes place in the conductivity of the external solution is

similar to that in the case of the strong mineral acids, but as Fig. 5 indicates the decrease in conductivity is less, which may be due to a smaller absorption of hydrogen ions, or to the greater exosmosis of electrolytes.

FIG. 5. Potato in Oxalic Acid.

Formic Acid.

Series 7 and 8.—In the case of formic acid considerably different results were obtained. Conductivity measurements of the external solution were made with acid of original concentration $\frac{N}{500}$, $\frac{N}{1000}$, $\frac{N}{2000}$. In all cases the conductivity showed at first a slight decrease, which speedily gave place to a rapid increase, this increase being greater the higher the concentration of the acid (cf. Fig. 6).

Measurements of the actual concentration of the hydrogen ion were made in the case of $\frac{N}{1000}$ acid.

Fig. 7 indicates the course of the absorption. It will be observed that during the first few hours the hydrogen ion is absorbed at almost the same rate as the hydrogen ions of nitric acid. After a time, however, a remarkable change occurs. This consists in the concentration of the hydrogen ion of the external solution increasing, a phenomenon which was never observed in the case of the inorganic acids.

FIG. 7. Potato in $\frac{N}{1000}$ Formic Acid.

FIG. 6. Potato in Formic Acid.

Acetic Acid.

Series 9 and 10.—The next acid of the series, acetic acid, was used in concentrations $\frac{N}{500}$, $\frac{N}{1000}$, $\frac{N}{2000}$, $\frac{N}{5000}$. It will be observed from Fig. 8 that the conductivity of the external solution changes in the same way as in the case of formic acid.

FIG. 8. Potato in Acetic Acid of various strengths.

In Fig. 9 is shown the rate at which the hydrogen ion is absorbed by potato tissue from $\frac{N}{1000}$ acetic acid. The curious rise in concentration of the hydrogen ion, after a few hours of continuous decrease, is shown again here, as in the case of formic acid.

The experiments described above show that considerable difference exists between acids, even in dilute solution, as regards their action on plant cells. The mineral acids on the one hand cause much less exosmosis, and presumably therefore produce much less injury than the two fatty acids employed on the other. Oxalic acid appears to occupy an intermediate position.

The earlier work referred to in the introduction to this paper is not therefore absolutely confirmed. Support is given to the contention that with organic acids the anion influences the action on plant cells, but, having

FIG. 9. Potato in $\frac{N}{1000}$ Acetic Acid.

regard to the different results with hydrochloric and nitric acids, it must be supposed that even with these the anion also has an effect. However, the acids containing 'nutrient' ions such as SO_4 and NO_3 , as well as hydrochloric acid, are sharply marked off from the fatty acids in respect of their power of producing exosmosis from the tissues.

A further conclusion which may be drawn from the curves given in this section, is that the rate of absorption of an acid depends on its

concentration, as has already been shown in the second paper of this series (13).

The experiments recorded in which the electrometric method of measurement was used show that an actual absorption of hydrogen ions does take place with all the acids examined. With the mineral acids on the one hand, and the fatty acids on the other, there is, however, a very noteworthy difference. In the case of the former there was a continuous absorption of acid as far as the experiments were carried; with the latter this continued reduction of the acidity of the external solution gave place after a time to a marked increase in the acidity. A discussion of this result follows later in this paper.

II. Experiments with Living Plants of *Vicia Faba*.

In order to determine whether the roots of living plants absorb acids in a similar way to potato cells, some experiments were made with Bean plants (*Vicia Faba*). Seeds were germinated in sawdust, and the young seedlings transferred to water-culture solutions, so that the roots of the experimental plants should be uninjured. Before an experiment the roots of the plants were well washed with distilled water, then the plants were placed with their roots in bottles containing 100 c.c. of acid.

Series 11. In this series, Bean plants were used with $\frac{N}{1000}$ nitric acid. Both the conductivity and the hydrogen-ion concentrations of solutions were measured after various intervals. Fig. 10 shows the change in conductivity, and Fig. 11 the rate of absorption of the hydrogen ion.

These results indicate that the absorption of acid by living Bean plants takes place in exactly the same way as the absorption by discs of potato tuber.

III. On the Part played by proteins in the Absorption of Acids by Potato Cells.

In order to obtain some information as to which of the various substances present in the cells of the potato are responsible for this rapid absorption of hydrogen ions, potatoes were ground up with sand and subjected to pressure. The expressed liquid so obtained was filtered off. The filtrate contained sugar and proteins, but no starch. The hydrogen-ion concentration of the liquid was measured, and was found to be only slight.

There was now added to 100 c.c. of an $\frac{N}{1000}$ acid solution 1 c.c. of the filtrate, and the hydrogen-ion concentration of the resulting liquid measured. The hydrogen-ion concentration had decreased considerably. An attempt

was made at a partial separation of the colloidal and crystalloidal substances in the expressed liquid, by filtering under pressure in Bechold's ultra-filtration apparatus through a 3 per cent. collodion filter. The filtrate so obtained would contain the crystalloids, but a large part of the colloids would be left behind on the filter. When 1 c.c. of the filtered solution was now added to 100 c.c. acid, there was produced a much smaller decrease in the hydrogen-ion concentration of the acid; thus it appears that the decrease in hydrogen-ion concentration, produced by the crude expressed liquid, is probably due to the action of the colloidal substances in the

FIG. 10. Bean in $\frac{N}{1000}$ Nitric Acid.

potato extract. Formic, acetic, and nitric acids were used in these experiments. The proteins in some of the solution obtained from the potato were precipitated by means of colloidal ferric hydroxide, and the solution then filtered. 1 c.c. of the filtrate added to 100 c.c. acid produced no effect on the hydrogen-ion concentration of the acid.

As the removal of protein from the solution also removes its power of reducing the hydrogen-ion concentration of acid solutions, it suggests that the absorbing substances of the living cells may be proteins. It is known that proteins react chemically with acids, and will therefore affect their hydrogen-ion concentration.

A solution of peptone in water was therefore prepared, and 1 c.c. of it

added to 100 c.c. of $\frac{N}{1000}$ acid. There was immediately produced a marked decrease in the hydrogen-ion concentration of the acid, very similar to that produced by 1 c.c. of potato extract.

To obtain further proof that in the case of the liquid obtained from the potato it is the protein that affects the acid, the proteins of the potato were extracted. These were found to act in the same way, in regard to

FIG. 11. Bean in $\frac{N}{1000}$ Nitric Acid.

the acid, as did the peptone solution and also the liquid expressed from potatoes.

It has been thought by some investigators that lipid substances play an important part in the absorption of substances by the cell, and lecithin has been suggested as particularly active in this regard. A colloidal solution of commercial lecithin in water was prepared, and 1 c.c. of this was added to 100 c.c. of $\frac{N}{1000}$ acid; measurements before and after the addition

of the lecithin showed that it had little effect on the hydrogen-ion concentration of the acid.

DISCUSSION.

The results of the experiments described in this paper lead one to conclude that acids in dilute solution, or at any rate their hydrogen ions, readily enter plant cells.

The curves obtained in the electrical conductivity experiments show that plant cells are affected in different ways by different acids, and also give an idea of the rate and the manner in which the acids affect the cells; but considering the complexity of the system, and the number of actions possible, it cannot be assumed that they give a definite measure of either the rate of absorption of the acid, or of the exosmosis of the substances in solution within the cell. They do suggest, however, that in dilute solution some acids can penetrate into the cells without doing much injury to them for some time, while others produce such an effect that some of the substances within the cell pass out almost as soon as the potato discs are immersed in the acid.

The hydrogen-ion concentration measurements show the actual rate of absorption of the hydrogen ions by the cells. From the curves, it can be seen that the hydrogen ions of various acids are absorbed in the same way for some time, but that, after some hours, acids such as formic acid produce results different from those produced by the mineral acids. The anions have obviously an effect, and determine whether the acid is toxic or comparatively non-toxic, toxicity being roughly indicated by the rate of exosmosis of electrolytes from the cell, as indicated by a comparison of the conductivity curves and those of hydrogen-ion concentration.

The increase in the hydrogen-ion concentration of the external solution which takes place after some hours in the case of formic and acetic acid is a curious fact which requires explanation.

It may be supposed that in penetrating into the cell, these acids, which are both chemically active, may react with some of the cell contents and produce other acids, probably organic acids which cannot normally pass through the limiting layer of the protoplast. But in entering the cell, the acid will have probably reacted with the outer layers of the protoplasm, and will have so altered their chemical constitution that the acids produced in the cell are able to diffuse out, and so increase the hydrogen-ion concentration of the external solution.

Considerable interest attaches to the mechanism by which acids enter the cell so rapidly. So far, opinion has been divided as to whether it is the protein or lipid substances which are active in absorption. The experiments described in the third section of the experimental part of this paper all appear to suggest that, in regard to absorption of acids, it is the protein

substances of the cell which are responsible. The only lipid substance examined, crude lecithin, gave no result similar to that obtained with living cells, whereas the resemblance between the action of living cells on acids and that of both peptone and the proteins extracted from potato is striking.

Perhaps too much stress should not be laid on this isolated experiment, for commercial lecithin is a very impure product, and may contain as much as 50 per cent. impurities (8). Nevertheless, even in such a case one would expect a reduction in the hydrogen-ion concentration of the acid, if lecithin absorbed acid in the same way as the cell.

Finally, it should be mentioned that the decrease in the hydrogen-ion concentration of the solutions containing plant tissue cannot be explained by the diffusion out from the cell of protein substances which then react with the acid in the external solution. Tests made for proteins in the external solution failed to reveal their presence there, even after forty-eight hours' immersion in dilute acid. Moreover, it would be extremely unlikely that complex compounds like proteins would diffuse out during the first few hours of immersion in dilute acid.

SUMMARY.

1. The hydrogen ions of all acids examined are rapidly absorbed by plant tissue from dilute solutions.

2. The anion of the acid plays a large part in determining the effect of the acid on the cell, the fatty acids standing in strong contrast to the mineral acids. In the case of the mineral acids the exosmosis of electrolytes produced is considerably less than in the case of formic and acetic acids.

3. Some evidence is brought forward which suggests that proteins may play an essential part in the absorption of acids by plants. No evidence has been obtained suggesting that lecithin is at all active in this regard.

BOTANY DEPARTMENT,
THE UNIVERSITY, LEEDS.

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