Studies in Permeability.

II. The Effect of Temperature on the Permeability of Plant Cells to the Hydrogen Ion.

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With four Diagrams in the Text.

IN the first of these papers we have indicated that the cells of potato tuber absorb hydrogen ions very rapidly, and in the succeeding article of this series it will be shown that this rapid absorption is a general characteristic of acids. It becomes of interest to discover whether this absorption is due to simple diffusion into the cell, whether it is due to adsorption, or whether the entrance of the acid is the result of its chemical combination with some substance or substances of the cell, for all information of this kind is likely to be of help in the elucidation of the mechanism of permeability. We have therefore examined the influence of temperature on the absorption of hydrochloric acid by potato cells, as these three processes, diffusion, adsorption, and chemical action, should all be influenced differently by alterations in temperature.

Method.

The method of investigation has been briefly indicated in a previous paper, here we may give further details.

The tissue used consisted of discs of potato tuber which were 1 cm. in diameter and weighed about 0·5 grm. They were washed in a gentle stream of tap water for about an hour, then rinsed in distilled water, and transferred to the acid solution.

The acid used was hydrochloric acid, made up by means of specific gravity tables to a strength of about $\frac{N}{10}$, which was diluted for use to 0·001 N. Subsequent titration with standard alkali showed that the stock

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acid solution was actually 0·110 N, so that the acid used in the experiments had a concentration of 0·0011 N. This low concentration was used as it is unlikely to damage the plant-cells for some time; a higher strength of acid is likely to be more dangerous in this regard.

The experiments were carried out in stoppered bottles. In each bottle was placed 100 c.c. of acid and 20 discs of potato. A number of such bottles were placed in a thermostat kept at the desired temperature, and at

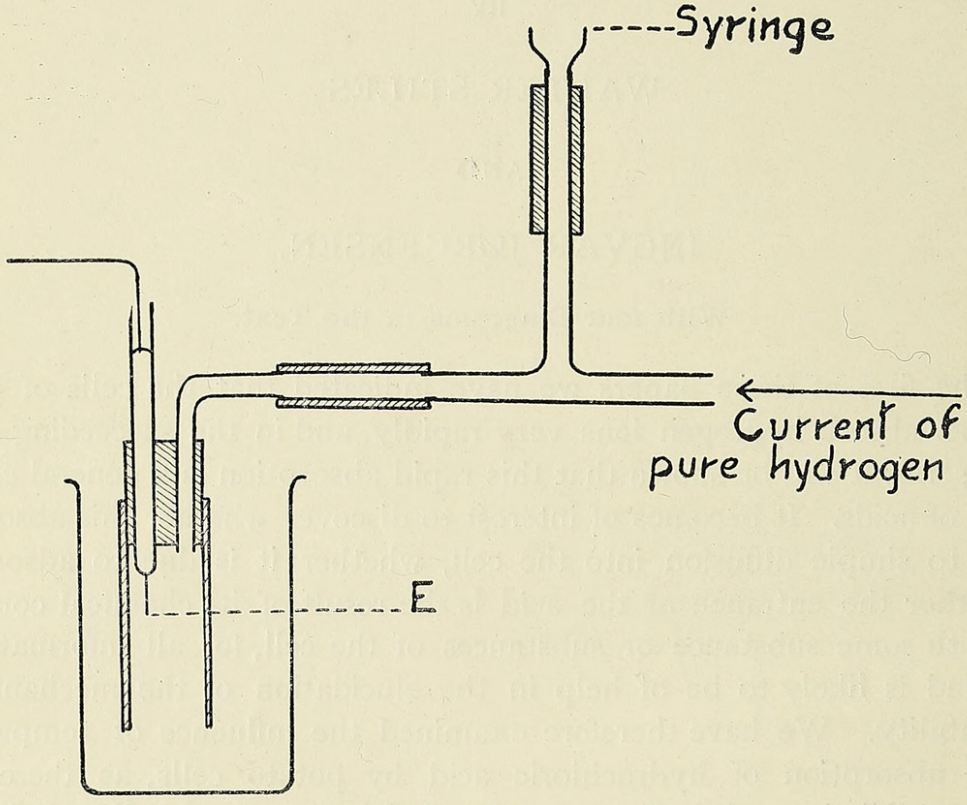

FIG. 1. This shows the form of hydrogen electrode used. The point electrode is shown at E. The liquid whose acidity is to be determined is placed in the larger vessel. Hydrogen is passed through the electrode vessel for a few seconds. By moving the plunger of the syringe backwards and forwards a few times while the gas is passing, the last traces of air are removed from the apparatus. The current of gas is stopped, and by pushing the plunger of the syringe in and out the electrode is wetted with the liquid. Finally the liquid is brought to such a level that the point just touches it. Equilibrium is very quickly attained with an electrode in good working condition.

various intervals of time the bottles were removed, usually in duplicate, the acid poured off, and the concentration of the acid solution measured.

In order to measure the acidity of solutions in this dilution, into which moreover various organic substances may have diffused out from the cell-tissue, the ordinary titration methods are useless. The measurement has therefore been made by means of the hydrogen electrode.

When a metal is immersed in a solution of one of its salts, an electromotive force is set up at the surface of contact of the metal and salt, and this E.M.F. is dependent upon the concentration of the metal ion in the

solution of the salt. The same is true also for an electrode of hydrogen immersed in an acid, i. e. a solution containing hydrogen ions.

As hydrogen electrode we used a modification of that suggested by Walpole (Bioch. Journ. 1913). The electrode vessel consisted of a small piece of glass tubing closed at one end with a rubber stopper. Through this stopper passed a glass tube with the electrode of platinized platinum wire sealed into the lower end, and a piece of capillary glass tubing connected to a T-piece. The other two ways of the T-piece were connected one to a small glass syringe, the other to an apparatus for generating hydrogen. This was carefully purified before passing into the electrode vessel.

The electrode vessel could be placed in a larger vessel containing the liquid whose acidity was to be determined (Fig. 1).

The electrode was charged in the usual manner as described by Walpole.

The hydrogen electrode was combined with an $\frac{N}{10}$ KCl-Calomel electrode, a 3·5 N solution of KCl being used as intermediate liquid. Kahlbaum's pure potassium chloride with certificate of guarantee was used and the mercury and mercurous chloride were carefully purified. The form of the calomel electrode is shown in the accompanying sketch (Fig. 2). The glass vessel itself is filled above the mercury and calomel with $\frac{N}{10}$ KCl saturated with calomel, and this solution occupies the tube between the vessel itself and the three-way tap. The intermediate liquid of 3·5 N KCl occupies the rest of the tube, and can be run out by turning the tap, or renewed from the same funnel. The tap is ungreased and kept closed, there always being enough liquid held by capillarity to ensure sufficient conduction.

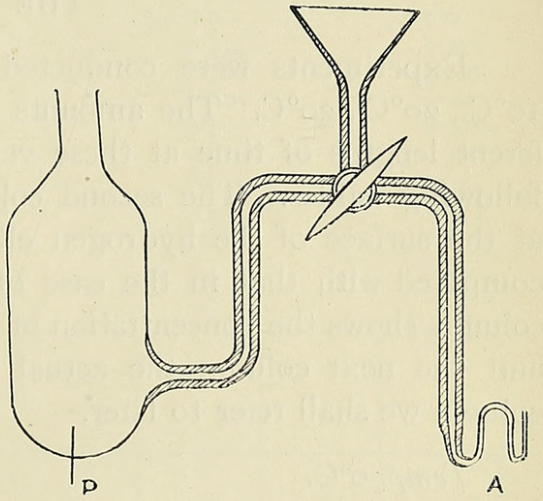

FIG. 2. The diagram shows the form of Calomel electrode used. The bent part at A actually lies in a plane at right angles to the rest of the apparatus. The platinum point P dips into a mercury cup.

The electromotive forces manifested by this combination were compared with a Weston Normal Cell manufactured by the Cambridge Scientific Instrument Company. The standard cell and the combination of calomel electrode and hydrogen electrode were compared with a 2-volt accumulator by Poggendorf's method. A capillary electrometer (enclosed pattern) was first used as a null instrument, but this was afterwards replaced by a delicate moving coil galvanometer manufactured by

R. W. Paul, and which proved a much more convenient and sensitive arrangement.

The hydrogen-ion concentrations of the various solutions were then calculated according to the formula

$$e_1 - e_0 = 2{\cdot}3026 \frac{RT}{F} \log \frac{C_0}{C_1}$$

where e_0 is the E.M.F. given with a liquid of hydrogen-ion concentration C_0 and e_1 the E.M.F. given with a liquid of hydrogen-ion concentration C_1. R is the gas constant, T the absolute temperature, and F signifies 1 faraday (= 96580 coulombs).

That the apparatus was giving correct readings was tested by the use of different concentrations of acids of known strength.

The Results.

Experiments were conducted at four different temperatures, 0°C., 10°C., 20°C., 30°C. The amounts of acid absorbed by the tissue after different lengths of time at these various temperatures are indicated in the following tables. The second column shows the increase of the E.M.F. at the surface of the hydrogen electrode in each of the acid solutions as compared with that in the case of the original 0·0011 N acid. The third column shows the concentration of the acid as calculated from this E.M.F., and the next column the actual absorption. The numbers in the last column we shall refer to later.

Temp. 0°C.

Time in hours.	*Increase of E.M.F. in volts.*	*Relative Concentration (C) of Hydrogen Ion in Solution.*	*Relative quantity of Hydrogen Ion absorbed.*	$-Log\ C.$
0·0	0·0	1·000	0·0	0·0
3·0	0·0069	0·751	0·249	0·124
6·0	0·0120	0·610	0·390	0·215
8·0	0·0172	0·493	0·507	0·307
8·0	0·0173	0·491	0·509	0·309

Temp. 10°C.

Time in hours.	*Increase of E.M.F. in volts.*	*Relative Concentration (C) of Hydrogen Ion in Solution.*	*Relative quantity of Hydrogen Ion absorbed.*	$-Log.\ C.$
0·0	0·0	1·000	0·0	0·0
0·5	0·0035	0·870	0·130	0·060
0·5	0·0027	0·898	0·102	0·048
1·0	0·0062	0·781	0·219	0·107
2·0	0·0084	0·709	0·291	0·149
2·0	0·0084	0·709	0·291	0·149
2·5	0·0145	0·561	0·439	0·251
4·0	0·0180	0·488	0·512	0·312
5·0	0·0231	0·399	0·601	0·399
5·0	0·0202	0·447	0·553	0·350
6·0	0·0256	0·361	0·639	0·443
6·0	0·0249	0·370	0·630	0·432

Temp. 20°C.

Time in hours.	*Increase of E.M.F. in volts.*	*Relative Concentration (C) of Hydrogen Ion in Solution.*	*Relative quantity of Hydrogen Ion absorbed.*	*– Log. C.*
0·0	0·0	1·000	0·0	0·0
1·0	0·0105	0·649	0·351	0·188
1·0	0·0117	0·619	0·381	0·208
2·0	0·0205	0·430	0·570	0·366
2·0	0·0219	0·407	0·593	0·390
3·0	0·0276	0·321	0·679	0·494
3·0	0·0269	0·331	0·669	0·480
4·0	0·0399	0 194	0·806	0·712
4·0	0·0421	0·177	0·823	0·752
5·0	0·0442	0·162	0·838	0·792
5·0	0·0513	0·121	0·879	0·917

Temp. 30°C.

Time in hours.	*Increase of E.M.F. in volts.*	*Relative Concentration (C) of Hydrogen Ion in Solution.*	*Relative quantity of Hydrogen Ion absorbed.*	*– Log. C.*
0·0	0·0	1·000	0·0	0·0
0·5	0·0102	0·667	0·333	0·176
0·5	0·0140	0·581	0·419	0·236
1·0	0·0232	0·398	0·602	0·400
1·0	0·0200	0·417	0·583	0·380
2·0	0·0418	0·190	0·810	0·721
2·0	0·0425	0·185	0·815	0·733
3·0	0·0660	0·0770	0·923	1·114
3·0	0·0720	0·0751	0·925	1·124

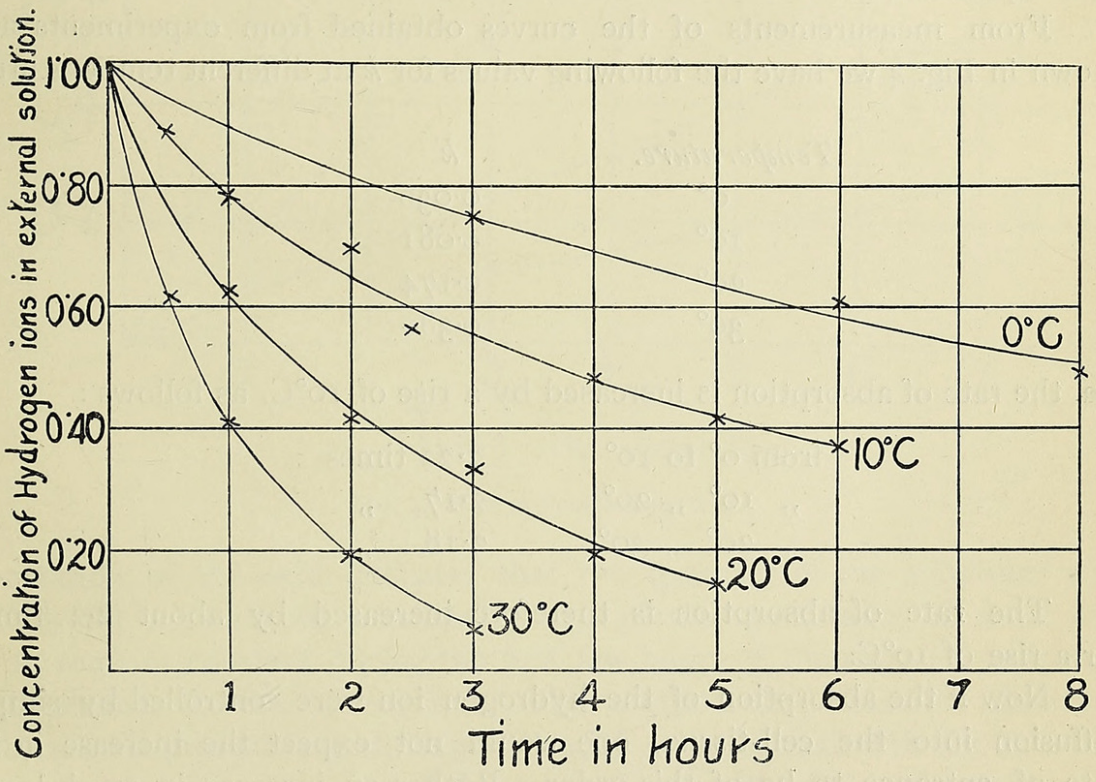

FIG. 3. For explanation see text.

The curves shown in Fig. 3 exhibit graphically the relation between the time the absorption has proceeded and the quantity of acid left in the solution at that time. All the curves strongly resemble exponential

curves in shape, and this is confirmed by plotting curves between the time and the logarithm of the concentration (see last column of tables and Fig. 4).

The relation between time and concentration is then given by the equation

$$-\log C = kt + k' \quad . \quad . \quad . \quad . \quad . \quad (1)$$

and if x represents the amount of acid absorbed at any time we have

$$-\log (A - x) = kt + k'$$

where A is the original quantity of acid present.

If this is taken as unity we have $k' = 0$, and $k = \frac{-\log (A-x)}{t}$.

The rate of absorption $\frac{dx}{dt}$ is then given by the equation

$$\frac{dx}{dt} = k (A - x) \quad . \quad . \quad . \quad . \quad . \quad (2)$$

i. e. the rate of absorption is proportional to the concentration at any time and to the constant k, and k has the same value in equation (2) as in equation (1).

From measurements of the curves obtained from experiments and shown in Fig. 4 we have the following values for k at different temperatures:

Temperature.	k
0°	0·036
10°	0·081
20°	0·174
30°	0·380

i. e. the rate of absorption is increased by a rise of 10°C. as follows:

from 0° to 10°	2·22 times
„ 10° „ 20°	2·17 „
„ 20° „ 30°	2·18 „

The rate of absorption is therefore increased by about 2·2 times for a rise of 10°C.

Now if the absorption of the hydrogen ion were controlled by simple diffusion into the cell-tissue, one would not expect the increase in its rate of entrance to be of this order. Rather an increase in much lower proportion would be expected, of about the order of 1 : 3.

As regards the effect of temperature on the rate of adsorption, the coefficient seems to be of the same order as that of diffusion; certainly the van't Hoff rule is not followed.

But it has been shown by van't Hoff and subsequent workers that the rate of many chemical reactions is doubled or trebled by a rise of 10°C. In the realm of plant physiology such a rise has been shown by F. F. Blackman in the case of carbon-assimilation in the leaf. The study of the effect of temperature on the absorption of the hydrogen ion would seem to indicate that this absorption is controlled by some chemical action in the cell, and is not the result of simple diffusion through the plasma-membrane, or of mere adsorption by the cell protoplasm.

That the numbers obtained experimentally show that the rate of the reaction depends merely on the temperature coefficient k, and the con-

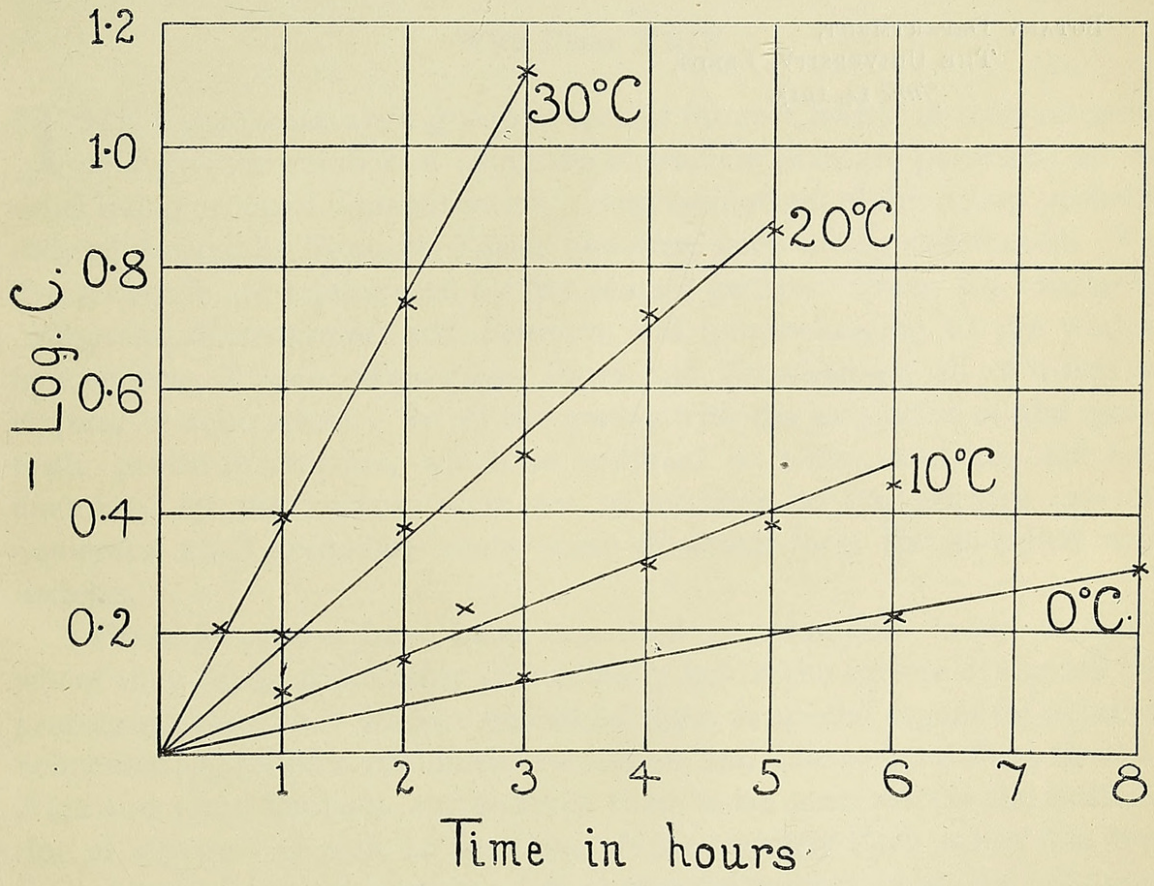

Fig. 4. For explanation see text.

centration of the acid indicates that the quantity of the substance with which the acid reacts, presumably the plasma-membrane, or some part of it, remains constant during the first few hours of the reaction, as it does not influence the rate of reaction. This suggests that either the absorbing substance is present in such large quantity as compared with the acid that the amount changed is small in comparison with the total amount, or that the substance formed as a result of the absorption is broken down again almost as soon as formed. Such a view of the plasma-membrane is held by Pauli and Szücs who regard the entrance of ions into the cell as due to the reversibility of such a reaction between ions and the plasma-

membrane. We feel, however, that more experimental evidence is required before such theories can be discussed adequately and with profit.

Summary.

1. The absorption of the hydrogen ion of hydrochloric acid in dilute solution by potato cells takes place according to a simple exponential relation between time and the concentration of the acid.

2. The rate of absorption of these ions by potato cells is increased about 2·2 times for a rise of 10°C. between 0°C. and 30°C.

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The University, Leeds,
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