Botany. — *On the protoplasm permeability to water during the recovery from plasmolysis.* By Iz. DE HAAN. (Communicated by Prof. J. C. SCHOUTE.)

(Communicated at the meeting of September 26, 1931.)

The object of this investigation was to determine whether the permeability of the protoplasm to water during the course of the recovery from plasmolysis is subject to changes. For this purpose I employed the plasmometric method of HÖFLER (1918).

§ 1. *Method.*

HÖFLER's plasmometric method is described in detail in the excellent publications of HÖFLER (1930) and of HUBER and HÖFLER (1930), in which they communicate their pioneer investigations on permeability to water. HÖFLER and HUBER demonstrated in these that the resistance of the protoplasm to the ingress of water into the vacuole or to the egress of water from the vacuole was the limiting factor with regard to the rapidity with which this process runs its course. A detailed discussion of their work cannot be embarked upon in this brief communication.

If it is required to determine the degree of the permeability of a membrane to water, it is necessary to determine the amount of water which, at a particular difference in pressure, passes through a particular area in a particular time.

If it is possible to measure these three values for the protoplast, the permeability to water per area-element being thus calculated, instead of that per total protoplast, a value is obtained which is comparable to HUBER and HÖFLER's "Filtrationsgeschwindigkeit".

In order to measure the permeability to water one can measure the change in volume during plasmolysis; in this case the amount of water withdrawn from the vacuole is determined. The change in volume of the vacuole which is recovering from plasmolysis may, however, also be measured, in which case the amount of water absorbed by the vacuole is determined.

Both these methods were employed by HUBER and HÖFLER.

In the case of cylindrical cells with suitable protoplasts these changes in volume may be fairly easily determined by the plasmometric method (HÖFLER, 1918).

With the aid of the degree of plasmolysis, i.e., $\frac{\text{volume vacuole}}{\text{volume cell}}$, the

diminution in concentration between the vacuole and the external solution may be calculated.

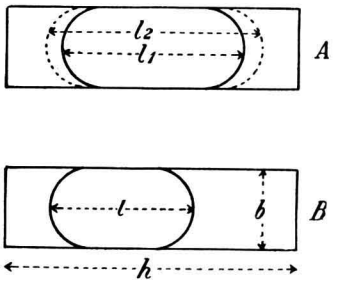

Fig. 1. [1]) Diagrammatic representation of the enlargement of the vacuole during recovery from plasmolysis.
The calculation of the degree of plasmolysis

$$g = \left(l - \frac{b}{3}\right) : h.$$

So far, now, as the determination of the area permeable to water is concerned, a difficulty arises which I have endeavoured to surmount by the choice of the object. I chose, as object, the epidermal cells of the inner epidermis of the bulb-scales of the onion.

This choice proved to be a happy one from several points of view; for it is possible to make a preparation from the epidermal cells without being obliged to injure any cells. Moreover, this object stands plasmolysis and recovery from plasmolysis very well. Part of a preparation of this kind is shown schematically in Fig. 2.

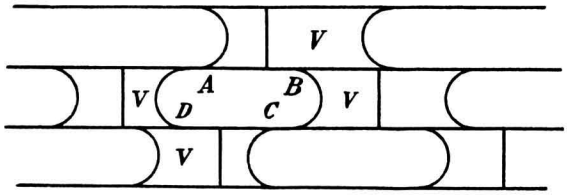

Fig. 2. Plasmolysed preparation, diagrammatic.

The walls of the epidermal cells contiguous to the neighbouring cells of the tissue have a good permeability to water and plasmolytic; the external wall, however, possesses a cuticle which is impermeable even to water.

One can easily convince oneself of this by letting a drop of water fall on a plasmolysed preparation, when the cells do not recover from plasmolysis.

If water or a hypotonic solution is present in the fore-space V (Fig. 2) then the area through which the plasmolysed vacuole can absorb water will be formed by the two hemispherical menisci in the cell, the two lateral areas *AB* and *CD* (Fig. 2), and by the surface of the vacuole adjacent to the inner wall. If the preparation be viewed from above, the inner wall contiguous to the subjacent tissue may be called the base of

[1]) According to HUBER and HÖFLER, 1930.

the vacuole, the part contiguous to the cutinized external wall, the upper surface. The entire surface is thus formed by the hemispherical menisci, the base, and the lateral surfaces of the vacuole. There is no reason in this case to assume that the water finds its way into the vacuole exclusively through the hemispherical menisci, as HUBER and HÖFLER, with their object Salvinia, gave grounds for supposing, and not through the other surfaces. This would even be very unlikely. The amount of water can thus be calculated from the changes in the degree of plasmolysis. The concentration of the vacuole can also be calculated with the aid of the degree of plasmolysis, whilst the area is to be determined in the manner described above. The calculation of the permeability to water may now be carried out, since HUBER and HÖFLER have established the fact that the rapidity of the recovery from plasmolysis is governed solely by the resistance of the protoplasm. Further the concentration of the liquid in the fore-space V (Fig. 2) must also be known. I claim to have succeeded in determining within what time the concentration of the plasmolytic in the fore-space is replaced by the deplasmolytic (water).

The following is a description of this (§ 4).

§ 2. *Arrangement of the experiment.*

I worked according to the method of recovery from plasmolysis. A small piece of epidermis was first plasmolysed in a saccharose solution 0.646 *n* (volume normal), after which the recovery from plasmolysis was investigated in double-distilled water. The epidermal cells of Allium cepa offer a particularly vigorous resistance to plasmolysis, but I tried to determine whether the recovery from plasmolysis in distilled water did not have a harmful effect on the protoplasm.

That this is not the case was proved by the facts that after the recovery from plasmolysis rotation was still observable, and that after recovery from plasmolysis the cells could again be normally plasmolysed.

In one or two cases I noticed an abnormally rapid recovery from plasmolysis, which I ascribe to a possible injury in the course of preparation; cells of this kind were not used for measurements.

A cinematographic film was taken, by means of a Zeiss Universal kinamo apparatus, of the course of recovery from plasmolysis, use being made of the microphot.

I further used a Leitz microscope with ocular 2 and objective 3.

An exposure was made every 30 seconds.

The film obtained was later projected on a white screen, so that I was enabled to measure the course of the recovery from plasmolysis of several cells from one preparation.

In this way I was also able to determine accurately the area of the vacuole which otherwise, when an ocular micrometer is used, is not feasible.

§ 3. *Calculation.*

The cells projected were measured with the aid of a glass ruler, which was divided into mm. I was able to measure accurately to within ½ mm. The enlargement was such that 1 mm. was equivalent to 1.86 μ.

The permeability to water was thus expressed in a figure which indicates the amount of water (measured in cubic mm. on the image projected) which finds its way into the vacuole in ½ minute through an area of 1 square mm. (again from the projected image) with a difference in pressure of 1 *n*. saccharose solution.

This figure was called the *permeability factor* and indicated by the letter *f*. This value corresponds to HUBER and HÖFLER's "Filtrationsgeschwindigkeit".

If it is desired to express the permeability to water in μ per hour, with a difference in pressure of 1 atmosphere, this can be converted, as the enlargement is known. This was done for a particular case.

A linear interpolation was used in calculating in order to obtain the average area and the mean concentration of two successive stages. This is sufficiently accurate, owing to the very short time intervals of ½ minute. (See HÖFLER's elementary calculation, HÖFLER 1930, p. 326).

I obtained the following result.

The quantity of water which finds its way into the vacuole in ½ minute is equal to the difference of the volumes of the vacuoles measured before and after the lapse of the ½ minute.

If we call these volumes: volume vacuole$_1$ and volume vacuole$_2$, then the quantity of water = volume vacuole $_2$ — volume vacuole$_1$.

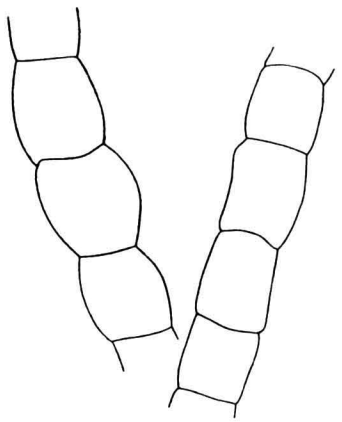

Fig. 3. Epidermal cells of the bulbscales of Allium cepa, in cross section.

The degree of plasmolysis (g) at a particular moment (1) = $g_1 = \frac{\text{volume vacuole}_1}{\text{volume cell.}}$

Volume vac$_2$ — volume vac$_1$ = (g_2—g_1) × volume cell.

The hemispherical area = $4\pi r^2$ (r = ½ breadth).

The lateral area see Fig. 2 = $O \times 2r$.

$O = AB + CD$.

The height of the lateral walls was assumed to be equal to the breadth (= $2r$) in view of the regular dimensions of the cells, even in transverse section. (See Fig. 3.)

The error that can be made here is that the lateral area (which forms only a small part of the total area) may be taken a little too large. This has practically speaking no effect on the permeability factor.

The length of the vacuole is indicated by l; stage (1), by l_1; stage (2), by l_2.

The base of the vacuole through which water can permeate $= (l - 2r) \times 2r$.

The average base during the course between two successive stages $\frac{l_1 - 2r + l_2 - 2r}{2} \times 2r$.

The concentration of the vacuole$_1 = \frac{1}{g_1} \times$ concentration threshold of plasmolysis.

Concentration threshold of plasmolysis $=$ G (final degree of plasmolysis) $\times$ concentration of the plasmolytic.

I thus obtain for f,

$$f = \frac{(g_2 - g_1 \times \textit{volume cell}}{\left(4\pi r^2 + \frac{l_2 + l_1 - 4r}{2} \times 2r + O \times 2r\right) \times \textit{average concentration vacuole}}.$$

§ 4. *The rapidity with which a sugar solution is washed out of the fore-space.*

In the above calculation it is assumed that pure water is present during the recovery from plasmolysis in the so-called fore-space (see V in fig. 2). On the strength of the following test I feel justified in assuming this to be the case.

I found, as the average of 27 preliminary tests, that the recovery from plasmolysis of epidermal cells of Allium in water, after plasmolysis in a saccharose solution of 0.646 *n.*, is completed in approx. 8 minutes at a temperature of 15 deg. C.

In these tests the preparation was moved several times to and fro by means of a pincette in a basin of water, after having been for 15 minutes in the plasmolytic; the recovery from plasmolysis is then traced in water on the microscopic slide.

It had previously been determined that the final degree of plasmolysis was reached after 15 minutes in the plasmolytic.

In order to determine the rapidity with which the sugar solution is washed out of the fore-space I then proceeded in the following manner. After 15 minutes the preparation was taken out of the plasmolytic, was then moved two and fro in water for 30 seconds, after which the water adhering to it was removed on a moist filter paper, and the recovery from plasmolysis was determined in liquid paraffin.

Before the preparation was examined in a drop of liquid paraffin on the microscopic slide, it was moved for a moment to and fro in a basin of liquid paraffin, in order to remove adherent small drops of water.

The course of recovery from plasmolysis is shown graphically in fig. 4.

In the graphic representation the degrees of plasmolysis are set up on the ordinate, and on the abscissa the time in minutes.

The two lines drawn give the course of recovery from plasmolysis of two adjacent cells from one preparation. This preparation was washed in water for 30 seconds. It can be seen that the recovery from plasmolysis

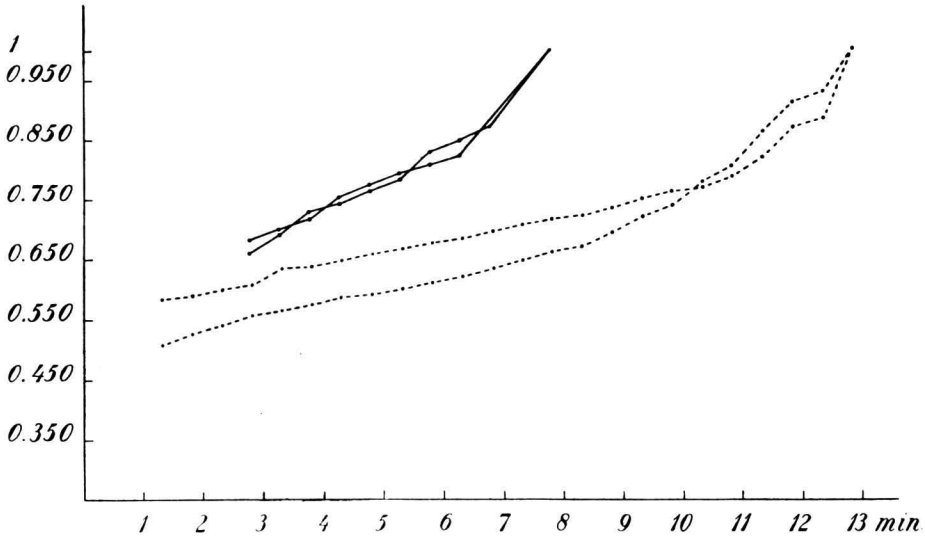

Fig. 4. The lines drawn indicate the course of the recovery from plasmolysis of two cells, which were plasmolysed for 15 minutes in a saccharose solution 0.646 *n*. They were then moved to and fro for 30 seconds in water, after which the water adhering to them was removed on moist filter paper; they were then put into liquid paraffin. Recovery from plasmolysis in liquid paraffin determined. Temperature 17 deg. The dotted lines give the recovery from plasmolysis of two cells which are treated in the same way as those above, with this difference that these were only 5 seconds placed in water.

took place in 7 minutes and 45 seconds, that is to say, as rapidly as in the case of cells which have *constantly* been in contact with water. The beginning of these curves is in the neighbourhood of 2 min. 45 seconds, i.e. 2 min. 45 sec. elapsed after the preparation had been removed from the plasmolytic before I was able to make the first exposure.

Fig. 4 also shows the course of recovery from plasmolysis of two cells from a preparation which was only placed in water for 5 seconds, and was then allowed to recover from plasmolysis in liquid paraffin. This is indicated by the dotted line.

These cells required a good 12 minutes to recover from plasmolysis. In this case I was able to make the first exposure after the lapse of 1 min. 20 sec.

That all the sugar is washed out of the fore-space in 30 seconds is proved by the rapidity of the recovery from plasmolysis, which is approximately equivalent to the recovery from plasmolysis in water. For if all the sugar were not removed from the fore-space the concentration in these fore-spaces would become greater and greater, owing to the fact that the vacuole absorbs water, as a result of which the diminution

ot concentration becomes smaller and therefore the course of recovery from plasmolysis slower.

On the other hand we see that the curves get steeper and steeper, which means that the recovery from plasmolysis is accelerated.

§ 5. *Recovery from plasmolysis in water.*

A preparation of the inner epidermis of the bulb-scale of Allium cepa was allowed to recover from plasmolysis for 15 minutes in a 0.646 *n* saccharose solution. It was then transferred into double distilled water, temperature 15—16 deg., in order to recover from plasmolysis. The course of recovery from plasmolysis was recorded by means of the cinematograph, the first exposure being made after 1 minute and 5 seconds.

The course of recovery from plasmolysis of two cells from one preparation is shown respectively by Fig. 5 and table I and by Fig. 6 and table II.

In the graph the degrees of plasmolysis are set up on the left-hand ordinates, and the times in which recovery from plasmolysis was complete on the abscissa.

The upper line indicates the course of recovery from plasmolysis. The course of this curve shows that the recovery from plasmolysis gets quicker and quicker. This may be due to the fact that the area of the vacuole through which water can enter becomes greater during the recovery from plasmolysis, whilst on the other hand the suction power of the vacuole becomes smaller. The constantly increasing rapidity of the recovery from plasmolysis may also be due to the fact that more water is admitted per area-element as the recovery from plasmolysis proceeds.

This latter should be demonstrated by the calculation of the permeability factor, which shows the amount of water admitted per ½ minute through a particular area, with a difference in pressure of 1 *n*. saccharose solution. The permeability factor was calculated during the course of the recovery from plasmolysis for every ½ minute. It was found that this increases during the course of recovery from plasmolysis.

For the purpose of a graphic representation the permeability factor was set up on the right-hand ordinate, and shown by means of a — - — - — - line.

The course of this line shows a marked rise as the recovery from plasmolysis proceeds.

The suggestion might be made that water enters not only by the areas of the vacuole adjacent to water, but that the water is then so rapidly absorbed by the cell-walls, that the vacuole is practically entirely surrounded by water. That this is the case, and that the upper surface of the vacuole, which lies firmly pressed against the upper wall, which is rendered impermeable by a cuticle, is also to be regarded as a surface which lets water through, seems to me unlikely.

This possibility, however, was also taken into account. The permeability factor was also calculated in case the entire vacuole surface should absorb

water. The permeability factor thus obtained was also graphically represented in fig. 5, and shown by means of .....

It is at once seen that this line also runs upward during the course of the recovery from plasmolysis.

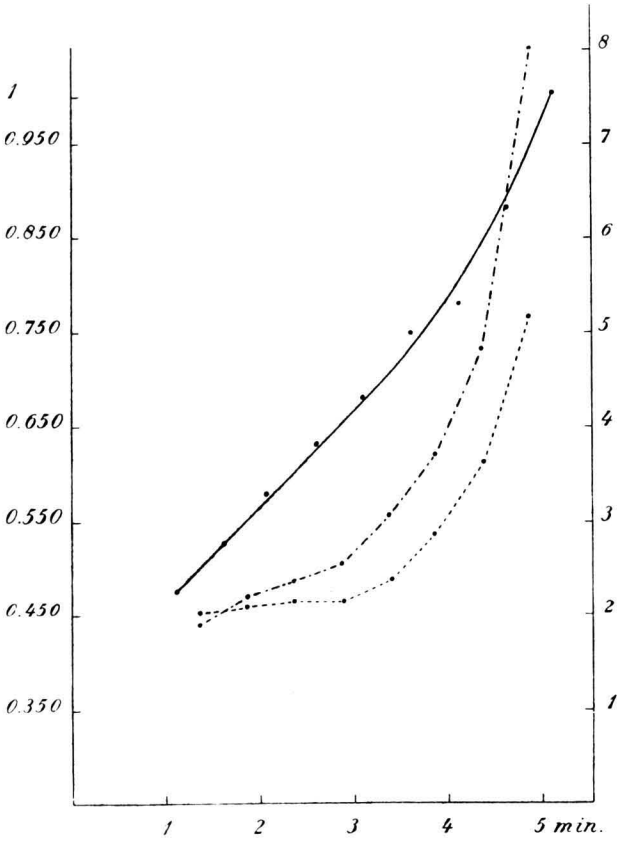

Fig. 5 The degrees of plasmolysis are set up on the left-hand ordinate, the time on the abscissa. The right-hand ordinate indicates the permeability factor. The line drawn indicates the course of the recovery from plasmolysis. The _ . _ . _ . line indicates the permeability factor calculated from the area adjacent to the water, the ..... line shows the permeability factor calculated from the total area of the vacuole.

This means therefore that, even if we assume the area which absorbs water to be as large as possible, an increase of the permeability to water during the course of the recovery from plasmolysis is to be noted.

I think it may be concluded from the course of the curves:

a. *that, in agreement with* HUBER *and* HÖFLER, *the resistance of protoplasm is limiting factor for the course of the recovery from plasmolysis.*

b. *that permeability increases during the recovery from plasmolysis.*

Finally, in order to be able to compare the permeability found by me with the figures found by HUBER and HÖFLER, and by LILLIE in 1915 in

the case of Arbacia eggs, I calculated, as they did, with what rapidity the water diffuses through the protoplasm, and expressed this, in μ per hour with a difference in pressure of 1 atmosphere.

As, however, the permeability to water is not the same at every moment, I took the mean of the permeability factors found, which mean was afterwards converted.

TABLE I.

Preparation plasmolysed in 0.646 n. saccharose solution, for 15 minutes, then allowed to recover from plasmolysis in double distilled water. Temperature 15—16.5 deg. C.

Length of the cell: 110. Breadth of the cell: 52. Preparation 20/5/31 No. 1 cell 4.

Time	Length of vacuole	Lateral area	Degree of plasmolysis[1])	Concentration vacuole	f. calculated with area adjacent to water	f. calculated with total vacuole area
0 min. 0 sec.			0.375	0.646		
1 „ 5 „	69	62	0.475	0.510		
1 „ 35 „	75	54	0.525	0.461	1.92	2.047
2 „ 5 „	81	47	0.577	0.420	2.202	2.164
2 „ 35 „	87	42	0.628	0.386	2.370	2.154
3 „ 5 „	92	28	0.678	0.357	2.563	2.150
3 „ 35 „	99	22	0.732	0.329	3.050	2.357
4 „ 5 „	102	35	0.795	0.305	3.744	2.885
4 „ 35 „	110	32	0.873	0.277	4.800	3.616
5 „ 5 „	—	20	1.00	0.242	8.040	5.146

The permeability factor was expressed in mm. of the image projected. The enlargement was such, that 150 mm. $=280\,\mu$, i.e. 1 mm. $=1.86\,\mu$.

If I now convert the averages found, I obtain the following figures.

Preparation: fig. 5.

f. average (calculated from area adjacent to water) $=35\,\mu$ per hour and 1 atmosphere difference in pressure.

Varying from $18.9\,\mu - 79.3\,\mu$ per hour and 1 atmosphere difference in pressure.

f. average (calculated from total area of the vacuole) $=28\,\mu$ per hour and 1 atmosphere difference in pressure.

Varying from $20.1\,\mu - 50.7\,\mu$ per hour and 1 atmosphere difference in pressure.

[1]) The figures for the degrees of plasmolysis are read off on the curves of fig. 5.

Preparation fig. 6.

f. average (calculated from area adjacent to water)	$= 33\,\mu$ per hour and 1 atmosphere difference in pressure.
Varying from $13.7\,\mu - 57\,\mu$	per hour and 1 atmosphere difference in pressure.

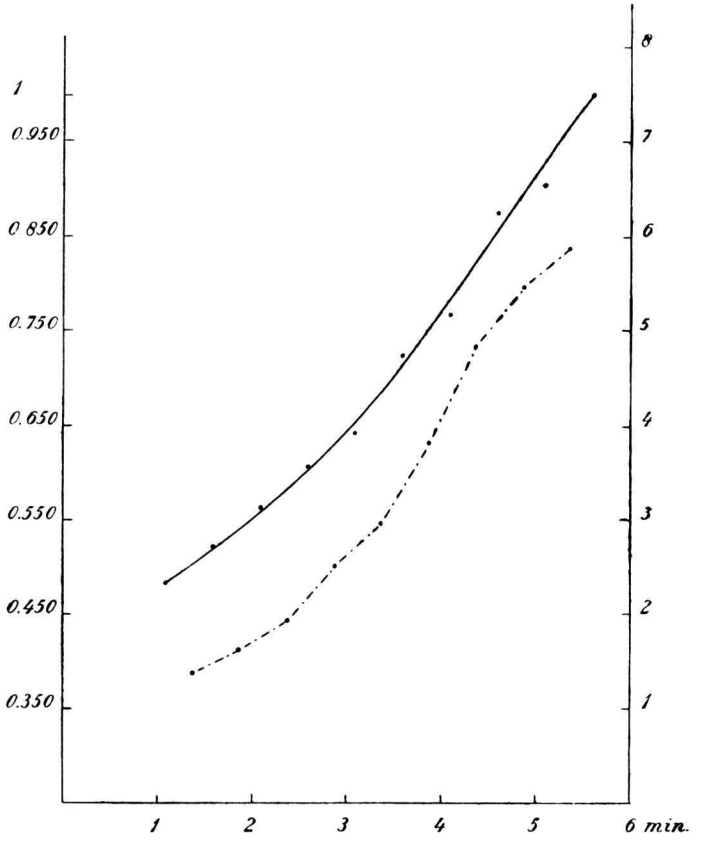

Fig. 6. The degrees of plasmolysis are set up on the left-hand ordinate, the time on the abscisse. The right-hand ordinate shows the permeability factor. The line drawn shows the course of the recovery from plasmolysis, the —.—.—. line indicates the permeability factor calculated from an area adjacent to water.

HUBER and HÖFLER found, in the case of Salvinia, $33\,\mu$ per hour and 1 atmosphere difference in pressure. LILLIE found $42\,\mu$ per hour and 1 atmosphere difference in pressure. The values found by me therefore agree pretty closely with theirs.

§ 6. *Discussion of the results.*

We have seen that the permeability to water becomes greater during the course of the recovery from plasmolysis.

What can this be due to?

We may assume, as does WALTER (1923), that the protoplasm behaves

like other swelling bodies, and thus loses water in concentrated solutions. and swells in water and diluted solutions.

Furthermore, a Q 10 of approx. 1 . 2 is characteristic of osmotic processes when a membrane is used which does not swell (copperferrocyanide) (KRABBE 1896, PFEFFER 1877 p. 85). The Q 10 which is found in the case of the absorption of water by plant tissue is 2 to 3 (VAN RYSSELBERGHE 1903, DELF 1916).

TABLE II.

Preparation plasmolysed in 0.646 n. saccharose solution, for 15 minutes, then allowed to recover from plasmolysis in double distilled water. Temperature 15—16.5 deg. C. Length of the cell: 206. Breadth of the cell: 45. Preparation 20/5/31 No. 1 cell 2.

Time	Length of vacuole	Lateral area	Degree of plasmolysis [1]	Concentration vacuole	f. calculated with area adjacent to water
0 min. 0 sec.			0.410	0.646	
1 „ 5 „	113.5	139	0.485	0.546	1.397
1 „ 35 „	123	123	0.520	0.509	1.620
2 „ 5 „	131.5	124	0.558	0.475	1.949
2 „ 35 „	138.5	132	0.602	0.440	2.518
3 „ 5 „	147	120	0.655	0.404	2.980
3 „ 35 „	162	135	0.715	0.370	3.831
4 „ 5 „	172	90	0.785	0.337	4.896
4 „ 35 „	186 vl.	70	0.857	0.309	5.466
5 „ 5 „	191 vl. [2]	65	0.930	0.285	5.794
5 „ 35 „	206	60	1.00	0.265	

With my own tests I also found a Q 10 of 2—3.

A Q 10 of 2—3 is characteristic of a swelling process; this was also found by VAN RYSSELBERGHE in 1903.

The result obtained by RISSE (1926) is of great importance; by model tests with swelling membranes he found that the permeability of colloids and of non-conducting crystalloids (dextrine, cane sugar) and to *water* depends on the degree of swelling of the membrane employed.

Further researches which should be mentioned are those of LUCKE and MACCUTCHEON (1926).

GELLHORN, in his new text-book (GELLHORN 1931) has the following remarks on this subject. "Es handelt sich hier um Veränderungen der Kolloïde hinsichtlich ihres Gehaltes an gebundenem Wassers, also um Quellungs- und Entquellungserscheinungen, und damit ist die Begründung

[1] The figures for the degrees of plasmolysis are read off on the curves of fig. 6.

[2] vl. means that the vacuole lies with one side against the transverse wall.

der Anschauung gegeben, dass eine völlig scharfe Trennung von osmotischen und Quellungsvorgängen unter bestimmten Bedingungen nicht möglich ist. Aus diesen und anderen Versuchen derselben Autoren folgt, dass die Geschwindigkeit der osmotischen Wasserbewegung vom Quellungsgrad der Zellkolloïde abhängt."

In view of the above data I consider the conclusion justified, *that the increase of the permeability to water during the course of the recovery from plasmolysis is caused by a swelling process. As the protoplasm swells, the permeability to water increases.*

Summary.

1. The plasma resistance determines the rapidity of the recovery from plasmolysis.

2. During the course of recovery from plasmolysis the plasma resistance becomes smaller and the permeability to water greater.

3. The increase in the permeability to water is caused by the swelling of the protoplasm.

4. The water penetrates the protoplasm of Allium cepa with a rapidity of approx. 34 μ per hour and a difference in pressure of 1 atmosphere.

These investigations were carried out in the Laboratory for Plant Physiology at the State University of Groningen. I wish to express my sincere thanks to Prof. Dr. W. H. ARISZ for his constant interest and constructive criticism.

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