INTAKE OF WATER BY PARENCHYMATIC TISSUE

by

DIRKJE E. REINDERS
(from the Botanical Laboratory of the State University, Groningen)
received for publication 12 Sept. 1941.

CONTENTS

PART I. EXPERIMENTS WITH POTATO DISCS.

INTRODUCTION.

The phenomenon that discs of various storage tissues increase in weight when in water, has already been investigated by STILES and JØRGENSEN (1917), who have made an investigation of the swelling of plant-tissues in water; they have worked with discs of potato and carrot. Immediately after being cut, the discs, with a diam. of 1.8 cm and a thickness of 2 mm, are put into water which is not aerated. From time to time the fresh-weight is determined, the swelling being expressed in % increase of the initial fresh weight. They have found that a great deal of water is taken in, especially at the beginning, in the first few hours. They state that changes in the weight of the discs is almost entirely due to the passage of water into or out of the tissue. The weight of the small amounts of dissolved substances which enter or leave the tissue is negligible in comparison with the weight of the water passing.

The phenomenon has also been noticed by STEWARD (1933) and BERRY and STEWARD (1934), who give, in table I, the fresh-weight of various storage-tissues at the beginning and at the end of a period of 91 hours in an aerated solution of 0.75 m.eq. KBR. There is in general an increase in the fresh-weight, the various storage-tissues, however, varying very much in this respect.

After the investigations were terminated (REINDERS 1940), the following papers have appeared. STEWARD, STOUT and PRESTON (1940) who have studied the influence of salts and that of oxygen on the metabolism of potato discs in aerated water, have also found an increase in the fresh weight. Furthermore BRAUNER, BRAUNER and HASMAN (1940) who also confirm the results of a preliminary paper (REINDERS 1938).

The object of this work is to investigate more closely this increase in weight in water. This is done in the first place with potato discs. Discs of various other storage tissues are also investigated, to admit of a comparison between these and the potato discs; the results, treated only briefly in the preceding paper (REINDERS 1940), are communicated more fully in Part II (page 87).

CHAPTER I.

§ 1. Material and method.

The experiments to be described are carried out with the tissue of the potato tuber (*Solanum tuberosum L.*). Only one variety of potato, the "Roode Star", is used. The Roode Star is a late, yellow-(-fleshed), durable, red-skinned potato for winter consumption, which is on sale practically everywhere. (see description in the 16th list of varieties of the "Instituut voor Plantenveredeling", Wageningen 1940).

Several other varieties of potatoes, with which a few provisional experiments are made, show the phenomenon of waterintake in the same way and also to about the same extent as the Roode Star, although the Roode Star has proved to be one of the most satisfactory. These are the summer potato varieties Eersteling and Present, and the varieties Borger and Souvenir, which, like the Roode Star, are winter potatoes. Eersteling is not so suitable for these experiments, the discs being very brittle and apt to break, and showing dead spots in most cases after a few days. Of the other kinds the Borger occasionally shows a somewhat stronger water-intake than the Roode Star. But Roode Star is to be preferred to all other kinds owing to its being obtainable practically everywhere and usable until late in spring.

The experiments are carried out over a period of two years, 1937—1938 and 1938—1939, the work being performed in both cases in the winter-season. Several experiments with hetero-auxin in anaerobic environment are made in November and December 1939 (Chapter III, § 6), as are also a few experiments on the influence of light. (Chapter I, § 5*d*).

The potatoes employed are always as far as possible of the same size and of a regular oblong shape. With a sort of cork-borer, a chromium-plated brass cylinder with a sharp edge, a cylinder of 3.4 cm in diameter is punched out in the longitudinal axis of the potato, this being then placed in the somewhat smaller, also chromium-plated, cylinder of a handmicrotome according to STEWARD (1930). The upper surface of this handmicrotome is fitted with a glass ring, fastened to it with putty, so that the tissue never comes into contact with brass. With this, discs of 1 mm in thickness are now cut, after which the middle piece is punched out with a chromium-plated corkborer of 1.7 cm in diameter.

In this way discs of 1 mm in thickness and with a diam. of 1.7 cm, cut perpendicularly to the morphological longitudinal axis of the potato, are obtained. The tissue consists of the star-shaped, entirely parenchymatic medullary tissue surrounded by parenchyma with dispersed groups of phloem-elements. A good exposition of the anatomy of the potato, with clearly coloured figs. of the various tissues is given by ARTSCHWAGER (1924).

The discs are from 6 to 7 cell-layers in thickness. The shape of the parenchyma-cells is irregularly isodiametrical. The diam. of the parenchyma-cells from the medullary tissue is 150—200 μ, usually 150—180 μ. The parenchyma-cells in the part containing the groups of phloem-elements are somewhat smaller, diam 100—150 μ, usually 100—120 μ. All the cells contain a great deal of starch. (According to the 16th list of varieties of the "Instituut voor Plantenveredeling", Wageningen 1940 the dry substance of the potato consists of approx. 75 % of starch). The parenchyma-cells of the part containing the phloem-groups have most starch, the parenchyma-cells of the central medullary tissue in general contain somewhat less starch. The diam. of the starch-grains differs widely, diam. 20—50 μ, the larger ones 30—50 μ, the smaller ones 10—20 μ. The cells near the phloemelements also contain many, but very small, starch grains, diam. 5—10 μ.

After the discs have been washed in running tap-water for a short time, from 20 to 30 min., to remove the contents of the cut cells, they remain for 24 hours in stagnant tap-water in the room in which the experiments are made. The next day the discs of one and the same potato are weighed separately on a HARTMANN-BRAUN torsion balance, after being superficially blotted off for 15 seconds between a double layer of soft filter-paper under a weight of 200 grammes (Baptiste 1935). On the scales of the balance is a thin cover glass, so that here, too, the discs do not come into contact with metal.

Series of 10 discs are then formed, so that these have the same weight. This average weight never diverges more than 0.2 to 0.3 mg. Care is further taken that the difference between the lightest and the heaviest discs of the same series is never more than $\pm$ 20 mg. In experiments in which more series of 10 discs are required than can be cut out of one potato, two potatoes are taken, and then each series is given an equal number of discs of the first and of the second potato. Care is then taken that when, e.g., each series is given 7 discs of the one potato, the average weight of all these groups of 7 discs is first equal, after which they are completed with 3 discs of the second potato, also to an equal average weight.

Potatoes are invariably used which are of as far as possible the

same size and of a regular oblong shape. This is of importance for the following reason. For normal oblong, fairly large potatoes, the variability of the initial dry weight of two series of 10 discs from the same potato with equal average initial fresh weight, is usually from 3 tot 4 mg, and does not exceed 5 mg (see table 1). In this table,

TABLE 1.

Variability of dry weight of two series, A and B, each of 10 discs from the same potato with equal average initial fresh weight. Directly after the composition of the series the initial dry weight is determined. Fresh weight and dry weight are stated in mg per 10 discs together. These experiments are made in April 1938.

exp. no.	initial fresh weight in mg		initial dry weight in mg		difference in mg
	A	B	A	B	
230 I normal sized potato	2743.2	2748.1	513	508	5
II small potato	2700.5	2700.8	518	533	15
231 normal sized potato	2663.3	2663.5	520	524	4
233 small potato	2628.2	2628.2	384	372	12
237 I normal sized potato	2563.8	2563.5	393	396	3
II " " "	2628.8	2630.8	403	399	4
242 I " " "	2583.2	2582.4	386	390	4
II small potato	2637.1	2638.5	555	529	26

however, we see that with smaller potatoes the variability is considerably larger. The differences between two series of the same potato amount to e.g. as much as 12, 15 and 26 mg. This will be due to the fact that the discs, which are selected for equal fresh weight, will consist of tissue differing morphologically. The discs nearer to the ends of the potato contain more parenchyma with phloem-groups, while in the discs from the middle of the potato a larger part of the surface is occupied by medullary parenchyma in which no phloem occurs. These cells are also somewhat larger in volume and also contain less starch. If, then, we take smaller potatoes, the discs are more likely to differ as regards the kind of tissue. Equal fresh weight is in this case no guarantee of equal dry weight. It is therefore important for the experiments always to take fairly large potatoes of an oblong shape, so that all the discs may be cut out of the central portion of tissue which is morphologically as homogeneous as possible.

After weighing, the discs are placed in experimental vessels, for which purpose glass-beakers with a capacity of 600 cm^3 (Jenaer Geräte-glas 20, model D) are used. These are filled with 500 cm^3

water or hetero-auxin solution, as the case may be. The water is distilled tap-water, distilled once over Jena-glass. On the brim of the glass-beaker is a paraffined plate of cork (*a*) with a large opening in the middle. In this opening a Jena-glass cylinder (*b*) is fixed, over the lower end of which coarse-meshed tulle is stretched. The diam. of this glass cylinder (lamp-glass) is 4.5 cm. The discs, in series of 10 per glass, are inside this at a distance of about 1 cm under the surface of the water. They are thus on a floor of stretched tulle. There are therefore 10 discs per 500 cm^3 water. This was chosen in agreement with STEWARD, who takes 40 discs to 2 litres. (STEWARD, BERRY and DROYER 1936).

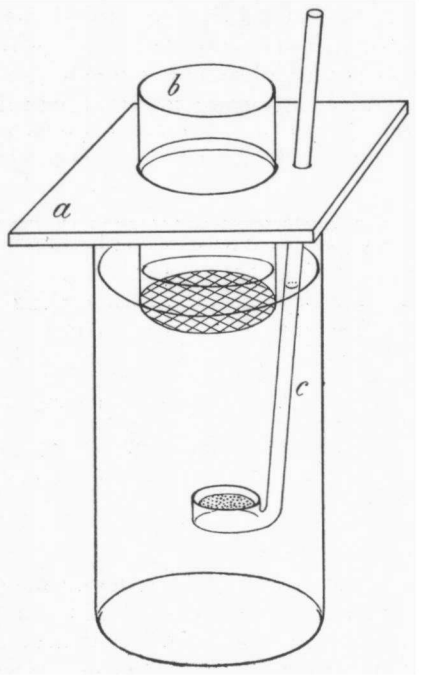


Fig. 1. Test-vessel. Description in the text.

The water is constantly aerated with air, which has previously passed through a couple of cottonwool filters and two wash-bottles filled with distilled water, so that the air is freed from dust and reaches the experimental glasses in a moist condition. The air is led in the experimental glasses through Schott's sintered glass-filters (*c*), whereby a proper aeration is promoted. It is of importance that the air should previously be made moist, as evaporation, to be noted especially in the first washbottle, is fairly large, and the experiments last a longer time.

The duration of the experiments, when not otherwise stated, is 8 days. The water-intake is determined every two days, by weighing the discs and expressing the increase in fresh weight in %. The water, or, as the case may be, the hetero-auxin solution, is renewed after every determination, i.e. every 48 hours. The experiments are made in a dark room for constant temperature at 21° C; only during the daytime while weighing is proceeding, is an electric lamp burning, the rest of the time the room is in darkness.

For the determination of the dry weight the discs are dried in an oven at 96° C. As it is found in former experiments that the dry weight has not yet become constant in 6 hours, the discs are invariably left in the oven for approx. 20 hours; the dry weight is then found to be constant in all cases.

At the end of the experiment, which lasts for 8 days, the glasswork is invariably cleaned with potassium bichromate and sulphuric acid, in which it remains for 24 hours. The tulle-gauzes are renewed each time, while the tulle is previously washed to remove the pap. The paraffined sheets of cork are held for a moment in a flame, so as to keep them as sterile as possible.

P_H measurements, if not otherwise stated, are carried out with the aid of the Merck Universal Indicator. Measurements of the resistance of the external solution, water or hetero-auxin solution, taken to get an impression of the exosmosis, are made with a Philips apparatus, the Philoscope. The cellconstant of the used platinum-electrode is 1.61. The specific conductivity can be calculated according: Specific conductivity $= \frac{1}{a\, R_x}$ (Ohm^{-1} cm^{-1}), in which formula a = the cellconstant of the electrode and R_x is the resistance in Ohm measured with the Philoscope. The resistance in ohm stated in the following paper is always the R_x.

§ 2. The increase in weight of the discs in water.

If potato-discs are put into water and their weight is determined from time to time, this is found constantly to increase (STILES and JØRGENSEN 1917; STEWARD, STOUT and PRESTON 1940). It makes a great difference whether the water is aerated or not. The increase in weight of the discs in aerated water has been found to be considerably greater than in unaerated water, a phenomenon which will be discussed in more detail in chapter II, § 1. It has further been found that in aerated water, at the ordinary temperature of 21° C, the discs can be kept alive for an indefinite period, the discs remaining turgescent and fresh. Not only for 8 days, which is the normal duration of the experiment, but also for a longer time, e.g. 5 weeks and more.

No particular precautions are taken to keep the discs sterile. TURNER (1938) works with discs of the carrot, also taking no particular precautions to keep them sterile. He remarks that plant-tissue, such as carrot-discs, after sufficient preliminary washing, remain in good condition for several weeks, even in distilled water. "Slices kept in well-aerated water are not readily attacked by bacteria". In unaerated water, on the other hand, the discs remain good

for a short time only. The water becomes foul and the discs flabby, in spite of the water being constantly renewed. This sometimes occurs in as little as two days, in other cases only after from 4 to 6 days; rarely the discs remain fresh for a longer period.

STILES (1927) also finds that discs of "storage tissues", e.g. of red beet, die within a few days in unaerated water, while the discs in aerated water remain in good condition for an indefinite time. Stiles has worked at a temperature of 20° C. The only case in which discs in aerated water do not remain good indefinitely, occurs in experiments at a higher temperature, 25° C and 30° C (Chapter II, § 4).

At a higher temperature, 30° C, but also at 25° C, the discs show signs of dying off after some time. It was found that the durability of the discs at 25° C can be favourably influenced by certain precautionary measures. If glass-work treated in the usual way is used, the discs show signs of deterioration at 25° C after about 4 days. The water is foul and turbid, while the discs are already in a bad condition; after 6 days most of the discs are quite flabby or have dead parts here and there. If, however, the glass-work with the already fixed tulle-gauze, after having undergone the usual cleansing, is further sterilized by heating to 110° C, the discs, as also the controls at 21° C, remain good for the entire duration of the experiment, 8 days. That is the case with the experiments at 25° C, in table 12 (p. 44). These experiments, both those in which the glass-work with the already attached tulle-gauze was not sterilized by heating and those in which this was done, are all carried out in March/April 1938. The reason why sterilisation of glass-work with tulle-gauze has a favourable effect, is therefore not to be sought for in the condition of the potatoes, which is equally good in all the experiments.

The sterilisation of the glass-work is applied in 1938. In the winterseason of 1938/1939 and also in Nov./Dec. 1939 this is omitted.

In a few experiments it is also noted whether this sterilisation of the glass-work with the already attached tulle-gauze possibly has an effect on the deterioration of the potato discs in unaerated water. This is done with the intention of perhaps applying it as a precautionary measure for sterility with the ordinary experiments with air-aeration. It is found, however, to have little influence on the durability of the discs, although in a few experiments the discs of the series with the glass-work treated in this way remain in a somewhat better condition than the discs of the control series, but the difference is really of little importance.

The covering of the experimental vessel at the top by means of a petri-dish laid on the lamp-glass is also not found to have a favourable effect on the durability of the discs in unaerated water, the discs

beginning to deteriorate just as quickly as the control series.

Let us now return to the phenomenon of the increase of weight in aerated water. A picture of the course of the increase in weight of potato discs in aerated water during the experiment-period is shown in fig. 3. The time in days is shown on the abscissa. The increase in the fresh weight in % of the initial fresh-weight is set out on the ordinate. The increase in weight is greatest in the first few days; the increase then gradually diminishes. The rapidity of increase of the first two-day period is maintained in the following two-day period also. The curve which indicates the course of the water-intake is practically a straight line for the first four days.

Gradually the water-intake becomes less strong, but an equilibrium is not reached. Normally the experiments always last for 8 days; only in a few cases are they continued for a longer period. With these experiments which are continued longer the water-intake still continues after the first 8 days, but the increase is then only very slight.

This increase in weight cannot be caused by anything but the fact that the tissue takes in water, for the simple reason that nothing else is available (STILES and JØRGENSEN 1917). But it should be noted that this increase in weight is not exactly equal to the water-intake, since the dry-weight decreases during the experiment (compare also STEWARD, STOUT and PRESTON 1940). At the end of the experiment the dry weight of the discs is determined. The initial dry weight of an equivalent series of discs with the same average initial fresh weight is determined immediately after the series had been made up. The discs are found to have lost a considerable amount of dry substance in the course of the experiment. The variability of the dry weights between two equivalent series is slight. (Table 1). In this way we obtain a reliable picture of the loss of dry substance during the experiment.

This loss of dry substance may be attributed to the respiration or to an exosmosis.

To get some idea of the exosmosis the conductivity of the water is found by determining the resistance of the external solution every other day, with each weighing of the discs. At the same time the p_H is noted. The resistance of the distilled tap-water, without potato discs in it, usually varies between 200 000 Ω and 300 000 Ω and remains, if aerated, constant. The p_H of distilled tap-water, without potato discs in it, is 4.5. If this water is aerated, still without potato-discs in it, the p_H remains practically constant. Only in the beginning, within as little as three hours, the p_H rises somewhat and then does not change any more. This is followed for three days. This slight

rise is probably attributable to the fact that less carbonic acid is dissolved in aerated water than in unaerated water.

In table 2 is given a survey of the experiments 384, 385 and 406, in which two series of 10 discs from the same potato are compared with one another. Of one series (A) the water is not renewed during the experiment. The discs of series A therefore remain 8 days in the same water. As a control, series B, is taken the ordinary case, with which at every observation, that is every other day, the water is renewed. Of the third series, (C), the dry weight is determined at the beginning of the experiment.

If we first pay attention to series A, with which the water is not renewed, we see that the resistance of the water decreases in the first few days. The fall is great in the first two-days' period and is continued in the following two days. After that, from the fourth to the sixth day, this fall changes into a slight rise, which continues in the final two-days' period to a more marked extent. The p_H rises in the first few days from 4.5 to 7.0 or 6.5. Later on the p_H falls, and again approaches 5.0. With series B, with which the water is renewed after each observation, we see the same thing in principle. In the first two-days period the resistance shows a marked decline. In the second period also, but the decline in the resistance is less marked. In the following period, from the fourth to the sixth day, the resistance changes only slightly, there is still a slight fall in experiment 406, but the resistance is really practically unchanged. (In experiment 384 and 385 there is a fall, but here the resistance of the fresh water on the fourth day is also uncommonly high). In the last two-days' period the resistance of the water in all experiments rises, and even to a fairly large extent. The p_H rises in the first two-days' period from 4.5 to 6.5 à 7.0. In the second period the p_H also rises, but the rise is less marked. In the following period, from the fourth to the sixth day, the p_H again rises, but only to approx. 6.0. In the final two-days' period practically no rise, or only a slight one, is to be observed in the p_H. These phenomena therefore point to the occurrence of exosmosis in the first few days of substances from the discs into the water, while later on there is a tendency to take in substances out of the water. The distilled water is therefore not absolutely pure water.

The water-intake for both series (table 2) is invariably equal, it differs hardly at all. The slight differences fall within the limits of variability (see § 3), although in all three cases the water-intake of the discs of the series with the water not renewed, is somewhat higher. The respiration measured as loss of dry substance is also exactly the same. It therefore really has no influence whether the

TABLE 2.

The water-intake, when the water is not renewed during the experiment.
Three series of 10 discs from the same potato. Of series A the discs remain 8 days long in the same water. Of series B the water is renewed after each observation. Of series C the initial dry weight is determined. Fresh weight and dry weight are stated in mg for 10 discs together. The water-intake is stated as increase of fresh weight in % of the initial fresh weight. The electrical resistance of the water is stated in Ω.

	exp. 384 (9-3-'39)			exp. 385 (9-3-'39)			exp. 406 (25-4-'39)		
	C	A water not renewed	B control	C	A water not renewed	B control	C	A water not renewed	B control
beginning of experiment									
dry weight	422			426			413		
p_H		4.0–4.5	4.0–4.5		4.0–4.5	4.0–4.5		4.5	4.5
electrical resistance		120 000	120 000		100 000	100 000		250 000	250 000
fresh weight		2401.9	2402.2		2505.8	2505.9		2423.4	2423.0
after 2 days			old new			old new			old new
p_H		6.5–7.0	6.5–7.0 4.5		7.0	7.0 4.5		7.0	7.0 4.5
electrical resistance		46 000	54 000 200 000		34 000	40 000 200 000		42 000	42 000 230 000
water-intake %		8.8	8.6		8.1	7.5		8.3	8.5
after 4 days									
p_H		7.0	6.0 4.5		7.0	6.0–6.5 4.5–5.0		7.0	6.5 4.5
electrical resistance		37 000	110 000 300 000		39 000	100 000 420 000		37 000	74 000 210 000
water-intake %		13.6	13.1		12.0	10.8		14.2	14.6
after 6 days									
p_H		6.0	5.0 4.5		6.0	5.0 4.5		7.0	6.0 4.5
electrical resistance		42 000	180 000 170 000		47 000	220 000 170 000		44 000	200 000 260 000
water-intake %		17.6	17.1		16.1	14.7		18.1	17.6
after 8 days									
p_H		5.0	4.5–5.0 —		4.5–5.0	4.5–5.0 —		6.0	4.5 —
electrical resistance		60 000	500 000 —		60 000	350 000 —		100 000	450 000 —
water-intake %		20.7	20.0		19.1	17.5		20.4	19.5
final dry weight		367	368		349	346		351	346
respiration		55 mg	54 mg		77 mg	80 mg		62 mg	67 mg

TABLE 3.

Water-intake and respiration after two, four, eight and sixteen days. (Exp. 408; see § 4 of this chapter). Each series exists of 5 discs of one potato and 5 of the second. Fresh weight and dry weight are stated in mg for 10 discs together. The

	A	B
beginning of experiment (27-4-1939)		
fresh weight	1157.1+1188.1=2345.2	1156.5+1189.3=2345.8
dry weight	400	—
p_H	—	4.5
electrical resistance	—	180 000
after 2 days		
water-intake %		8.3
dry weight		378 (22)
p_H		7.0 —
electrical resistance		39 000 —
after 4 days		
water-intake %		
dry weight		
p_H		
electrical resistance		
after 6 days		
water-intake %		
p_H		
electrical resistance		
after 8 days		
water-intake %		
dry weight		
p_H		
electrical resistance		
after 10 days		
water-intake %		
p_H		
electrical resistance		
after 12 days		
water-intake %		
p_H		
electrical resistance		
after 14 days		
water-intake %		
p_H		
electrical resistance		
after 16 days		
water-intake %		
dry weight		
p_H		
electrical resistance		

water-intake is stated as increase of fresh weight in % of the initial fresh weight. The respiration is stated ( ) as loss of dry substance in mg. The electrical resistance (in Ω) is stated before left and after renewal of the water right, so is the p_H.

C	D	E
1157.6+1189.2=2346.8 — 4.5 180 000	1156.9+1188.6=2345.5 — 4.5 180 000	1158.2+1189.1=2347.3 — 4.5 180 000
7.4 — 7.0 4.5 40 000 230 000	9.0 — 7.0 4.5 34 000 230 000	8.7 — 7.0 4.5 40 000 230 000
13,4 355 (45) 6.5 — 82 000 —	14,6 — 6.5–7.0 4.5 82 000 240 000	14,4 — 6.5–7.0 4.5 72 000 240 000
	18,7 6.5 4.5 190 000 280 000	18.6 6.5 4.5 210 000 280 000
	21,3 345 (55) 4.5 — 380 000 —	20.8 — 4.5 4.5 430 000 250 000
		22.0 4.5 4.5 260 000 180 000
		23.2 4.5 4.5 350 000 222 000
		23.8 4.5 4.5 290 000 220 000
		24.5 320 (80) 4.5 — 285 000 —

water is renewed or not. Nearly all the experiments, however, are carried out with renewal after each observation, and, as we shall see in chapter III, it actually does make a difference for the experiments on the effect of hetero-auxin.

There is therefore seen to be some exosmosis in the first few days, although not later. But this does not give us the certainty that the loss of dry substance is really to be ascribed to respiration, and is not caused by this exosmosis which makes itself felt at the beginning.

In § 4, where the loss of dry substance is determined after two, four, eight and sixteen days, the resistance of the external solution is also determined in each case (table 3). It is now found that, as described above, the resistance in the first few days shows a marked decline, which in the following period becomes less, and after the lapse of approx. 6 days changes into a rise in the resistance. There is here, in the later periods, from the fourth to the eighth day and from the 8th to the 16th day, a loss of dry substance, even a marked one (tables 3 and 6), while no exosmosis takes place in this time. On the contrary, the resistance of the water rises, showing that substances are being taken in from the water by the discs. We see, therefore, that the loss of dry substance is not due to exosmosis so that we may be sure that it is actually respired. The loss of dry substance in the first 4 days of the experiment is greater than later on. This is most probably due to the respiration being strongest in this period. But it is not to be said with certainty whether exosmosis does not contribute to this, even if only to a slight extent.

We now therefore take it that the loss of dry substance is due chiefly to the respiration, and can now enquire what effect this has on the increase in the fresh weight. An idea of this can be obtained from the following consideration.

STEWARD, WRIGHT and BERRY (1932) have found that the respiration of discs of potato remains constant for 4—6 days. They work e.g. with potato discs of 3.4 cm diam. and 0.82 mm thickness, and take 60 of these discs to 2 litres aerated distilled water, temp. 23.2° C. They have found an average value of 0.2000 mg. CO_2 production per gramme tissue per hour for the respiration. Let us try to compare this with the respiration, measured as loss of dry substance, in the present investigation; supposing that the material respired is glucose. According to BRAUNER, BRAUNER and HASMAN (1940) glucose predominates among the amount of soluble sugars occurring in the potato tuber. Starting from a respiration of 0.2000 mg CO_2per gramme tissue per hour, we get a CO_2 production per hour of 0.5 mg for 10 discs of 1 mm thickness and 1.7 cm diam, having a mean

fresh weight of approx. 2500 mg. So in 4 days (96 hours) this becomes $96 \times 0.5 = 48$ mg per 10 discs. $C_6H_{12}O_6 + 6\,O_2 = 6\,CO_2 + 6\,H_2O$. (Mol. wt. of glucose is 180). 180 grammes glucose corresponds, therefore, to 6 gramme-mol. CO_2, that is, therefore, to 264 grammes CO_2. In 4 days 48 mg CO_2 is lost by 10 discs, that is, therefore, $48/264 \times 180 = 33$ mg glucose.

In my experiments (table 6) the average respiration of dry substance is 33 mg per 10 discs in 4 days. According to table 22 the average loss of dry substance of the controls in 4 days is 45 mg, while the average respiration according to the tests of table 8 is 47 mg in 4 days.

STEWARD (1937) states that there is still uncertainty as to the substratum and the course of the respiration. There is here indeed an agreement between the results obtained by STEWARD, who determines the production of carbonic acid, and my tests, in which the loss of dry substance is determined, if, when converting these, we start from the fact that glucose (or at any rate a sugar of that empirical formula) is the substratum for the respiration. This, it seems to me, tends to show that glucose, at any rate in the case of the potato, is actually likely to be the substratum for the respiration.

The loss of dry substance with my experiments is of the same rate of greatness as the respiration with the experiments of STEWARD. It is as a rule, however, somewhat higher, as has been shown. (33 mg. glucose with the exp.'s of STEWARD; 33, 45 and 47 mg. with my experiments). It is not impossible that this difference, perhaps partly, may be ascribed to the fact that substances disappear into the filter-paper during the blotting of the discs. For STEWARD, STOUT and PRESTON (1940) have the opinion that rather significant amounts of organic substance are transferred to the drying papers used to remove surface water. The discrepancies in the balance sheet are ascribed to this cause for the greater part. Small amounts possibly disappear in the form of volatile organic compounds as is mentioned by STEWARD, STOUT and PRESTON. However, the figures mentioned above clearly show, that these causes may play a small part only. The loss of dry substance, therefore, though it may not be an absolute criterion as are determinations of the CO_2-production and the oxygen consumption, is a quite reliable criterion for the respiration.

In certain cases an R.Q. = 1 was found for potato tissue. (NOVY 1925; PRINGSHEIM 1935). BOSWELL and WHITING (1938) have found that the respiration of potato discs consists of two components, both of which have an R.Q. = 1.0. But here at the same time synthetic processes also take place, e.g. synthesis of protein, growth occurs. Cell-divisions e.g. are seen, at which occasion new walls and new protoplasm are formed; thus substances are fixed in an insoluble

form. (STEWARD 1932 and 1937; STEWARD and PRESTON 1939; STEWARD, STOUT and PRESTON 1940). It is therefere probable that the R.Q. for the total gas-exchange will not be exactly 1 in these experimental conditions, but that it will have a somewhat different value. In practice, however, the synthetic processes will not play a large part in proportion to the entire respiration. For this calculation we will assume that only a respiration of glucose (M.W. = 180) occurs, and that this is entirely broken down to carbonic acid and water. According to:

$$C_6H_{12}O_6 + 6\ O_2 = 6\ CO_2 + 6\ H_2O.$$

The CO_2 will pass into the air, and we may suppose that the water remains in the discs. A respiration of one gramme-molecule glucose means thus a diminution in the fresh weight of $6 \times 12 = 72$ g. (At. wt. of carbon is 12).

The reduction of the fresh weight by respiration will thus be equal to 72/180 or 2/5 × the loss of dry substance. A respiration of e.g. 60 mg dry substance then means a diminution in the fresh weight of 2/5 × 60 mg = 24 mg. As the average fresh weight of 10 potato discs together is approx. 2500 mg, that is thus approx. 1.0 %.

The actual water-intake in 8 days is therefore about 1 % larger than the increase of the fresh weight indicates. This hypothesis is worked out (REINDERS 1938), where in table 1 is calculated for four tests how much the actual water-intake then is in mg for 10 discs together. In table 6 (p. 24), too, is calculated according to this hypothesis to what loss in fresh weight a loss of dry substance by respiration is equivalent. This loss in fresh weight, converted into %, is then added to the increase in fresh weight found. In this way we obtain the actual water-intake. (last column, table 6).

We must, however, remember that this water-intake is not the same thing as the increase of the water-content of the tissue. The water which is produced from the glucose during respiration, is supposed to remain in the discs. And this therefore increases the total amount of water in the discs. In this investigation no further account is taken of this total increase, but only of the amount of water taken in from outside. It should further be remarked that, when in the rest of this investigation the term water-intake is used, the increase in fresh weight is meant by it. Without, therefore, taking into account the loss of weight by respiration, as is done only in table 6, this only amounting to approx. 1 %.

The question now is, how this water-intake is brought about. In part II this will be dealt with more fully. All that is investigated here

is whether it might not be merely an infiltration of the tissue. In view of the researches of VAN DER PAAUW (1935), we have to take into account the possibility that the so-called water-intake might to a greater or lesser extent be nothing else than the replacement of the air from the intercellulars by water.

A priori this seems very unlikely, since it is shown by the experiments in which a hetero-auxin solution is infiltrated (chapter III, § 3) that this has no influence whatever on the action of the hetero-auxin on the water-intake. The curve for the series with infiltrated hetero-auxin coincides completely with that of the series to which hetero-auxin is added in the ordinary way.

In view of this possibility, however, the following investigation is carried out. Series of 10 discs of the same mean fresh weight are formed in the usual way. The air is then withdrawn from the discs of one series (A), by evacuating them with the aid of a suction-pump in a little glass-beaker of water for about 20 minutes. When the air is again admitted to the bell, the discs are infiltrated with water. They are then again weighed and, as we see in figure 2, they have increased somewhat, but little, more than the controls, which have, of course, also become heavier in that time, owing to the ordinary intake of water. These experiments are summarized in table 4.

In my preceding paper (REINDERS 1940) I have already pointed to the improbability that infiltration might be the explanation of the

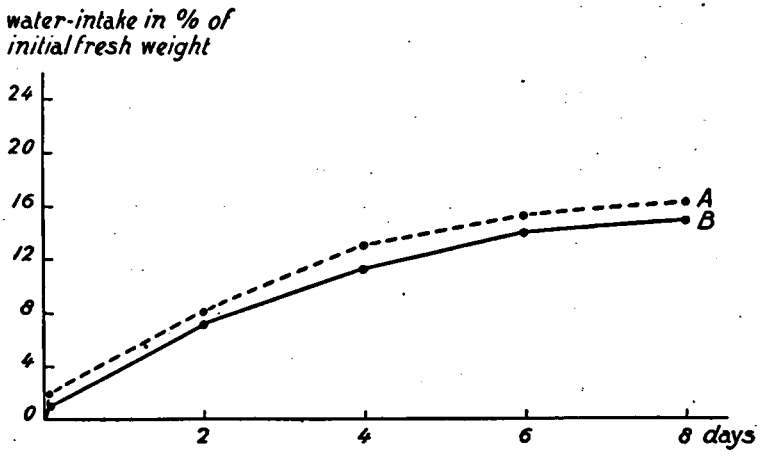


Fig. 2. The water-intake after the air has been removed from the discs by infiltration (exp. 320, table 4).
A: infiltrated.
B: control.

TABLE 4.

The water-intake after the air has been removed from the discs of series A by infiltration.
The water-intake is stated in % of the initial fresh weight. Fresh weight and dry weight are stated in mg for 10 discs together. The loss of dry substance is stated in mg for 10 discs together and in % of the intitial fresh weight.

exp. no.		initial fresh weight	water-intake as increase in % of initial fresh weight after:						dry weight mg		los of dry substance	
		mg	½ hour	2 d.	4 d.	6 d.	8 days	after-infiltr.	ini-tial	final	mg	%
312	A infiltrated	2429.3	1.9	8.1	13.3	16.1	17.3	16.9	—	361	89	19.8
(24-11-'38)	B control	2429.9	1.8	7.8	12.8	16.8	17.7	—	—	360	90	20.0
	C	2427.8	—	—	—	—	—	—	450	—	—	—
320	A infiltrated	2477.5	1.9	7.9	12.8	15.1	16.1	15.5	—	409	79	16.2
(6-12-'38)	B control	2477.8	1.1	7.0	11.3	13.9	14.8	14.9	—	411	77	15.8
	C	2478.8	—	—	—	—	—	—	488	—	—	—
321	A infiltrated	2402.3	1.9	9.5	15.7	20.3	22.4	—	—	—	—	—
(7-12-'38)	B control	2403.2	1.7	9.0	15.6	19.9	22.2	—	—	—	—	—
397	A infiltrated	2531.1	1.9	10.8	18.3	21.5	22.6	—	—	—	—	—
(7- 4-'39)	B control	2531.4	0.9	9.7	15.7	20.5	22.6	—	—	—	—	—
401	A infiltrated	2451.7	1.6	9.2	15.2	18.3	19.9	19.9	—	313	64	17.0
(12- 4-'39)	B control	2452.2	1.2	8.2	12.9	16.0	180.	—	—	312	65	17.2
	C	2450.7	—	—	—	—	—	—	377	—	—	—
404	A infiltrated	2455.9	1.6	12.2	19.7	23.5	25.2	25.6	—	383	80	17.3
(19- 4-'39)	B control	2455.9	1.2	12.0	18.2	22.8	25.4	25.7	—	392	71	15.3
	C	2457.1	—	—	—	—	—	—	463	—	—	—

total water-intake, the increase in the fresh weight being very considerable (20 to 30 % in 8 days), and the intercellulars of the potato tuber being extremely small, as is plainly to be seen microscopically. In 1940 BOSWELL and WHITING (according to BRAUNER, BRAUNER and HASMAN, 1940; the original publication of BOSWELL and WHITING in the Annals of Botany not being available to me) determine that the ratio: intercellular volume/cell volume for potato tissue is approximately 6.5 ml./100 g fresh weight. With a complete infiltration, the intercellular volume quite filled with water, this means thus an increase of weight of about 6.5 %. So in no case this can explain an increase of weight of about 20 % or more, as is found in the present investigation. The increase caused by infiltration found in the present investigation is much smaller, average approx. 0.5 %. However, as also BRAUNER, BRAUNER and HASMAN mention, it may be expected that the infiltration factor must be altogether negligible with tissue discs, which are left submersed in water for a longer time before the starting of the experiment, because then all injectable spaces must have been already filled. This may have taken place, as the discs in the present investigation are submersed in water during 24 hours before being used.

The course of the water-intake of the infiltrated series in the following days is further exactly like that of the controls. At the end of the test infiltration is again performed, while in a few tests the control series is also infiltrated. The weight of the control discs is hardly affected at all by this. The weight of the infiltrated series also remains practically constant; in a few cases, test 312 and test 320, there is a slight loss of weight. Probably these discs have got into a somewhat less good condition, and there will be more marked exosmosis than with the controls. That there actually is a somewhat stronger exosmosis with the infiltrated discs is shown in tests 401 and 404, in which the conductivity of the external solution is determined. (table 5). Both of these series are found to have behaved alike in the first 6 days, the resistance diminishing after every renewal, most in the first two days, and the values are invariably exactly alike for series A and B. From the 6th to the 8th day, however, this changes as usual, into an increase of the resistance, but series A (infiltrated) does not increase quite as much as the control. This suggests a somewhat stronger exosmosis, at any rate if we consider that both exosmosis and intake continually take place; or perhaps it is only a slighter intake. In any event it is an indication that the infiltrated discs are in a somewhat less favourable condition than the control discs.

The respiration is exactly alike in the two series. (table 4, last

TABLE 5.

Determination of the conductive capacity of the external solution of the experiments 401 and 404 of table 4. The electrical resistance is stated in Ω.

exp. 401				exp. 404			
		infiltrated A	control B			infiltrated A	control B
12-4-'39		260 000	260 000	19-4-'39		230 000	230 000
14-4-'39	old	53 000	53 000	21-4-'39	old	39 000	38 000
	new	235 000	235 000		new	250 000	250 000
16-4-'39	old	90 000	90 000	23-4-'39	old	58 000	58 000
	new	250 000	250 000		new	300 000	300 000
18-4-'39	old	160 000	160 000	25-4-'39	old	200 000	200 000
	new	230 000	230 000		new	200 000	200 000
20-4-'39	old	350 000	390 000	27-4-'39	old	300 000	330 000

column), the infiltrated discs have lost the same amount of dry substance as the controls. Only in test 404 is the loss of dry substance in the case of the infiltrated discs somewhat larger than in that of the discs of the control series. The somewhat larger exosmosis in the last few days of the test, in the case of the infiltrated discs, shown by the determination of the conductivity of the external solution, is therefore not of great importance.

These tests have therefore shown that the water-intake, as found here with potato discs, is not due to the replacement of air from the intercellulars by water. It will therefore really be an intake of water by the cells of the tissue themselves.

§ 3. The variability of the water-intake and of the respiration.

The variability of the water-intake is found to be very slight (figure 3), the values diverging in the most unfavourable cases only a trifle more than 1%. The variability of the respiration is also very slight. This difference is not, as a general rule, larger than 5 mg. It falls within the limits of the margin of error indicated by the variability of the initial dry weight (table 1), which also as a rule does not diverge more than 5 mg.

With exp. 284 the water-intake in 8 days of two equivalent series amounts to 18.5 % resp. 17.2 % of the initial fresh weight; the respiration amounts to 78 resp. 80 mg (average 17.9 % of the initial dry weight). With exp. 301 this water-intake is 22.0 % and 21.2 %

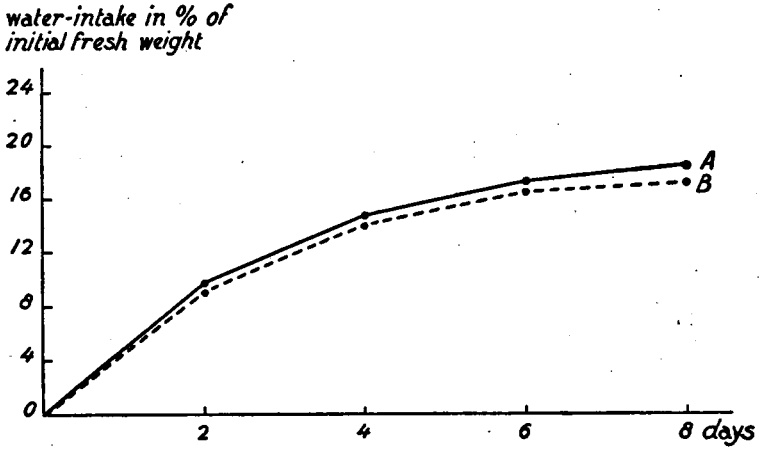


Fig. 3. The variability of the water-intake (exp. 284). A and B are two series of 10 discs from the same potato with equal average initial fresh weight.

respectively; the respiration being 57 and 62 mg resp. (average 15.7% of the initial dry weight). With exp. 305 this water-intake is 18.0 % and 18.0 % resp.; the respiration amounting to 72 resp. 69 mg (average 16.8 % of the initial dry weight).

Between the potatoes in the various tests there are no great differences, either as regards the water-intake or as regards the respiration. As far as these few tests permit of a judgment, a vigorous water-intake does not accompany a vigorous respiration. The most marked water-intake is seen in the second mentioned test, while it is precisely here that the respiration is the slightest.

§ 4. The respiration in course of time.

In the above we have gained an impression of the course of the water-intake. The water-intake is strongest in the first few days; later on the increase becomes less marked. We will now try to obtain an impression of the course of the respiration in course of time, to see whether this perhaps does run parallel to the intake of water. STEWARD, WRIGHT and BERRY (1932) have found, that the respiration of potato discs remains constant during more than six days.

For this purpose a number of series of discs are taken, the dry weight of which is determined after a period in aerated water of a varying number of days. The initial dry weight of series A is immediately determined. Of the others the water-intake is determined,

TABLE 6 (cf. table 3).

The respiration, measured as loss of dry substance (stated in mg and in % of the initial dry weight), in course of time. Fresh weight and dry weight are stated in mg for 10 discs together. The water-intake is stated as increase of fresh weight in % of the initial fresh weight. The water-intake after 2 days is stated as the average of the series B, C, D and E; after 4 days as the average of the series C, D and E; after 8 days as the average of the series D and E; after 16 days the % stated is that of series E alone.

	exp. 398 (6-4-'39)			exp. 400 (12-4-'39)			exp. 408 (27-4-'39)			average of the 3 experiments				
initial fresh weight	2536.9			2474.4			2346.1			2452.5				
initial dry weight	387			366			400			384				
	water-intake	loss of dry substance		water-intake	loss of dry substance		water-intake	loss of dry substance		water-intake	loss of dry substance		loss of fresh weight by respiration	real water-intake
	%	mg	%	%	mg	%	%	mg	%	%	mg	%	mg %	in %
after 2 days	7.7	14	3.6	9.2	9	2.5	8.4	22	5.5	8.4	15	3.9	6 0.2	8.4+0.2= 8.6
after 4 days	12.4	34	8.8	13.8	20	5.5	14.1	45	11.2	13.4	33	8.5	13.2 0.5	13.4+0.5=13.9
after 8 days	17.0	55	14.2	18.1	42	11.5	21.1	55	13.7	18.7	51	13.1	20.4 0.8	18.7+0.8=19.5
after 16 days	20.3	79	20.4	19.5	62	17.0	24.5	80	20.0	21.4	74	19.1	29.6 1.2	21.4+1.2=22.6

while they are stopped after 2, 4, 8 and 16 days respectively for the determination of the dry weight. In this way we get an impression of the respiration after varying periods.

In table 6 we see that the respiration in the first 4 days is practically constant. The respiration, however, then becomes somewhat less. The respiration from the 5th to the 8th day inclusive is less than that of the first 4 days, remaining approximately constant only in test 400. After 8 days, however, the respiration in all the tests distinctly decreases. We see here, thus, the same thing as with the intake of water, which is also strongest in the first few days, practically with constant rapidity in the first 4 days and then becoming less. After 8 days the water-intake is only very slight.

Like the tests in the previous §, these tests do not give the impression that there is a direct connection between the intake of water and respiration. In table 6 we see that tests 398 and 408 have respired pretty much the same amount after 8 and also after 16 days, while the water-intake of test 408 is about 4 % more than that of test 398. And test 400 has a % water-intake approximately equal to that of test 398, while the respiration with test 400 is slighter than with test 398. It is true that test 400 is somewhat exceptional; during the entire duration of the test the respiration of test 400 is slighter than

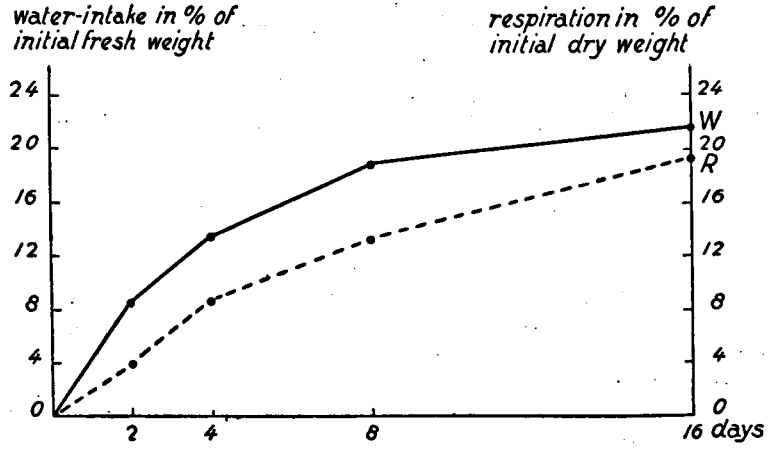


Fig. 4. The water-intake and the respiration in course of time. (as average of the exp.'s 398, 400 and 408 of table 6).
W indicates the water-intake as increase of the fresh weight in % of the initial fresh weight.
R indicates the respiration as loss of dry substance in % of the initial dry weight.

that of the other two tests, while the water-intake shows the highest % at the beginning and ends later as the lowest of the three tests. In figure 4 the course of the respiration and that of the water-intake in course of time is graphically represented.

§ 5. The influence of various factors connected with the method.

In the discussion of the method it is stated that aerated distilled water is used, which is renewed after each observation, while the number of discs per series amounts to 10. These and a few other factors are examined in the following experiments.

a) *Strong or weak aeration.*

Whether the water is strongly or weakly aerated makes little or no difference. With one test there is no difference whatever, the water-intake of series A (strong aeration) being 19.1 % and that of series B (weak aeration) 19.3 % of the initial fresh weight in 8 days. With two other tests there is a slight difference in favour of the stronger aeration. With one of them the water-intake of series A (strong aeration) is 21.2 % and that of series B (weak aeration) 20.2 %; with the other the water-intake of series A (strong aeration) amounts to 27.2 % and that of series B (weak aeration) to 25.1 % of the initial fresh weight in 8 days. Practically, however, it falls within the limits of variability. Evidently, therefore, the provision of oxygen is not limiting with weaker aeration.

In all the following tests a moderately strong aeration is applied.

b) *Tap-water or distilled tap-water.*

A few tests are made for the sake of comparison of the water-intake in distilled tap-water and in ordinary tap-water. The difference, although not great, is invariably in one direction, the tap-water series being in every case behind the control in distilled tap-water. The difference between the two does not remain quite equal during the test. With three exp.'s the water-intake of series A (tap water) is 23.0 %, 20.4 %, 18.5 % of the initial fresh weight in 8 days and that of series B (distilled water) is 23.3 %, 21.8 % and 22.7 % respectively. Probably the slighter water-intake in tap-water is due to the inhibitory action of the calcium content of the tap-water. In experiments on the influence of salts on this process of water-intake (chapter II, § 5), Ca is found to have an inhibitory action, while potassium has a beneficial effect.

STILES-JØRGENSEN (1917) also find that the water-intake in tap-

water is smaller than in distilled water. The maximum reached is lower, and is reached earlier. This is the case both for potato-discs and for discs of carrot. They find that the weight of the discs in tap-water diminishes after a few days, whereas this is not the case in distilled water. This diminution is much greater in the case of potato-discs than in that of carrots.

As the composition of tap-water during the entire experiment will not always be exactly the same, and as it is better for the comparison of the tests that the water shall have the same composition, all the other tests in this work are carried out with self-distilled tap-water. This water is distilled once over Jena-glass. Some tests are made in which double distilled water is compared with distilled tap-water, but this is found to make no difference.

c) *Number of discs per series.*

In a few of the tests observations are made as to a possible influence of the number of discs per series.

STEWARD, WRIGHT and BERRY (1932) have found that the respiration of potato discs is slighter if 120 discs are taken to 2 litres water, than if 60 discs are taken to the same volume of water. The CO_2 production amounts to 0.2112 mg CO_2 per gramme tissue per hour in cases where 60 discs are taken, whereas when 120 discs are taken this amounts to 0.1807 mg CO_2 per gramme tissue per hour.

A series with the usual number of 10 discs and a series of 5 discs are taken from one potato. The water-intake of these, measured as increase in fresh weight, is followed. With two exp.'s the water-intake of series A (5 discs) is found to be 23.8 % and 23.2 %, while that of series B (10 discs) amounts to 24.3 % and 23.0 % respectively. As we see, it makes no difference whether 5 or 10 discs are taken in a series; the increase in the fresh weight is the same in both cases.

We may therefore say that 10 discs, under these experimental conditions, have absolutely no hindering effect on one another. For this reason the remaining tests are carried out with 10 discs per series. The average of a larger number always gives, if possible, a still more accurate picture, especially in view of the figures for the loss of dry substance and the differences in these e.g. owing to the influence of hetero-auxin.

d) *The influence of light.*

As is stated in the description of the method, the tests take place in a dark room at a constant temperature. For a few hours daily, however, during the weighing and handling of the tests, a lamp of

78 Watts (100 decalumens) is burning at a distance of from 1 to 1.5 metres from the test-vessels. The rest of the time it is dark.

It is not very likely that the light will have any effect on this nongreen tissue. But it is, of course, not altogether out of the question, that light might have an influence on the water-intake process, e.g. by a possible effect on the water-permeability. (JÄRVENKYLÄ 1937; L. and M. BRAUNER 1938, 1940; LEPESCHKIN 1940). STEWARD (1932) investigates the influence of light on the bromine-absorption with potato-discs. He has found that light has no influence, the bromine-absorption being practically as strong in the dark as under illumination with a lamp of 1000 Watts at a distance of a few feet above the experimental vessels.

To obtain some data on this point I have made the following experiments in December 1939. A couple of series of 10 discs each are taken from the same potato, one of which standing at the ordinary place in the room at approx. 1 to 1.5 metres distance from the lamp. Instead of for a few hours a day, the lamp now burns continuously. The other series is in the same room, in a light-tight closed chest, so continuously in the dark. With four experiments the water-intake in 8 days of series A (dark) amounts to 16.8 %, 18.8 %, 16.7 % and 19.3 % of the initial fresh weight, while that of series B (light) is found to be 18.1 %, 20.3 %, 16.9 % and 20.9 % respectively.

So it is seen that the difference between the light and dark series is very slight, falling well within the limits of variability. However, it is precisely the water-intake of the series in the light that is the greater. This may be a coincidence, but it is more likely to be attributable to the difference in temperature existing between the two series. The temperature of the room is constant, viz. 21° C. During these tests, however, the temperature is read off close above the test-vessels. The temp. measured above the tests in the light shows a minimum of 21.2° C and a maximum of 22,0° C. For the temp. above the tests in the dark, which are on the floor in a chest, the lowest value measured is 20.1° C and the highest 21.2° C. This amounts to saying that the temp. of the test-vessels standing in the dark is regularly about 1.0° C. lower than that of the test-vessels in the light. To my mind the difference in water-intake between light and dark series in favour of the discs in the light is fully accounted for by this. At a higher temp. the water-intake is stronger than at lower temperatures. (chapter II, § 4).

These tests therefore give no indication of any influence of light on the intake of water.

e) *Influence of hydrogen ion concentration.*

The fact that it makes no difference whether the water is renewed or not (table 2, p. 13), while at every renewal the p_H is considerably altered, the p_H of the fresh water being much lower than that of the old water, is in itself an indication that the p_H evidently has little influence.

Moreover, another test is made, in which the water of one series is rendered slightly acid with HCl to a p_H of 3.8. (This p_H is colorimetrically determined with bromine-phenol-blue as an indicator), The p_H is kept as constantly as possible at this value, by occasionally adding a slight amount of hydrochloric acid, during the first two days a few times a day. With test 378 the water of the acidified series is renewed on the 2nd day and on the 6th day, and with test 370 every day. The water of the control is never renewed. The p_H of the control rises in one day to 6.5 or 7.0, and remains at this point for several days. In the last few days the p_H again falls to 4.5 or 5.0.

With experiment 370 the water-intake in 8 days is the same (22.5 % of the initial fresh weight) for both series, while with exp. 378 the water-intake of series A (acidified) is 20.7 % and that of series B (control) is 20.9 %. So series A (acidified) behaves exactly like the control in respect of the water-intake. The water of the acidified series does, however, become somewhat turbid, in the case of test 378 on the 6th day and in test 370 as early as the third day, while here an odd disc has a dead spot. A degree of acidity of this kind is therefore evidently somewhat harmful, but these tests at any rate clearly confirm the notion that the p_H has no particular influence. The loss of dry substance is considerably larger in the acidified series than with the controls, which is probably caused by exosmosis from the dying parts. In test 378 the loss of dry substance of the acidified series is e.g. 15 mg more than that of the control, and in test 370 it is as much as 30 mg. more. The difference in water-intake between acidified series and control is really somewhat larger than is indicated by the difference in the increase in fresh weight. But no significance should be attached to this for the influence of the p_H on the intake of water. The differences are so slight that we may say that the water-intake of the controls and of the acidified series is precisely equal.

§ 6. Summary.

If potato discs are put into aerated water their weight constantly increases. The increase is greatest in the first few days, later on the increase gradually becomes less marked.

It has been made probable that this increase in weight is caused

by an intake of water by the cells of the tissue. Infiltration at which the air from the intercellulars is replaced by water has no influence on the progress of the increase in weight.

During the stay in aerated water the discs loose a considerable amount of dry substance. By determinations of the conductivity of the external solution it has been made probable that this loss in dry weight is caused, if not entirely then indeed chiefly, by the respiration. In periods when no exosmosis is to be indicated a loss of dry weight takes place indeed.

The variability of the water-intake with two series of equal value from the same potato is very slight; so is the variability in respiration.

In the first four days the respiration, determined as a loss of dry substance, goes on with a practically constant intensity, in the following periods it gradually diminishes.

The water-intake is not noticeably increased by stronger aeration.

The water-intake in tap-water is a little less than in distilled water. The course of the curve indicating the water-intake, however, is of the same kind.

The number of discs per test-vessel has no influence on the water-intake. The water-intake is just the same no matter if 5 or 10 discs per series are taken.

The light, at least the lamp of 78 Watts (100 decalumens) burning in the room at a distance of 1 to 1,5 m from the test-vessels, has no influence on the course of the process of water-intake with potato discs.

The p_H of the water has no influence on the water-intake.

CHAPTER II.

§ 1. The water-intake and the respiration in an environment without oxygen.

In order to determine whether the water-intake is dependent on the supply of oxygen (compare also KRAMER 1940), tests are made in an anaerobic environment. In these the increase in weight in an anaerobic environment is compared with that in aerated water and with that in unaerated water.

In order to obtain an environment without oxygen, the test-vessel with the potato discs is placed inside an anaerobic jar (McIntosh and Fildes). Pure nitrogen is first passed through for half an hour, this is also passed through the water in which are the potato discs by means of a sintered glass filter. For a short time hydrogen is

then passed through, after which the final remains of the oxygen are bound to the hydrogen by means of a palladium catalysator. A reduced aqueous methyleneblue solution, in a test-tube placed in the jar, remains colourless.

By way of control the content of oxygen of the water treated in this way is determined with Van Dam's micro-Winkler method (VAN DAM, 1935). Determinations are made half an hour after the vessel has been closed and also after the discs have been in it for 2 days. After half an hour 0.25 cc, 0.00 cc and 0.30 cc oxygen per litre are found in three different tests. After two days 0.00 cc oxygen

TABLE 7.

Water-intake of 3 series of 10 discs of the same potato, each in 500 cm^3 distilled water. Series A is aerated. Series B is not aerated. Series C is in water free from oxygen; at ↓ these are taken out of the anaerobic jar and are again aerated with air in the ordinary way.

exp. no.		water-intake as increase in % of initial fresh weight after:							
		1	2	3	4	5	6	7	8 days
169	A control	—	10.3	—	19.2	—	—	—	—
(19-1-'38)	B unaerated	—	3.8	—	8.1	—	—	—	—
	C without O_2	—	2.0	—	1.6	—	—	—	—
177	A control	4.8	9.1	12.8	15.5	18.0	20.6	—	—
(28-1-'38)	B unaerated	2.9	4.4	6.7	8.6	—	—	—	—
	C without O_2	1.1	1.1↓	1.6	5.1	10.0	15.7	—	—
180	A control	5.1	9.6	13.7	17.0	19.6	21.7	—	—
(5-2-'38)	B unaerated	3.6	5.1	—	—	—	—	—	—
	C without O_2	2.3	1.8↓	2.7	6.9	12.3	16.7	—	—
186	A control	4.1	8.4	12.2	15.8	18.6	20.6	—	—
(12-2-'38)	B unaerated	2.7	4.9	7.2	9.2	12.3	14.6	—	—
	C without O_2	0.2	−0.5↓	−0.2	3.0	7.3	10.9	—	—
211	A control	6.0	11.4	15.2	18.8	21.7	23.7	24.6	—
(18-3-'38)	B unaerated	—	—	—	—	—	—	—	—
	C without O_2 (nitrogen passing through)	2.1	2.8	3.6↓	6.3	11.0	16.3	20.3	—
190	A control	6.5	12.7	18.0	23.3	26.8	29.0	30.0	31.0
(19-2-'38)	B unaerated	4.5	7.7	11.4	—	—	—	—	—
	C without O_2	2.9	3.6	3.5	1.0↓	0.3	3.6	7.6	10.3
200	A control	5.6	10.8	15.5	18.8	21.4	23.0	23.8	24.5
(4-3-'38)	B unaerated	3.7	4.9	6.4	—	—	—	—	—
	C without O_2	2.4	3.4	3.6	3.6↓	4.6	9.2	14.6	18.7

per litre is found, that is to say that not a trace of blue colouring can be observed with starch. If the water has been deoxygenated solely by passing nitrogen (purified commercial nitrogen gas) through it, this not being followed by binding with hydrogen, the water still contains 0.093 cc oxygen per litre after the discs have been in it for two days. The oxygen content of the water aerated with air, in which potato-discs are allowed to remain, is 6.18 cc per litre.

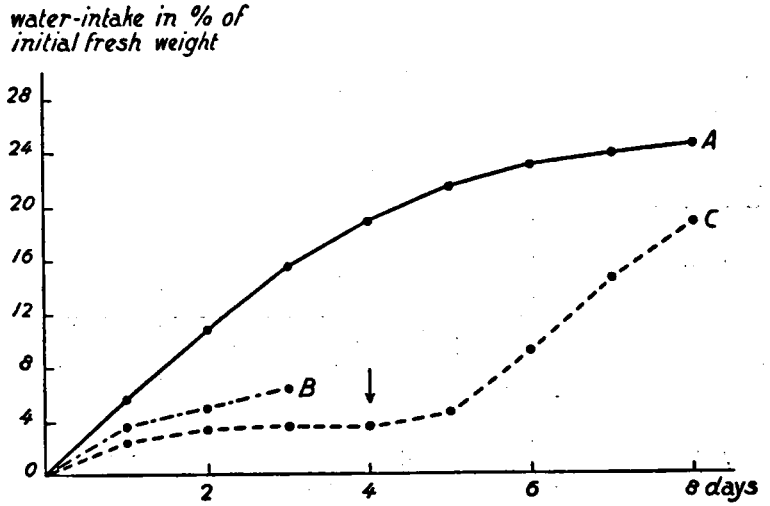


Fig. 5. The influence of the oxygen supply on the water-intake. (exp. 200 of table 7).
A: water aerated with air.
B: water not aerated.
C: the test-vessel is in an anaerobic jar; again aerated with air after 4 days (at ↓).

In table 7 the tests of winter 1938, of which test 200 is represented graphically in fig. 5, are summarized. Here 3 series are taken in each case. Series A, the control, is continuously aerated with air; series B is not aerated and in series C the water is freed from oxygen in the manner described above. In all the experiments the oxygen is removed in this way, except in test 211, where purified nitrogen flows through continuously for three days. The methylene-blue solution is not quite colourless in this case.

It is seen that the intake in unaerated water (B) is considerably less than in aerated water (A), while the discs in the unaerated water

do not long remain in a healthy condition either, the water becoming turbid and the discs flabby, indicating the progressive breakdown of the tissue, in spite of the water being repeatedly renewed. In aerated water, however, the discs, as is always the case, remain in good condition during the entire duration of the experiment. They can even be kept alive indefinitely, e.g. a month or longer. (tabel 14, p. 57). Curve C of fig. 5 shows the increase in weight in an environment without oxygen. Here we see, as in nearly all the tests in table 7, that there is still a slight increase in weight in the first 24 hours. After that the weight remains practically constant. No more water, therefore, is taken in. The slight increase in weight in the beginning is probably caused by the fact that the tissue still has some oxygen at its disposal. Infiltration can not be the cause; the increase in weight possible by infiltration (see fig. 2, p. 19) is much less than the increase found here. STEWARD, STOUT and PRESTON (1940) have found that with a reduced metabolism the tissue retains a higher sugar concentration. However, BRAUNER, BRAUNER and HASMAN (1940) have stated a constant decrease of the sugar content from the very beginning of the experiment. So it is very unlikely that a somewhat higher sugar concentration may cause the initial slight water-intake.

It is possible to leave the discs 2 days or 4 days in the environment without oxygen without any deleterious effect worth mentioning. If the discs (series C) are then put back again into normal conditions, i.e. if they are then again aerated with air (tests 177—180—186—211—190—200 of table 7), the weight again begins to increase. In the first 24 hours the increase is slight, but after that it becomes more marked and continues with almost the same speed as the controls at the beginning. That is the case even with test 190, where we see a slight loss in weight from the third to the fourth day.

If, however, instead of at the beginning of the test, the potato discs are put into an environment without oxygen later on, after they have been aerated with air in the ordinary way for 4 days first, they are no longer able to tolerate this. There is a marked loss of weight in the course of the first day on which they are in an anaerobic environment, which is continued in the next 24 hours. If the discs are then again put into the environment that is aerated with air, the decrease comes to a standstill, and there is a tendency to recover, that is, the weight begins to increase again, but only to a slight extent. With one exp. the water-intake of series B amounts to 16.8 % during the first 4 days with air-aeration; during the stay in an anaerobic environment this falls to 10.8 % on the first day, on the next day continued to 3.1 %. On the 6th day aeration is again

applied, the fresh weight still falling to 0.5 % during the next day, during the last day of the experiment a slight tendency to recover is perceptible, the water-intake measured being 0.7 %. The water-intake of series A (control) amounts to 17.8 % after 4 days, increasing to 26.1 % in 8 days. With another exp. the water-intake of series B amounts to 18.7 % during the first 4 days with air-aeration, falling to 9.6 % and further to 4.8 % during the next two days in an anaerobic environment. The water-intake of series A (control) amounts to 19.0 % after 4 days, increasing to 23.9 % in 6 days.

The water, although, as in all cases, it is renewed every other day, becomes very turbid in the two last days. At the end of the experiment the discs show soft spots here and there, while the other portions make the impression of being fairly turgescent. The second test is not continued any further; on the 6th day the water is pretty foul and turbid, while the discs are somewhat limp and no longer in good condition.

Table 8 gives a summary of several tests in an environment without oxygen, the dry weight being in all cases determined. In all the tests the vessels are deoxygenated in the manner described above. With regard to the intake of water, we see an entire confirmation of the previous tests. (Table 7). With tests 258 and 262 there is still no difference in dry weight to be seen between the control and the anaerobic series after two days. With tests 371 and 372, which are made more in the winter season, a difference in respiration between the anaerobic series and the control is to be seen after two days, in test 371 in fact a very large one. The amount of dry matter lost after two days, is distinctly less than the amount lost after 4 days. Series B (anaerobic) has therefore in all cases except in test 383 lost considerably less dry substance than the control. Owing to this, of course, the difference in water-intake between the control and the anaerobic series will be somewhat larger than the difference in increase in the dry weights indicates.

In the experiments 383 to 407 inclusive, in table 8, the electrical resistance of the water is also measured, in order to get an impression of the difference in exosmosis between the anaerobic series and the control. In the first period the fall in the resistance is about equal in both, but in the second two-days' period the fall in the resistance in the anaerobic series is somewhat greater than in the control. (See table 9). This indicates a stronger exosmosis. It is conceivable that exosmosis of alcohol occurs with the anaerobic series, viz. of the alcohol which will have been produced during the anaerobic (intermolecular) respiration. But it is not certain that this is the cause. It may also be caused by exosmosis of substances from

TABLE 8.

Water-intake and respiration in an environment without oxygen.
Series A: control, aerated with air.
Series B: in anaerobic environment.
Series C: determination of initial dry weight.
Fresh weight and dry weight are stated in mg for 10 discs together. The water-intake is stated as increase of the initial fresh weight in %. The respiration is stated as loss of dry substance in mg.

exp. no.	at the beginning of the experiment		after 1 day	after 2 days			after 4 days		
	fresh weight mg	dry weight mg	water-intake %	water-intake %	dry weight mg	respiration mg	water-intake %	dry weight mg	respiration mg
258 A control	2646.4	—	—	11.9	433	—	—	—	—
B without O_2	2646.2	—	—	3.4	429	—	—	—	—
(20-5-1938)									
262 A control	2713.1	—	—	13.5	487	—	—	—	—
B without O_2	2712.2	—	—	3.9	491	—	—	—	—
(24-5-1938)									
371 A control	2479.4	—	4.6	7.8	408	25	—	—	—
B without O_2	2479.5	—	3.3	4.0	428	5	—	—	—
C	2479.9	433	—	—	—	—	—	—	—
(21-2-1939)									
372 A control	2470.1	—	5.3	9.3	442	30	—	—	—
B without O_2	2469.6	—	2.9	3.6	451	21	—	—	—
C	2471.2	472	—	—	—	—	—	—	—
(21-2-1939)									
383 A control	2433.9	—	—	9.1	—	—	14.1	362	33
B without O_2	2434.3	—	—	3.5	—	—	2.4	368	27
C	2435.1	395	—	—	—	—	—	—	—
(8-3-1939)									
402 A control	2416.0	—	—	9.5	—	—	15.6	361	56
B without O_2	2415.6	—	—	4.0	—	—	2.7	384	33
C	2415.1	417	—	—	—	—	—	—	—
(14-4-1939)									
405 A control	2455.2	—	—	8.8	—	—	15.3	411	57
B without O_2	2454.6	—	—	3.6	—	—	1.6	430	38
C	2455.9	468	—	—	—	—	—	—	—
(20-4-1939)									
407 A control	2493.9	—	—	9.9	—	—	15.8	393	42
B without O_2	2493.1	—	—	3.7	—	—	2.8	406	29
C	2494.9	435	—	—	—	—	—	—	—
(26-4-1939)									

TABLE 9.

Exosmosis in an anaerobic environment compared with the exosmosis when aerated with air. (Determinations of some experiments of table 8).
Series A: control, aerated with air.
Series B: in anaerobic environment.
The electrical resistance of the external solution is stated in Ω.

exp. no.	af the beginning of the experiment		after 2 days				after 4 days	
	new		old		new		old	
	p_H	resistance	p_H	resistance	p_H	resistance	p_H	resistance
383 A control		170 000		44 000		210 000		90 000
B without O_2		170 000		39 000		210 000		64 000
402 A control	4.5	260 000	7.0	45 000	4.5	230 000	6.5	74 000
B without O_2	4.5	260 000	5.5	46 000	4.5	230 000	5.5	34 000
405 A control	4.5	270 000	7.0	39 000	4.5	260 000	6.5	67 000
B without O_2	4.5	270 000	5.5	42 000	4.5	260 000	5.5	34 000
407 A control	4.5	255 000	7.0	46 000	4.5	200 000	6.5	68 000
B without O_2	4.5	255 000	5.5	48 000	4.5	200 000	5.5	38 000

the cells of the dying parts. If this is so, the loss of dry substance of the anaerobic series with this test-period of four days will be somewhat greater than agrees with the respiration. As we see in table 9 the increase of the p_H with the anaerobic series is only very slight compared with that of the control. This is in accordance with HOAGLAND and BROYER (1940), who state that aeration produces a very remarkable alkalization in unbuffered culture solutions of growing barley seedlings, while aeration with pure nitrogen-gas causes practically no change of the initial low p_H-value.

§ 2. The influence of aeration with pure oxygen.

STEWARD (1933) investigated the respiration of potato discs at 23.2° C, by determining the production of CO_2, with various % oxygen. While he has found that the respiration was limited by an oxygen % below 20.9 %, he found that an oxygen % higher than 20.9 %, up to 100 %, practically does not affect the respiration. The CO_2 production is practically the same with all oxygen % above 20.9 %. TURNER (1938), has determined the respiration, the consumption of oxygen, of discs of carrot, 1 mm in thickness, in aerated water. He has aerated with air and also with 100 % oxygen. He has

found that the oxygen consumption and also the R.Q. of discs from water that is aerated with 100 % oxygen does not differ to any extent worth mentioning from the oxygen consumption and the R.Q. of discs from water that is aerated with ordinary air. The respiration of carrot discs is therefore not affected by aeration with higher oxygen percentages.

In this § a few tests will be described in which observations are made, in an environment rich in oxygen, of the water-intake and the respiration, measured as loss of dry substance. This is obtained by aerating with 100 % oxygen (extra pure) instead of with air; this oxygen is first passed through two wash-bottles of distilled water. So the water is equilibrated with pure oxygen.

With 4 experiments the water-intake of series B (rich in oxygen) is found to be 11.7 %, 10.6 %, 17.0 % and 11.6 % of the initial fresh weight; while that of series A (control) amounts to 16.9 %, 12.3 %, 19.4 % and 15.6 % respectively. So the water-intake of series B (rich in oxygen) is somewhat slighter than that of the control in all tests. A higher oxygen % is therefore not conducive to a higher intake, but on the contrary somewhat detrimental to it. Also the dry weight at the end of the experiment is for the series with a plentiful supply of oxygen somewhat lower than that of the control, but the differences are in point of fact of no significance. In one test, which lasts only for 2 days, the difference is only 3 mg.; this falls within the limits of variability. In another test the dry weight of the series with an ample supply of oxygen is 8 mg lower than that of the control, at the end of 4 days. This might seem to indicate a somewhat more vigorous respiration, although this will have to be confirmed by further experiments. It might also be due to a somewhat stronger exosmosis owing to a detrimental influence of the high content of oxygen.

With a microscopic examination of the discs at the conclusion of the test the disappearance of the starch from the cell-layers on the surface of the discs is already to a certain extent visible. (Cf. § 6). With series B (rich in oxygen) this is less distinct than with the control, series A.

§ 3. The intake of asparagine, aerated with air and under anaerobic conditions.

It has been seen that the intake of water by potato discs, in agreement with STEWARD's tests on bromium-intake and those of HOAGLAND and BROYER on the intake of salts by root-tissue, is a process which only occurs with a plentiful supply of oxygen. ARISZ and OUDMAN (1938) have also found that the intake of asparagine by the

leaves of Vallisneria is dependent on a good supply of oxygen.

On occasion of this a few tests are made as to the intake of asparagine in the case of potato discs. The variability of the content of nitrogen [1]) of discs of the same potato is first determined. After the usual preliminary treatment of 24 hours' washing in tap-water, 3 samples are taken, each of 5 discs with equal fresh weight of the same potato. The nitrogen content of these samples is found to be 1109 γ, 1120 γ, and 1146 γ respectively; so we see that this may diverge 37 γ.

In addition to this (the variability at the beginning of the test) the nitrogen content of 2 series of 5 discs is also determined at the end of 2 days, during which time one group is aerated with air and the other has been in an environment without oxygen. In one test the anaerobic group is found to contain 18 γ nitrogen less than the control, and in the other test 28 γ more. These differences fall within the margin of error. We can therefore be certain that no differences in the nitrogen content occur in consequence of the different treatment, viz. aeration with air or a period spent in an environment without oxygen.

The difference in nitrogen content at the beginning of the test and after a period in aerated water of for instance two days, is not determined.

A few tests are then made on the intake of asparagin from various concentrations. The following concentrations are used: 1/20 mol., 1/40 mol., 1/80 mol., 1/160 mol., 1/320 mol., and 1/640 mol. In each case 3 series of 5 potato discs are taken. Of one the nitrogen content is immediately determined, the two others are given water for the first 24 hours, in one of these cases the water being substituted for the asparagine solution during the following 24 hours. The water or, as the case may be, the asparagine solution, is aerated in the ordinary way with air. At the same time the water-intake of the discs is determined. The experiment is then terminated, the discs are well rinsed, for three times five minutes, each time in pure distilled water, after which the nitrogen contant is determined. The period of intake is therefore 24 hours, viz. the second 24 hours after the commencement of the experiment. This is chosen because in an anaerobic environment at first some water-intake occurs. This is possibly due to oxygen being still present in the tissue and in the intercellulars. In view of this the second period is chosen for the intake of asparagine, as only then has the tissue come as far as possible to a state of repose,

1) The nitrogen-determinations are carried out by Miss J. W. E. VAN WEERDEN, analyst.

judging, at any rate, by the water-intake which then does not go on any more.

Observations, however, are made in a few experiments as to whether the asparagin-intake with normal aeration with air is different during the first period and the second period of 24 hours. This is found not to be the case. As much asparagin is taken in in the first period of 24 hours as in the second one (viz. one test: increase 127 γ and 137 γ nitrogen respectively per disc and another test: increase 150 γ and 163 γ nitrogen respectively per disc). Both are calculated with respect to the nitrogen content of the control at the end of the experiment after 2 days in aerated water. The intake of asparagine thus goes on constantly, at any rate in the first 2 days, as is also the case with the water-intake.

In table 10 the results of the intake from various concentrations are summarized, the second 24 hours after the commencement of the test being taken as intake period. We see that less nitrogen is taken in from the strongest concentrations than from the weaker ones. The nitrogen intake is most favourable from the weaker solutions, such as e.g. 1/320 mol.

In all the tests we see that the stronger concentrations have a fairly strong inhibitive effect on the water-intake of the discs. The stronger the concentration, the stronger is the inhibition. During the first 24 hours, when all the series are in water, the water intake of all of them is pretty well equal, but in the following 24 hours the water intake of the discs in the stronger asparagine solutions lags very noticeably behind. The inhibitory effect of the weakest concentrations, 1/640 mol. and also 1/320 mol., is still only very slight.

To determine whether this inhibition is due to osmotic causes or to a special influence of the asparagine, a few tests are made, in which, in addition to the series with asparagine, another series is treated with a sugar solution of similar concentration. Both glucose and saccharose are tried. In tests 226 and 234 1/40 and 1/80 mol. asparagine, i.e. 3.75 g. and 1.875 grammes asparagine per litre respectively, are used. For comparison glucose solutions which also contain 3.75 g. and 1.875 g. glucose per litre are used. In test 236 saccharose is used instead of glucose. The results are shown in table 11. As is seen, neither these sugar solutions nor glucose nor saccharose have any influence on the increase in the fresh weight of the discs. The inhibitory effect on the increase in the fresh weight, as has been seen with the asparagine solutions, is therefore not due to osmotic causes, but will probably be attributable to a toxic effect of the asparagine solution.

TABLE 10.

Intake of asparagine from solutions of different concentration.
Series A: 5 discs in 500 cm³ of water.
Series B to E inclusive: 5 discs, during the first 24 hours in 500 cm³ of water, during the next 24 hours (at ↓) in 500 cm³ of asparagine solution.
At the end of the experiment: Nitrogen content of the control series (A) is stated in γ nitrogen per disc. The asparagine intake is stated as the increase in γ nitrogen per disc. Aeration in the usual way.

exp. no.	initial fresh weight in mg per disc	increase of fresh weight in % of initial fresh weight after: 24 hours	48 hours	control quantity N in γ per disc	increase of nitrogen in γ per disc from: 1/20 mol. asparagine	1/40	1/80	1/160	1/320	1/640
219 A	277.6	5.6	11.4	1068.8	—	—	—	—	—	—
(26-3-'38) B	277.7	5.7 ↓	9.4	—	123.4	—	—	—	—	—
220 A	270.2	7.0	13.3	979.6	—	—	—	—	—	—
(29-3-'38) B	270.3	6.8 ↓	10.3	—	—	122.4	—	—	—	—
226 A	269.2	5.8	12.0	961.0	—	—	—	—	—	—
(6-4-'38) B	268.8	5.2 ↓	7.4	—	—	195.0	—	—	—	—
D	268.9	5.2 ↓	8.7	—	—	—	279.0	—	—	—
234 A	254.0	5.5	12.0	1048.0	—	—	—	—	—	—
(17-4-'38) B	253.6	5.2 ↓	8.8	—	—	100.0	—	—	—	—
D	254.0	5.3 ↓	9.3	—	—	—	138.0	—	—	—
236 A	258.0	4.0	9.8	739.0	—	—	—	—	—	—
(20-4-'38) B	256.8	4.8 ↓	6.6	—	—	119.0	—	—	—	—
D	256.7	4.0 ↓	6.8	—	—	—	121.0	—	—	—
241 A	272.6	5.0	12.0	832.0	—	—	—	—	—	—
(27-4-'38) B	272.6	4.6 ↓	8.5	—	—	—	167.0	—	—	—
C	273.0	4.3 ↓	10.1	—	—	—	—	214.0	—	—
D	273.0	5.0 ↓	11.2	—	—	—	—	—	172.0	—
E	272.7	4.7 ↓	11.2	—	—	—	—	—	—	138.0
285 A	258.1	5.3	9.0	617.0	—	—	—	—	—	—
(28-6-'38) B	258.2	5.0 ↓	6.5	—	—	—	93.0	—	—	—
C	258.1	5.2 ↓	7.5	—	—	—	—	120.0	—	—
D	258.2	5.3 ↓	8.4	—	—	—	—	—	147.0	—
E	258.2	5.7 ↓	9.3	—	—	—	—	—	—	147.0

TABLE 11.

Water-intake of potato discs in aerated solutions of sugar and asparagine. A, B, C, D and E are series, each of 5 discs, with the same average initial fresh weight of the same potato.

	increase of fresh weight in % of initial fresh weight after:			
	24 hours		48 hours	
	exp. 226	exp. 234	exp. 226	exp. 234
A. water	5.8	5.5	12.0	12.0
B. for the first 24 hours water, for the next 24 hours asparagine 1/40 mol. (3.75 g per l)	5.2	5.2	7.4	8.8
C. for the first 24 hours water, for the next 24 hours glucose (3.75 g per l)	6.0	5.0	11.9	12.7
D. for the first 24 hours water, for the next 24 hours asparagine 1/80 mol. (1.875 g per l)	5.5	5.3	8.7	9.3
E. for the first 24 hours water, for the next 24 hours glucose (1.875 g per l)	6.0	5.3	12.4	13.3

	exp. 236	
A. water	4.0	9.8
B. for the first 24 hours water, for the next 24 hours asparagine 1/40 mol. (3.75 g per l)	4.9	6.6
C. for the first 24 hours water, for the next 24 hours sucrose (3.75 g per l)	3.7	8.6
D. for the first 24 hours water, for the next 24 hours asparagine 1/80 mol. (1.875 g per l)	4.0	6.8
E. for the first 24 hours water, for the next 24 hours sucrose (1.875 g per l)	4.4	10.5

A difference is seen in the colour of the discs. The discs assume a distinctly browner tint in the asparagine solution than the controls in water in the course of the 24 hours. The discs in the sugar solutions do not do so, and behave exactly like the discs in water. If we examine the brown colour of the discs in the asparagine solution it is found to consist of a dense covering of the surface with coarse, somewhat vaguely outlined spots. This change of colour occurs in the concentrations 1/20, 1/40, and 1/80 mol. asparagine to pretty well the same extent.

Moreover in these strong concentrations the water also usually becomes somewhat turbid.

In order to find out whether here, too, as is the case with Vallisneria, the intake of asparagine is a process depending on oxygen,

a few tests are made in an environment without oxygen. There are then 3 series of 5 discs. Series A is the control, which remains in water for 2 days, and is aerated with air in the same way as series B, which is given water for the first 24 hours, this being replaced the next 24 hours by asparagine solution. Series C is kept in an anaerobic environment for the entire duration of the experiment: the first 24 hours it is given water, this being replaced in the following period by asparagine.

In a preliminary test (test 220, cf. table 10) it is seen that 1/40 mol. asparagine, which exerts a somewhat inhibitory influence on the water-intake with air aeration in the control also, is very deleterious in an anaerobic environment. During the period spent in the asparagine solution a very marked loss of weight occurs, the weight even falling below the original weight, (the fresh weight rose in the first 24 hours in water by 2.4 %, and then fell in the asparagine 1/40 mol. to —2.8 %). The nitrogen analysis shows that there is a considerable loss, viz. 151.2 γ per disc; (the figures are 828.4 γ per disc, while the control contains 979.6 γ per disc). The concentration 1/160 mol., which with aeration with air has little or no deleterious effect (tests 241 and 285 of table 10), is found to cause a loss of weight in anaerobic conditions. Water-intake with aeration with air (series A, constant supply of water) is 6.8 % after 24 hours and 12.1 % after 48 hours; the water-intake of series B (first 24 hours water, then asparagine 1/160 mol.), likewise aerated with air, is 7.0 % after 24 hours and 12.1 % after 48 hours. The water-intake of series C in an environment without oxygen (first 24 hours water and the following 24 hours 1/160 mol. asparagine), is 3.1 % after 24 hours, and has fallen to the original weight after 48 hours (increase in weight 0.00 %). As to the asparagine intake with this experiment, the nitrogen increase of series B (aeration with air) is 289 γ per disc and that of series C (without oxygen) 31 γ per disc. In another test it is shown that asparagine in the concentration 1/320 mol. has no deleterious effect in anaerobic conditions either. The water-intake with aeration with air, (series A, the whole time in water) is 4.7 % after 24 hours and 9.3 % after 48 hours. The water-intake of series B (first 24 hours water, then asparagine 1/320 mol.), likewise aerated with air, is 4.4 % after 24 hours and 9.0 % after 48 hours. The water-intake of series C, in an environment without oxygen (first 24 hours water, then 1/320 mol. asparagine) is 3.3 % after 24 hours and 3.4 % after 48 hours. There has therefore been no loss of weight.

Two more tests were made with 1/640 mol. asparagine. This is given during the second period of 24 hours. It is seen that there is a great difference in the asparagine-intake. Series B (aerated with

air) shows an increase of 188 γ and 137 γ nitrogen per disc respectively; series C (anaerobic environment) shows an increase of 20 γ and 15 γ nitrogen per disc respectively.

The intake of asparagine in the case of potato discs is therefore also a process which occurs only when the tissue is well supplied with oxygen and can respire sufficiently. These tests are therefore a confirmation of the results found by ARISZ and OUDMAN with Vallisneria. Only the stronger asparagine concentrations, which can be safely used with Vallisneria, are found to be unsuitable for potato tissue, so that it is necessary to confine oneself to weaker concentrations.

§ 4. The influence of temperature.

In addition to those at 21° C, a few tests are made at other temperatures, viz. at 25° C, at 30° C, and also at 2° C and 11° C.

The air used for aeration is in all cases brought up beforehand to the required temperature. The air, before reaching the test vessels, is led through a long lead pipe, a good 10 metres long, wound around

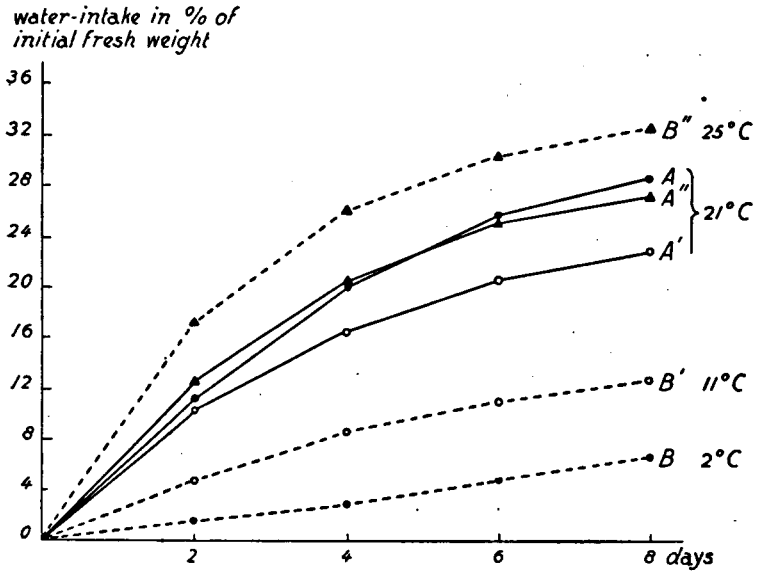


Fig. 6. Influence of temperature on water-intake.
2° C: A and B are the average values of 4 experiments of table 12.
11° C: A′ and B′ are the average values of the 3 experiments of table 12.
25° C: A″ and B″ are the average values of the 5 experiments of table 12.

a bottle, which is also inside a thermostat or refrigerator, as the case may be. The temperatures are measured in the water of the test-vessels in which are the discs.

It is impossible to work at 30° C, the water becoming foul and turbid, and the discs dying after only a few days. STILES and JØRGENSEN (1917) also point to the deleterious effect of fairly high temperatures.

In table 12 the results are summarized. We see just as STILES and JØRGENSEN (1917) that the water-intake (measured as increase of fresh weight in %) is stronger according as the temperature is higher.

TABLE 12.

Water-intake of potato discs at different temperatures.
Series A: control at 21° C.
Series B: test-series at 25° C, 11° C and 2° C respectively.
The respiration is stated as loss of dry substance in % of the initial dry weight.

exp. no.	water-intake as increase in % of initial fresh weight after: 2	4	6	8	10	12	14 days	respiration %
25° C average of 5 experiments								
A	12.5	20.3	24.8	27.1	—	—	—	—
B	17.1	25.9	30.3	32.4	—	—	—	—
11° C average of 3 experiments								
A	10.3	16.4	20.5	22.8	—	—	—	15.5
B	4.7	8.6	11.0	12.7	—	—	—	6.0
2° C After 8 days (at ↓) series B with exp. 163 and 178 is removed to 21° C.								
163 A control	11.1	20.6	26.6	29.1	31.2	32.2	32.9	—
B	1.6	2.5	4.4	5.7↓	10.2	16.1	20.5	—
178 A control	13.1	22.2	26.6	29.6	30.6	31.0	31.2	—
B	1.0	3.0	5.4	8.0↓	14.2	19.9	24.0	—
average of 7 (3 as to the respiration) exp.'s								
A	10.1	18.5	23.9	26.7	—	—	—	15.7
B	1.5	2.7	4.2	5.9	—	—	—	3.7

This is clearly shown in fig. 6. The intake at the lower temperature proceeds with constant rapidity during the entire duration of the experiment, the course of the curve of the water-intake being almost a straight line. At the higher temperature the intake-velocity is practically constant only in the first 4 days, the increase being subsequently slighter.

With a few experiments at the lower temperatures 2° C and 11° C the dry weights are also determined at the end of the test, while the initial dry weight is also known by a third series of discs.The respiration, the loss of dry substance, as is to be expected, is slighter according as the temperature is lower. At the lower temperatures it is also visible that less starch has disappeared than with the controls at 21° C.

In tests 163 and 178, series B, after a period of 8 days at 2° C, is transferred to 21° C. As is to be expected, the water-intake becomes more marked from this moment. But the rise of the curve is less steep than that of the control at the beginning; the water-intake is therefore not so vigorous as the intake of the control at the beginning of the test.

§ 5. The influence of salts on the water-intake.

In this chapter experiments will be treated with which the influence of potassium chloride and of calcium chloride on the water-intake of potato discs are studied.

STEWARD, STOUT and PRESTON (1940) have stated that potato discs in an aerated dilute potassium bromide solution gain more water than the controls did in aerated water, whereas discs in aerated dilute calcium bromide solution show a water-intake significantly less than that of the control discs. The results of BAPTISTE (1935), who has stated that a pre-treatment with KCl and solutions of other monovalent cations promotes the water-permeability of the tissue of potato discs, while $CaCl_2$ and other divalent cations give a decrease, have to be called in question. In the first place it is not the real permeability which he has examined, as besides the rapidity of the water-intake also the water-capacity of the tissue is influenced. Moreover, the control discs, treated beforehand with water only, don't reach the initial weight any more. The weight reaches an equilibrium, which indicates that the tissue may be damaged by the treatment of desiccation; healthy tissue goes on to take in water continuously as is shown in the present investigation. Finally, the concentration (1 atmosphere, that is about 44 m aeq. as to KCl), used by BAPTISTE, is fairly strong, so that it will have withdrawn water from the tissue osmotically (see below). It is true, he has used

the same concentration with all the examined salts, so that his experiments can very well be compared to one another at least.

It has also to be mentioned here that a treatment before-hand with $CaCl_2$, in general with di- and trivalent cations, inhibits the exosmosis with various storage tissues (INGOLD 1931) and reduces the absorption of ammonium by potato discs (ASPREY 1933, 1935), whereas monovalent salts, such as KCl, promote both processes.

Experiments: After the treatment beforehand, as usual during 24 hours in tap water or as the case may be in distilled water, equivalent series of 10 discs each from the same potato are composed in the usual way.

At the beginning of the experiment the salt solution is applied to one series during a few hours, while the other series is used as a control. Both are aerated in the usual way. The salt solution is given only during the first two hours, or, as to KCl in some cases during the first 5 or 17 hours; afterwards the salt-solution is replaced by distilled water. The water-intake is studied only during 24 hours by determining the fresh weight of the discs at the end of the period during which the salt solution is applied, two hours after that, in most cases also after another two hours and at the end of the experiment. The results are summarized in table 13.

With the pre-treatment in the usual way, during 24 hours in tap water, we see in this table that the discs in a KCl solution of the concentration 100 m aeq., applied during the first two hours, decrease considerably in weight during this period, probably because the concentrated salt solution withdraws water osmotically from the tissue. Also STEWARD, STOUT and PRESTON (1940) have found that the stronger KCl solutions (50 m aeq.) mask the specific influence of the potassium by their osmotic effect. L. and M. BRAUNER (1940) also have stated a smaller water-intake from 0.1 n. KCl (= 100 m aeq.) solution by carrot discs than from distilled water; that they did not state a loss of weight will be due to the fact that the tissue is used immediately after cutting the discs, so being not saturated with water as is the case when the discs are treated beforehand with a 24 hours' stay in tap water. In the next two hours, when the KCl is replaced by water, this is recovered and in the following hours the water-intake of the discs of the KCl-series is even somewhat greater than that of the control. At the end of the experiment this promotion is fairly well gone; an after-effect of a detrimental influence of the strong salt solution may be the cause of this. Also $CaCl_2$ in the concentration 100 m aeq., applied during the first two hours, withdraws water. The loss of weight, however, caused by it is much smaller than that caused by KCl in the same concentration. In the

TABLE 13.

The influence of KCl and of $CaCl_2$ on the water-intake of potato discs.
Two equivalent series of 10 discs each are taken from the same potato. The discs are treated beforehand as usual for 24 hours in tap water, or, instead of this in distilled water.
Series A: at the beginning of the experiment a salt solution is applied, KCl or $CaCl_2$ respectively, in various concentrations and during a various number of hours. Afterwards the salt solution is replaced by distilled water.
Series B: control, in distilled water during the entire experiment.
The duration of the experiment is 24 hours. Aeration in the usual way. These experiments were made from December 1936 to April 1937.

		number of experiments	average initial fresh weight in mg per disc	water-intake in % of initial fresh weight after: 2 hrs.	4 hrs.	5 hrs.	6 hrs.	7 hrs.	17 hrs.	19 hrs.	24 hrs.
KCl											
pre-treatment in tap water											
100 m aeq. 2 hrs.	A	3	243.6	−2.4	2.1	—	3.4	—	—	—	5.3
	B		243.6	1.2	2.1	—	2.5	—	—	—	5.1
40 m aeq. 2 hrs.	A	3	233.1	−0.4	2.1	—	3.0	—	—	—	6.4
	B		233.1	0.9	1.4	—	1.9	—	—	—	4.5
10 m aeq. 2 hrs.	A	4	241.4	1.2	2.4	—	3.3	—	—	—	6.8
	B		241.4	1.0	1.7	—	2.1	—	—	—	5.0
5 hrs.	A	5	242.0	—	—	1.2	—	3.0	—	—	7.0
	B		242.0	—	—	1.5	—	2.3	—	—	5.2
17 hrs.	A	4	232.2	—	—	—	—	—	2.6	5.0	6.7
			232.2	—	—	—	—	—	2.7	4.0	4.8
1 m aeq. 2 hrs.	A	6	239.5	1.5	2.2	—	2.8	—	—	—	5.2
	B		239.5	1.3	1.9	—	2.5	—	—	—	4.8
5 hrs.	A	5	239.2	—	—	2.3	—	3.4	—	—	5.9
	B		239.2	—	—	2.5	—	3.2	—	—	5.4
17 hrs.	A	5	234.8	—	—	—	—	—	2.9	4.4	5.2
	B		234.8	—	—	—	—	—	3.2	4.2	4.8
pre-treatment in distilled water											
10 m aeq. 2 hrs.	A	5	249.7	1.1	2.3	—	3.0	—	—	—	4.8
	B		249.7	1.6	2.5	—	3.0	—	—	—	4.8
1 m aeq. 2 hrs.	A	4	243.5	1.7	2.8	—	3.5	—	—	—	5.6
	B		243.5	1.1	1.8	—	2.5	—	—	—	4.6
0.1 m aeq. 2 hrs.	A	5	250.9	1.6	2.2	—	3.1	—	—	—	4.7
	B		250.9	1.5	2.2	—	3.0	—	—	—	4.7
$CaCl_2$											
pre-treatment in tap water											
100 m aeq. 2 hrs.	A	3	242.3	−1.0	1.4	—	1.8	—	—	—	4.6
	B		242.3	1.4	2.2	—	2.6	—	—	—	5.2
40 m aeq. 2 hrs.	A	4	237.4	0.1	1.1	—	2.0	—	—	—	4.5
	B		237.4	1.4	2.0	—	2.5	—	—	—	5.2
30 m aeq. 2 hrs.	A	2	243.3	0.7	1.7	—	2.4	—	—	—	5.1
	B		243.3	1.8	2.4	—	3.2	—	—	—	6.0
10 m aeq. 2 hrs.	A	3	240.9	1.1	2.0	—	2.7	—	—	—	5.3
	B		240.9	1.6	2.4	—	3.2	—	—	—	5.7
1 m aeq. 2 hrs.	A	3	243.2	1.2	1.6	—	2.3	—	—	—	4.9
	B		243.2	1.3	1.9	—	2.5	—	—	—	5.1
pre-treatment in distilled water											
10 m aeq. 2 hrs.	A	3	251.0	1.1	2.0	—	2.6	—	—	—	5.1
	B		251.0	1.9	2.6	—	3.1	—	—	—	5.6
1 m aeq. 2 hrs.	A	3	243.3	1.3	1.7	—	2.4	—	—	—	5.0
	B		243.3	1.4	1.8	—	2.5	—	—	—	5.3

following period, when the salt solution is replaced by distilled water, the weight of the discs increases considerably, but it remains below that of the controls and the difference is maintained practically till the end of the experiment.

KCl in the concentration 40 m aeq. turns out to withdraw water from the discs during the period of application also, but the loss of weight is much smaller than with 100 m aeq. In the following hours the water-intake is promoted compared to that of the control, the difference with the control still increasing till the end of the experiment. Discs to which $CaCl_2$ in the concentration 40 m aeq. is applied during the first two hours of the experiment, don't show a loss of weight; however, there is practically no increase of weight either, in contrast with the control. In the next period, when the $CaCl_2$ is replaced by distilled water, the difference in weight between both series diminishes rapidly; the total water-intake, however, during the further course of the experiment is smaller than that of the control. Discs, treated with $CaCl_2$ 30 m aeq. behave fairly well just as those treated with 40 m aeq., but the diminution of the water-intake during the period of application is much smaller.

KCl in the concentration 10 m aeq., given during the first two hours, promotes the water-intake with respect to that of the control, small at first and still increasing during the experiment. The final promotion is practically as great as that with 40 m aeq., though it remains uncertain if with 40 m aeq. the effect may be somewhat disturbed by a detrimental influence. The water-intake of discs which were in a solution of $CaCl_2$ 10 m aeq. during the first two hours, is somewhat less than that of the controls, the difference with the control remaining fairly well constant during the experiment.

KCl 10 m aeq. applied for a longer time, viz. for 5 hours or 17 hours, causes during that period a small inhibition of the water-intake with regard to that of the control, in the case of the 17 hours' stay there is practically no difference at all with the control. After the salt solution has been replaced by water, a promotion is to be seen, the difference with the control gradually increasing during the experiment; the final promotion at the end of the experiment is about as large as with a two hours' stay in the salt solution. Why an inhibition of the water-intake occurs at first when the discs remain in the salt solution during a longer period is difficult to say with certainty, probably a single-salt solution may be somewhat detrimental to the tissue.

KCl in the concentration 1 m aeq., applied for the first two hours causes a slight promotion of the water-intake, fairly irregular with the various experiments, but the average being quite regular. The

promotion is much weaker than that of the concentration 10 m aeq. The water-intake of discs to which $CaCl_2$ 1 m aeq. was given during the first two hours of the experiment, is somewhat inhibited with regard to that of the controls. The strength of the influence differs less from that of the concentration 10 m aeq. than in the case of KCl.

If the KCl 1 m aeq. is applied for a longer time, namely for 5 hours or 17 hours, the water-intake during that period is slightly inhibited compared to that of the control. After the salt solution has been replaced by water, a slight promotion becomes evident; the final promotion at the end of the experiment is about as great as when the salt solution is given for two hours only.

As to the nature of the influence of the salts, these experiments justify the conclusion, that it is an influence on the water-capacity of the tissue more than on the water-intake itself. This becomes evident especially from the experiments with $CaCl_2$, with which only in the initial hours an inhibition of the water-intake is to be seen, while during the further course of the experiment the difference with the control practically remains constant. Also with KCl 1 m aeq. this is the case. With KCl 10 m aeq., however, the difference with the control increases continuously during the experiment; but we may assume that also in this case a constant difference with the control results. It is to be regretted that no intermediate observations are made, the experiment should probably have been continued somewhat longer.

Besides these experiments with the usual treatment beforehand in tap water for 24 hours, also some experiments are carried out with which the discs are treated beforehand in distilled water for 24 hours. These experiments are made with KCl as well as with $CaCl_2$; the salt solution is given only during two hours at the beginning of the experiment, while only the concentrations 10 m aeq. and weaker are used.

As is to be seen in fig. 7 (exp.'s of table 13), in the case of $CaCl_2$ it practically makes no difference, either with the concentration 10 m aeq. or with the concentration 1 m aeq. whether the treatment beforehand has taken place in distilled water or in tap water. In both cases a small inhibition of the water-intake with regard to that of the control occurs; with the concentration 10 m aeq. the inhibition being somewhat greater than with the concentration 1 m aeq. In the initial hours of the experiment, as to the concentration 10 m aeq., however, the inhibition is somewhat greater if the discs are treated beforehand with distilled water than with tap water.

With KCl, however, there is a marked difference. When treated beforehand with distilled water, the concentration 10 m aeq. does

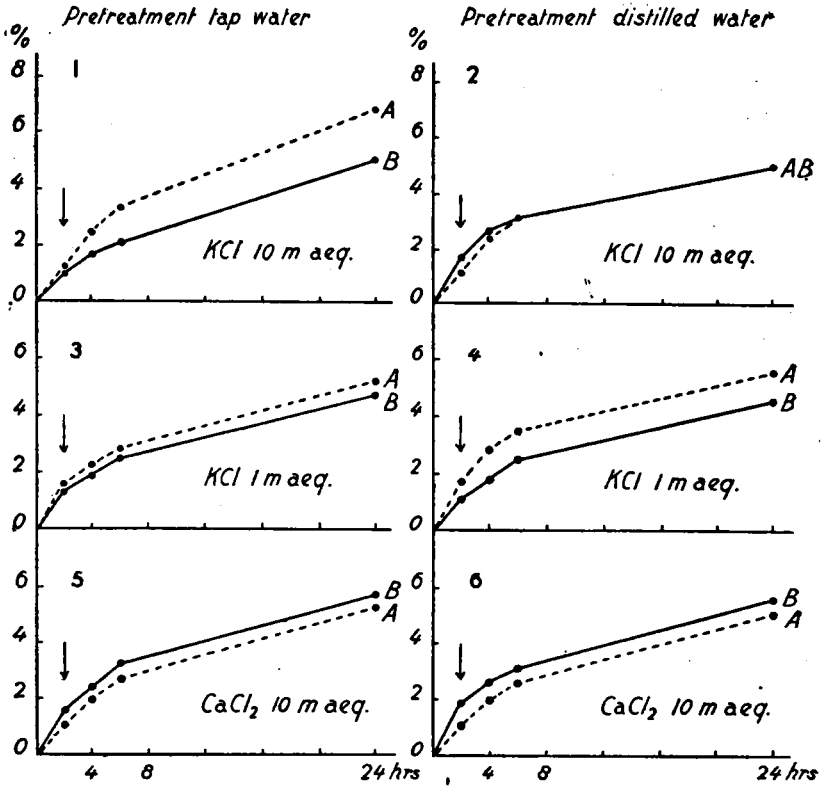


Fig. 7. Influence of salts on water-intake (exp.'s of table 13). Abscissa: time in hours. Ordinate: water-intake in % of initial fresh weight.
1. and 2. The discs are treated during 2 hours in an aerated solution of KCl 10 m aeq.; afterwards (at ↓) aerated distilled water is applied.
3. and 4. The discs are treated during 2 hours in an aerated solution of KCl 1 m aeq.; afterwards (at ↓) aerated distilled water is applied.
5. and 6. The discs are treated during 2 hours in an aerated solution of $CaCl_2$ 10 m aeq.; afterwards (at ↓) aerated distilled water is applied.
Pretreatment tap water, no. 1, 3 and 5.
Pretreatment distilled water, no. 2, 4 and 6.

not exert any influence, the water-intake being as great as that of the control; in contrast with the discs treated beforehand in tap water, the concentration 10 m aeq. increases the water-capacity rather strongly. The concentration 1 m aeq. on the contrary, which has only a slightly promoting influence when the discs are treated

beforehand in tap water, has a distinctly stronger effect when the discs are treated beforehand with distilled water. A tenfold weaker concentration, 0.1 m aeq., does not exert any influence, the water-intake being equal to that of the control discs.

Salts, principally the Ca salts present in the tap water, may probably be the cause of this difference. Probably the tissue is influenced in such a way that it may resist the somewhat poisonous single-salt solutions better, which may explain that KCl 10 m acq. shows no influence when the discs are treated beforehand in distilled water; the poisonous action and the influence promoting the water-intake neutralize each other. Besides depoisoning the Ca-salts of the tap water may probably have a somewhat condensing influence on the plasma-membranes (DE HAAN 1935), which may be the cause that the influence of KCl 1 m aeq. is somewhat stronger when the tissue is treated with distilled water than when it is treated with tap water beforehand. If the Ca-salts of the tap water are actually the cause of the different behaviour of the tissue as to KCl, it is also easy to understand that as to $CaCl_2$ it is fairly indifferent, whether the treatment beforehand has taken place in tap water or in distilled water.

So it is stated here that calcium reduces the water-capacity of the potato tissue, while potassium as a rule increases this, though this is not the case during a somewhat longer stay in the salt solution; the treatment beforehand also playing a part.

§ 6. The outward appearance of the discs and the occurrence of cell-divisions.

A short time after they are put into aerated water, the exterior of the discs becomes a little brownish, which tint becomes more intense as time goes on. This discoloration is said by STEWARD to be a sign of health.

A second phenomenon is the occurrence of small brown spots, which begin to make their appearance after two days. After 4 days they are more clearly outlined, and remain so. With some potatoes these spots occur as a fine sprinkling, with others they are much coarser. They occur over the entire surface of the disc, but with some potatoes they are found chiefly in the central medullary tissue, with others more in the parenchyma, which contains phloem-groups.

In unaerated water the discs become brown much more slowly and less intensely than in aerated water. The spots never occur in unaerated water, at all events, not in the limited time that the experiments last.

In an environment without oxygen no brown discoloration occurs, nor do the spots appear; the discs remain perfectly white to the end.

To what this brown colouring is due, and what the occurrence of these brown spots really is, cannot be said with certainty. Probably it is a phenomenon of oxydation (STEWARD and PRESTON 1940), as it does not occur in an anaerobic environment, and also proceeds so much more slowly in an unaerated environment. The data found with the temperature tests (§ 4) also point in the same direction. At 11° C the discs become only very slightly light-brown in colour, while practically nothing is to be seen of brown spots. At 2° C the discs remain quite white.

The occurrence of cell-divisions. From HABERLANDT's investigations (1913; 1930) it is known that substances from the phloem are required if cell-divisions are to occur. Pieces of potato-tissue without phloem show no cell-divisions in HABERLANDT's experiments. The cell-divisions occur more plentifully according as the cells are situated closer to the leptome-groups. HABERLANDT's tests are carried out in moist air.

With a microscopic investigation of the discs, after these have been in aerated water, I have found, as did STEWARD, WRIGHT and BERRY (1932), a fairly strong demolition of starch in the superficial zone, while in most cases an almost entire meristem has developed. In the most favourable cases this has formed from 3 to 4 new cell-walls in one original cell. In less favourable cases only the cells in the vicinity of the phloem-groups show regular cell-divisions, while they occur more sporadically at other places. After a period of two days in aerated water no cell-divisions are to be observed yet; four days after the commencement of the experiment they begin to occur, and only in the vicinity of the phloem-groups, while usually only one or two new walls are formed per cell. After 8 days the maximum number of cell-divisions is practically reached. In experiments lasting 4—6 weeks, practically no more cell-divisions occur than there were after one week.

The occurrence of numerous cell-divisions is not accompanied by a vigorous water-intake. There are tests with a strong water-intake both with few cell-divisions and with many. In tests with a comparatively weak water-intake well-developed cell-divisions are also found in some cases, in other experiments it is accompanied by only slightly developed cell-divisions. Cell-division and cell-elongation do not, therefore, coincide.

It makes little difference whether the water is aerated strongly or weakly (cf. p. 26). But there is perhaps a little difference, in the sense that with strong aeration the divisions occur to a somewhat greater

extent than with weak aeration, but the difference is hardly appreciable. In unaerated water scarcely any starch demolition is to be seen, and no cell-divisions occur (with the exception of an odd one or two close to the phloem-elements). KNY (1889) already has found that oxygen is necessary to form cell-divisions.

No starch demolition is to be seen in an environment without oxygen, either, while here, too, no cell-divisions occur.

Discs which have first been for two days in an environment without oxygen, and the following 4 days have been aerated with air, show, when examined microscopically after the experiment, rather fewer, but still almost as many cell-divisions, as the controls which have been continuously aerated with air. In test 186 (table 7), e.g., we find that in series B, (unaerated), no cell-divisions have occurred, while in series C (in an environment without oxygen for the first two days, and then aerated with air), this is the case, and to almost as great an extent as in the control series A, which has been continuously aerated with air. But the water-intake of series C is only 10.9 %, and that of series B 14.6 %. With discs, however, which have been without oxygen for the first 4 days, and which have then been aerated with air for 4 days, no further cell-divisions occur. After a period of 4 days in the anaerobic jar the discs have therefore practically lost the capacity for cell-division. (exp. 190 and exp. 200 in table 7).

The occurrence of cell-divisions is therefore connected with a good aeration and with the capacity of the tissue to react to this.

The formation of cell-divisions is not promoted by a period spent in an environment amply supplied with oxygen (§ 2). At the end of the experiment cell-divisions are already clearly visible in the control-series A, while with series B (rich in oxygen) practically no cell-divisions have occurred, these being plainly less far advanced than with the control.

The occurrence of cell-divisions is affected by the temperature. At lower temperatures practically no cell-divisions occur. At 25° C there is practically no difference to be seen as compared with the control; in fact the cell-divisions at 25° C are somewhat less developed than is the case with the control at 21° C.

§ 7. Summary.

The water-intake is greatest in water aerated with air. In unaerated water the water-intake is considerably less, the discs beginning to deteriorate within a few days. In an anaerobic environment no water-intake occurs; during the first 24 hours the fresh weight shows a slight increase, later on it remains constant. If, after a stay

of a few days in an environment without oxygen, aeration with air is again applied, the tissue goes on taking in water; at the beginning, however, the weight increases only to a slight extent, later on the increase becomes more intense. If in the beginning the discs are aerated with air during a few days, followed by a stay in an anaerobic environment they are no longer able to tolerate this. There is a marked loss of weight and the discs become flabby.

The respiration, measured as loss of dry substance, is considerably less under anaerobic conditions than when aeration is applied.

Aeration with 100 % oxygen does not accelerate the water-intake; on the contrary it is somewhat inhibitory, though the difference is very slight. The respiration is not influenced by it either.

The intake of asparagine in the case of potato discs is also an active process. Considerably more asparagine is taken in from solutions aerated with air than from solutions in an environment without oxygen.

The water-intake is also greatly dependent on temperature. The higher the temperature, the more water is taken in. Also the respiration is greater as the temperature is higher. Temperatures higher than 25° C cause the dying of the tissue already within a few days.

Calcium reduces the water-capacity of the potato tissue, while potassium as a rule increases this, though this is not the case during a somewhat longer stay in the salt solution; the pre-treatment also playing a part.

In an anaerobic environment no cell-divisions occur, nor in unaerated water either; on the other hand in aerated water they do occur, to the greatest extent near the phloem-groups. The occurrence of numerous and well-shaped cell-divisions need not go together with a vigorous water-intake. Cell-division and cell-elongation do not, therefore, coincide. There are no more cell-divisions when the water is equilibrated with 100 % oxygen; they then are developend to a clearly slighter extent than is the case when the water is aerated with ordinary air. The cell-divisions occur moie frequently as the temperature is higher. They are developed best at 21° C; at 25° C they are, though the difference is small, developed to a somewhat slighter extent than is the case at 21° C.

CHAPTER III.

THE INFLUENCE OF HETERO-AUXIN (INDOLE-3-ACETIC ACID).

§ 1. Introduction.

In the preliminary publication (REINDERS 1938) the fact has been communicated that hetero-auxin has a favourable effect on the intake of water by potato-discs. The increase in the fresh weight in hetero-auxin solution is much stronger than the increase of the fresh weight in distilled water. Besides this promoting influence on the water-intake, hetero-auxin also increases the loss of dry substance. With the discs in the hetero-auxin solution a much greater loss of dry substance is observable than with the controls in distilled water.

These tests will now be reproduced in a somewhat more detailed form, while a few points have meanwhile received support from new data. In a few tests the conductivity of the external solution is determined, in order to see whether the greater loss of dry substance of the discs, under the influence of the hetero-auxin, is really caused by an influence on the respiration, or whether it is perhaps due to a stronger exosmosis. This is not found to be the case.

§ 2. The effect of hetero-auxin in various concentrations on the water-intake and on the respiration.

Hetero-auxin solutions [1]) of various strengths are used. The concentrations 1 to 1000 and 1 to 10.000 have a deleterious effect, the discs becoming flabby and are soon dying.

A number of tests are carried out with the concentrations 1 to 10^5, 1 to 10^6 and 1 to 10^7. A number of series of 10 discs, with the same initial fresh weight, are composed in the usual way. Series A is given distilled water, series B is given hetero-auxin solution. The initial dry weight of series C, if present, is determined. Aeration always takes place in the same fashion. The observation is made every other day, the discs being then weighed one by one on the torsion-balance. After each observation the hetero-auxin solution and the water of the control are renewed. At the end of the experiment the final dry weight of the discs is determined.

A picture of the influence of concentrations, 1 to 10^6, and 1 to 10^7

1) The hetero-auxin, a pure chemical product of indole-3-acetic acid (melting-point 166.5°—167.5°), is supplied by Prof. Dr H. J. BACKER, Director of the Org. Chem. Laboratory at Groningen, for which kindness I pronounce my sincere thanks.

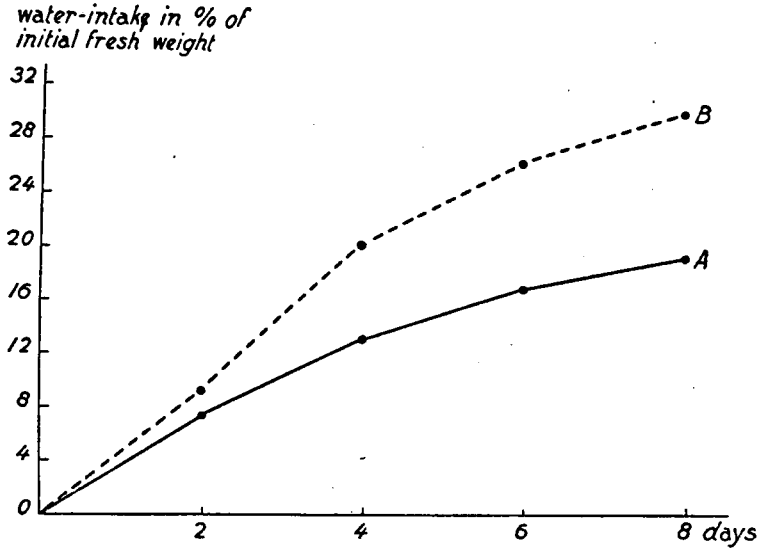


Fig. 8. The influence of hetero-auxin, concentration 1 to 10^6, on the water-intake. (exp. 376 of table 15).
A: distilled water.
B: hetero-auxin solution.

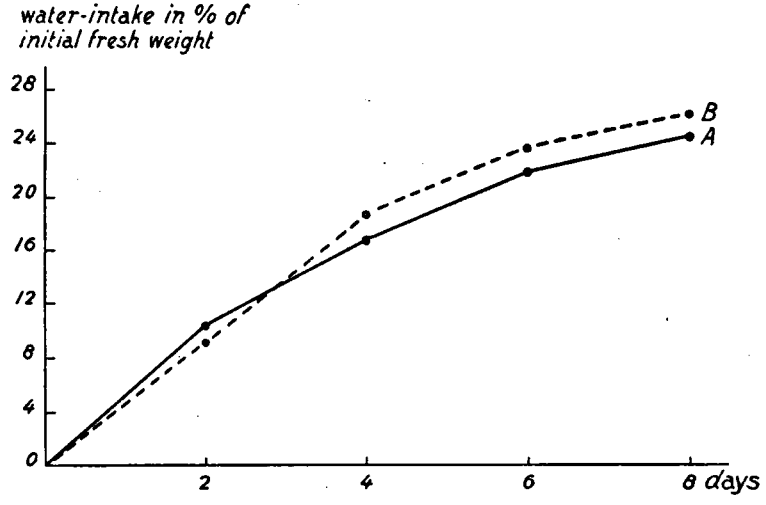


Fig. 9. The influence of hetero-auxin, concentration 1 to 10^7, on the water-intake. (exp. 235 of table 15).
A: distilled water.
B: hetero-auxin solution.

on the course of the water-intake is given by figs. 8 and 9. The course is of such a character that the increase in weight of the discs in the hetero-auxin solution (series B) in the first two days, differs little from that of the controls, (series A), while in the following days a typical promotion of the water-intake due to the hetero-auxin occurs. The concentration 1 to 10^5 has the greatest effect, the effect of the concentration 1 to 10^6 is a trifle weaker, while the concentration 1 to 10^7 only has a very slight influence.

The course of the water-intake in the first two days may differ. In some case, viz. in most tests, but not in all, in the season 1937—1938, it is of such a nature that the water-intake of the discs in the hetero-auxin solution is somewhat, but plainly, less than that of the controls in water. In many cases, in most of the tests of the season 1938—1939, though not in all, the water-intake of the discs in the hetero-auxin solution is somewhat, but plainly, larger than that of

TABLE 14 (exp. 216).

Influence of hetero-auxin on water-intake; duration of experiment 5 weeks.
Series A: distilled water.
Series B: hetero-auxin 1 to 10^6.
Both are aerated in the usual way and are renewed after each observation. The initial fresh weight is stated in mg for 10 discs together. The water-intake is stated as the increase of the initial fresh weight in %.

		water-intake as increase in % of initial fresh weight		
		A	B	difference B—A in %
initial fresh weight	23-3-'38	2633.8 mg	2634.1 mg	
	25-3-'38	12.7	9.9	-2.8
	27-3-'38	21.3	24.8	3.5
	29-3-'38	26.1	33.1	7.0
	31-3-'38	28.3	36.8	8.5
	2-4-'38	29.2	38.2	9.0
	4-4-'38	29.8	39.2	9.4
	6-4-'38	30.1	40.1	10.0
	8-4-'38	30.1	40.9	10.8
	10-4-'38	30.1	45.4	15.3
	12-4-'38	30.0	47.6	17.6
	14-4-'38	—	—	—
	16-4-'38	30.6	51.1	20.5
	18-4-'38	—	—	—
	20-4-'38	31.4	53.5	22.1
	22-4-'38	—	—	—
	24-4-'38	—	—	—
	26-4-'38	32.3	52.0	19.7

TABLE 15.

Water-intake and respiration in hetero-auxin solutions. (concentrations: 1 to 10^5; 1 to 10^6 and 1 to 10^7).
Series A: distilled water.
Series B: hetero-auxin solution.
Fresh weight and dry weight are stated in mg for 10 discs together. The water-intake is stated as increase of fresh weight in % of the initial fresh weight. The respiration is stated as loss of dry substance, in mg for 10 discs and in % of the initial dry weight.

exp. no.	initial fresh weight	water-intake as increase in % of initial fresh weight after:				dry weight in mg		respiration	extra respiration caused by hetero-auxin	
	mg	2	4	6	8 days	initial	final	mg	mg	%
1 to 10^5										
227 (9-4-'38)										
A control	2763.9	15.1	23.3	28.1	30.5	—	411	—	—	—
B hetero-auxin	2763.5	10.3	30.1	37.4	42.9	—	364	—	47	—
244 (1-5-'38)										
A control	2538.0	12.2	21.6	28.3	30.5	—	378	71	—	15.8
B hetero-auxin	2539.0	9.8	27.6	36.8	43.6	—	338	111	40	24.7
C	2536.1	—	—	—	—	449	—	—	—	—
249 (9-5-'38)										
A control	2580.9	10.6	16.6	20.3	22.7	—	404	53	—	11.6
B hetero-auxin	2580.7	9.1	24.9	31.5	35.6	—	351	106	53	23.0
C	2580.6	—	—	—	—	457	—	—	—	—
1 to 10^6										
240 (26-4-'38)										
A control	2717.9	10.1	20.9	25.4	28.4	—	360	—	—	—
B hetero-auxin	2718.0	8.8	28.2	36.2	40.2	—	351	—	9	—
245 (2-5-'38)										
A control	2664.2	10.8	15.4	18.5	20.3	—	362	—	—	—
B hetero-auxin	2661.3	8.8	17.6	22.5	24.2	—	350	—	12	—
248 (9-5-'38)										
A control	2705.5	11.0	17.8	20.9	23.0	—	468	—	—	—
B hetero-auxin	2705.1	8.5	20.2	27.5	31.0	—	452	—	16	—
253 (17-5-'38)										
A control	2641.4	10.1	16.9	21.8	24.2	—	370	—	—	—
B hetero-auxin	2642.5	8.8	21.2	27.1	30.7	—	361	—	9	—
256 (18-5-'38)										
A control	2730.8	7.9	12.6	16.9	19.5	—	358	—	—	—
B hetero-auxin	2719.1	7.9	18.8	25.3	29.0	—	342	—	16	—

TABLE 15 (*continuation*).

exp. no.	initial fresh weight	water-intake as increase in % of initial fresh weight after:				dry weight in mg		respiration	extra respiration caused by hetero-auxin	
	mg	2	4	6	8 days	initial	final	mg	mg	%
332 (3-1-'39)										
A control	2351.4	7.7	11.7	15.4	16.9	—	305	70	—	18.7
B hetero-auxin	2350.7	10.3	22.4	29.3	38.3	—	283	92	22	24.5
C	2352.8	—	—	—	—	375	—	—	—	—
335 (5-1-'39)										
A control	2428.8	7.9	13.6	15.9	17.3	—	357	69	—	16.2
B hetero-auxin	2428.7	9.1	22.9	30.5	35.2	—	324	102	33	23.9
C	2429.0	—	—	—	—	426	—	—	—	—
369 (18-2-'39)										
A control	2438.7	10.7	16.7	21.3	23.8	—	396	54	—	12.0
B hetero-auxin	2438.7	10.1	24.9	30.8	34.2	—	374	76	22	17.0
C	2438.1	—	—	—	—	450	—	—	—	—
376 (27-2-'39)										
A control	2495.5	7.4	13.0	16.7	19.1	—	409	69	—	14.4
B hetero-auxin	2495.7	9.1	20.0	26.1	29.7	—	397	81	12	17.0
C	2496.0	—	—	—	—	478	—	—	—	—
382 (6-3-'39)										
A control	2361.4	7.8	11.7	14.5	16.9	—	332	58	—	14.9
B hetero-auxin	2361.2	8.7	20.2	24.6	26.6	—	312	78	20	20.0
C	2361.5	—	—	—	—	390	—	—	—	—
1 to 10^7										
235 (19-4-'38)										
A control	2630.9	10.5	16.8	21.9	24.4	—	397	—	—	—
B hetero-auxin	2630.9	9.2	18.7	23.5	26.1	—	400	—	-3	—
247 (8-5-'38)										
A control	2563.4	10.8	16.3	19.3	21.4	—	374	—	—	—
B hetero-auxin	2563.3	8.5	16.7	20.7	23.5	—	368	—	6	—
393 (30-3-'39)										
A control	2647.7	8.3	12.9	16.5	17.4	—	384	82	—	17.6
B hetero-auxin	2647.8	7.4	13.3	17.0	18.7	—	395	71	-11	15.2
C	2647.5	—	—	—	—	466	—	—	—	—

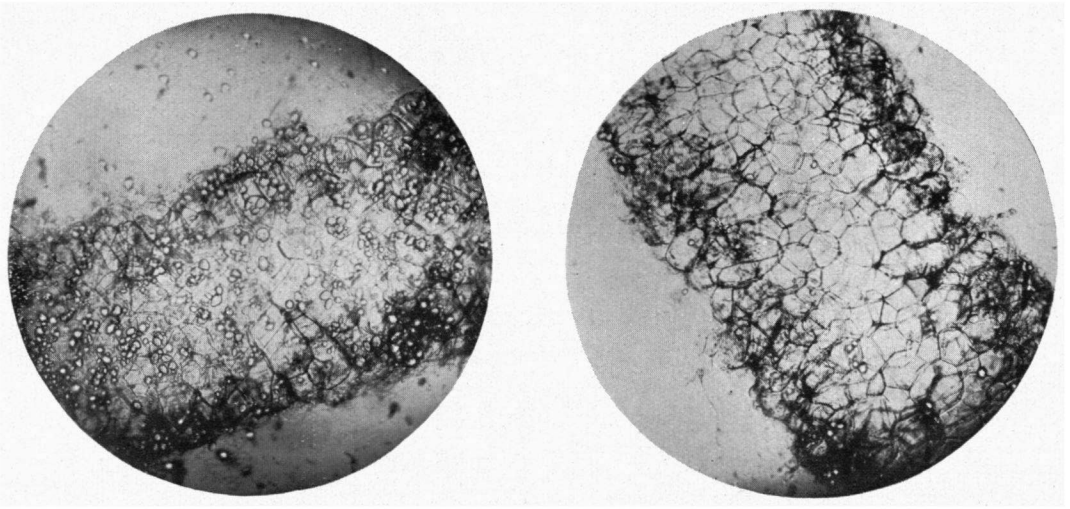
the controls in the first two days also. In many cases, however, no difference in the water-intake is observable in the first two-days' period either, between the discs in the hetero-auxin solution and that in water.

The promotion of the water-intake by the hetero-auxin is strongest in the second two-days' period; after this the curves which show the course of the water-intake diverge still further in by far the most cases; hetero-auxin thus continues to have a promoting effect during the further course of the process, but the strongest effect is nevertheless to be found in the period from the second to the fourth day. Later on, if the water-intake itself becomes less strong, the favourable effect of the hetero-auxin on this also apparently becomes less strong. With test 216, which is continued for a month (table 14), the difference between the hetero-auxin series and the control continues to increase somewhat for quite 4 weeks; only during the last week is this no longer the case.

The form of the curve which shows the course of the water-intake, is remarkable. This form points to the co-operation of two influences, a favourable one, which in some cases makes itself felt immediately, and is in all the experiments very pronounced in the second period, and an inhibitory one, which manifests itself in a reduced water-intake in the first period and in a slighter promotion of the water-intake in the first period than in the second period.

These experiments regarding the effect of the concentration are summarized in table 15. We see there that, in addition to the favourable influence on the water-intake, a great difference in dry weight is also observable at the end of the experiment, that is, therefore, after 8 days. The discs from the hetero-auxin solution have lost much more dry substance than the controls. The strongest concentration, 1 to 10^5, has the greatest influence, giving an extra loss of 40—53 mg dry substance, i.e. approx. 50 mg. The concentration 1 to 10^6 gives an extra loss of dry weight that is considerably less, while the concentration 1 to 10^7 has practically no influence. It seems not impossible that the concentration 1 to 10^7 has a slight inhibitory influence?

A miscroscopic examination of the discs at the end of the experiment shows that, under the influence of the hetero-auxin, a visibly larger amount of starch has disappeared out of the cells. This is as a rule plainly visible after the lapse of as little as 8 days. In test 216, however, (table 14), which is continued for 5 weeks, the cells of the discs from the hetero-auxin series are practically empty, a few starch grains being only sporadically present. In the cells of the control series a good deal of starch is still present. (microphotograph).



Microphotograph (exp. 216, table 14).
Disappearance of starch by hetero-auxin.
Left: control.
Right: hetero-auxin 1 to 10^6.
Duration of the experiment 5 weeks.

There are a few exceptions. In tests 289 and 368, in table 17 (§ 4) hetero-auxin 1 to 10^6 has no effect on the loss of dry substance, the final dry weight of the discs of the hetero-auxin series being practically equal to that of the control-discs. In test 392, table 18 (§ 5), hetero-auxin 1 to 10^6 apparently has no influence on the loss of dry substance. And in test 389 of this same table the loss of dry substance in the case of the hetero-auxin series is as much as 12 mg less than that of the control; here, then, there is an inhibition instead of a promotion.

With these tests of table 15 a few differences are noticeable between the tests in the season 1937—1938 and those in the season 1938—1939. In the first place the initial fresh weights of the potato discs in 1938—1939 are in general somewhat lower than in 1937—1938. In the second place the total water-intake in 8 days of the controls, that is, therefore, in ordinary distilled water, is as a rule lower in the season 1938—1939 than that in 1937—1938. Moreover, in the experiments in table 15 with hetero-auxin 1 to 10^6, of which a number of tests from both seasons are placed side by side, there is a difference in this sense, that the influence of hetero-auxin on the loss of dry substance makes itself felt somewhat more strongly in the season 1938—1939 than in the tests of the season 1937—1938. In 1937—1938 the loss of dry substance under the influence of hetero-auxine 1 to 10^6 is about 12 mg greater than that of the control, ranging from 9 to 16 mg. In 1938—1939 the loss of dry substance of the hetero-auxin series is approx. 22 mg more than that of the control, ranging from 12 to 33 mg.

In the tests in table 18 (§ 5 of this chapter), nothing is to be seen of any difference in this sense, the extra loss of dry substance under the influence of the hetero-auxin 1 to 10^6 being about equal for the tests of both seasons.

Under the influence of hetero-auxin, therefore, more dry substance is lost. If more dry substance is lost, this naturally also has an effect on the fresh weight. As a matter of fact, therefore, the favourable effect on the water-intake is somewhat greater still than is shown by the figures for the increase in fresh weight.

It is not certain that this extra loss of dry substance is really due to respiration. It may, for instance, be caused by exosmosis.

In order to obtain data on this point, observations are made as to the conductivity of the external solution in the experiments of § 5 (and § 6) in this chapter, while at the same time the p_H determined. In these tests the resistance of the old and the new hetero-auxin solution and of the old and the new water of the control is determined every other day (table 16). If we take particular notice of series A,

TABLE 16.

Exosmosis and absorption of potato discs in water and in a solution of hetero-auxin. Determinations of p_H and of the electrical resistance of the experiments of table 18.

Series A: continuously distilled water.
Series B: continuously hetero-auxin 1 to 10^6.
Series C: first two days hetero-auxin 1 to 10^6, then distilled water.
Series D: first two days distilled water, then hetero-auxin 1 to 10^6.

The electrical resistance is stated in Ω.

	beginning of experiment		after 2 days			after 4 days			after 6 days			after 8 days	
	p_H	resistance	p_H		resistance	p_H		resistance	p_H		resistance	p_H	resistance
			old	new	old new	old	new	old new	old	new	old new	old	old
exp. 387													
A	4.5	200 000	7.0	4.5	34 000 300 000	6.0	4.5	130 000 240 000	4.5	4.5	320 000 200 000	4.5	360 000
B	4.5	150 000	7.0	4.5	37 000 220 000	6.0	4.5	100 000 200 000	5.0	4.5	230 000 160 000	5.0	220 000
C	4.5	150 000	7.0	4.5	33 000 290 000	6.0	4.5	130 000 240 000	4.5	4.5	390 000 200 000	4.5	340 000
D	4.5	200 000	7.0	4.5	38 500 220 000	6.0	4.5	100 000 200 000	5.0	4.5	180 000 160 000	5.0	170 000
exp. 389													
A	4.5	90 000	6.5	4.5	40 000 240 000	6.0	4.5	80 000 180 000	5.0	4.5	180 000 190 000	4.5	410 000
B	4.5	90 000	6.5	4.5	35 000 160 000	7.0	4.5	70 000 190 000	5.0	4.5	220 000 150 000	5.0	260 000
C	4.5	90 000	6.5	4.5	35 000 240 000	6.5	4.5	66 000 180 000	5.0	4.5	240 000 190 000	4.5	400 000
D	4.5	90 000	6.5	4.5	40 000 160 000	6.5	4.5	70 000 190 000	5.0	4.5	210 000 150 000	5.0	260 000
exp. 390													
A	4.5	400 000	7.0	4.5	48 000 180 000	6.5	4.5	77 000 360 000	5.0	4.5	190 000 210 000	4.5	390 000
B	4.5	280 000	7.0	4.5	43 000 150 000	6.5	4.5	66 000 220 000	5.5	4.5	250 000 160 000	5.0	340 000
C	4.5	280 000	7.0	4.5	43 000 180 000	6.5	4.5	85 000 380 000	5.0	4.5	300 000 200 000	4.5	400 000
D	4.5	400 000	7.0	4.5	50 000 150 000	6.5	4.5	64 000 250 000	5.5	4.5	190 000 160 000	4.5	370 000
exp. 391													
A	4.5	280 000	7.0	4.5	47 000 180 000	6.5	4.5	90 000 220 000	5.0	4.5	220 000 200 000	4.5	420 000
B	4.5	200 000	7.0	4.5	39 000 150 000	6.5	4.5	83 000 170 000	5.0	4.5	320 000 150 000	4.5	430 000
C	4.5	200 000	7.0	4.5	40 000 180 000	6.5	4.5	110 000 220 000	5.0	4.5	280 000 200 000	4.5	420 000
D	4.5	280 000	7.0	4.5	45 000 140 000	6.5	4.5	85 000 170 000	5.0	4.5	310 000 150 000	4.5	420 000
exp. 392													
A	4.5	160 000	6.5	4.5	52 000 160 000	6.5	4.5	87 000 78 000	5.0	5.0	180 000 41 000	5.5	63 000
B	4.5	130 000	6.5	4.5	45 000 120 000	6.5	4.5	88 000 66 000	5.0	5.0	260 000 40 000	6.0	55 000
C	4.5	130 000	6.5	4.5	48 000 160 000	6.5	4.5	100 000 78 000	5.0	5.0	250 000 42 000	5.5	66 000
D	4.5	160 000	6.5	4.5	54 000 120 000	6.5	4.5	92 000 66 000	5.0	5.0	200 000 40 000	6.0	60 000

the whole time in water, and of series B, the whole time in hetero-auxin 1 to 10^6, we find that in both series in the first two-days' period the resistance falls to a marked extent, and to about the same degree. As a matter of fact the fall of the hetero-auxin series is even smaller, since the initial value is also lower, but the substance hetero-auxin will of course also be taken in. In the second two-days' period the same thing occurs, only the fall in the resistance is considerably slighter. Absolutely, the fall in the hetero-auxin series is again slighter than that of the control. In the following two-days' period a rise in the resistance is observable in test 387, instead of a fall. This rise is absolutely larger with the control (A) than with the hetero-auxin series (B.) In test 390 the hetero-auxin series, however, shows a very slight rise, while the control, on the other hand, still displays a fall in the resistance. In test 391 there is a marked rise in the resistance in the case of the hetero-auxin series, while the control does not show any change in the resistance. This seems confusing, but we may say, that in general there is an inclination to an increase of the resistance in this third two days' period. Substances, therefore, are taken up. At any rate the absorption has evidently become preponderant over the exosmosis, which preponderates in the first few days, if there is then any absorption. (STILES 1927). In the last two-days' period a marked rise in the resistance occurrs in all tests, both with the hetero-auxin series and with the control. In tests 387 and 389 the rise in the case of the hetero-auxin series is less marked than with the control.

If, now, we first consider the course of the p_H, we find that with both series there always occurs a marked rise in the p_H in the first few days, but less later on, while in the last few days of the experiment the p_H changes but little with respect to that of the fresh water or hetero-auxin solution. Here and there the rise of the p_H in the case of the hetero-auxin series is rather more than that of the control series, e.g. in the two last two-days' period of test 387 and also in the last period of test 389. This is accompanied only by a slight rise in the resistance, while with the control in water, where the p_H has changed little if at all, a strong rise in the resistance does occur. It is hardly likely that there is only a slight absorption with the hetero-auxin series in this case; there is no reason to suppose that the p_H of this would alter, while the p_H does not do so with a strong absorption. This actually indicates the existence of exosmosis. In test 387 there is therefore a stronger exosmosis with the hetero-auxin series than with the control, and it may very well be that the greater loss of dry substance is partly caused by this. But it is not probable, for in test 389 it is just the same, and here, exceptionally, the hetero-auxin has

not acted favourably on the loss of dry substance; on the contrary, the loss of dry material with all series is slighter than with the control (See table 19). With the other tests of table 16 (tests 390, 391 and 392) the rise in the resistance in both series occurs to about the same extent, in test 391 even absolutely more markedly with the hetero-auxin series than with the control. From these data it is seen, therefore, that as a rule no stronger exosmosis occurs under the influence of hetero-auxin, and if this is sometimes the case, this is not the cause of a greater loss of dry material, at any rate certainly not in test 389.

We may therefore conclude, with a very great chance of being right, that the extra loss of dry substance that occurs under the influence of hetero-auxin, is caused by a beneficial effect of the hetero-auxin on the respiration.

It is, however, still possible that hetero-auxin exerts an influence on the exosmosis of substances which perhaps will have but little influence on the conductivity of the water; it is conceivable that e.g. an extra exosmosis of sugars occurs under the influence of hetero-auxin. To determine this, Fehling's test-solution is used as a reagent on the evaporated hetero-auxin solution at the end of each two-days' period. This reaction, however, yields in every case a negative result. This is also the case with the hetero-auxin solution, which is never renewed during the test, so that the discs remain in it for 8 days instead of for 2 days. (§ 4 of this chapter). Here, too, no sugars are found with Fehling's solution in the evaporated solution at the end of the experiment.

The conclusion, I think, is justified that the favourable influence of the hetero-auxin on the loss of dry substance is actually a favourable influence on the respiration. Respiration-tests, by determining the consumption of oxygen or the production of carbonic acid, may throw light on this.

If, then, we assume that the extra loss of dry weight occurring under the influence of the hetero-auxin, is due to the respiration of carbo-hydrates, then the loss of one gramme-molecule (180 g) of glucose will result (as has already been discussed on page 18) in a loss of fresh weight of 6 gramme-atoms C, that is $6 \times 12 = 72$ g. That is, therefore, 2/5 of the dry weight lost.

An extra respiration of approx. 10 to 12 mg dry substance owing to hetero-auxin in the concentration 1 to 10^6 therefore makes a difference of $2/5 \times 10 =$ approx. 4 mg in the fresh weight, that is approx. 0.16%, since the fresh weight of 10 discs together is approx. 2500 mg. The difference is thus not great, at any rate not for this concentration.

In the preliminary publication (REINDERS 1938) a calculati was

made with every test as to how much the extra loss of fresh weight is owing to the extra respiration under the influence of hetero-auxin while then the actual extra water-intake of the hetero-auxin series is shown in the last column of table IV.

For the concentration 1 to 10^5 the extra respiration is approx. 50 mg more than that of the control. That means, therefore, an influence on the fresh weight of $2/5 \times 50 =$ approx. 20 mg. That is thus approx. 0.8 %, as 10 discs together have a fresh weight of approx. 2500 mg. A difference of this magnitude might account for the inhibition of the water-intake in the first few days in some cases, but usually the difference in weight between the control-dices and the discs in the hetero-auxin solution is greater than 1 %. It is also assumed that the difference in respiration will manifest itself in its full strength in these first few days, which is very unlikely to be the case. We revert to this point in § 5. It will there be seen that the influence on the respiration in the first few days practically is not evident at all; this occurs only in the further course of the experiment.

As is seen by the course of the curve, there is therefore an inhibitory effect on the water-intake, which co-operates with a favourable one, the inhibitory effect being most manifest at the beginning of the test. This inhibitory effect is not accounted for by the extra loss of dry weight as a result of the effect of the hetero-auxin on the respiration. The nature of this inhibitory influence remains therefore unknown.

In addition to the influence of hetero-auxin on water-intake and on the respiration, the influence on size of cells and cell-division has also to be discussed. The discs of the hetero-auxin series, at the end of the experiment, make the impression of being somewhat thicker than the discs of the control, while the cells also seem somewhat larger when examined microscopically. This in itself is not surprising, for the discs in the hetero-auxin solution have also taken in so much more water, for which room has to be found. In a few cases, but not by any means in all the tests, the cells of the dividing cell-layer are somewhat more strongly stretched vertically on the surface of the discs in the case of the discs of the hetero-auxin series than is the case with the discs of the control series. This is observed only in tests with the concentration 1 to 10^5. In the tests with the concentration 1 to 10^6 this phenomenon is not observed.

If we notice the cell-divisions in the cell-layers under the cut surface, we see that as a general rule fewer cell-divisions have occurred in the case of the hetero-auxin solution than with the discs of the control in water. This is so with all the concentrations used,

1 to 10^5, as also 1 to 10^6 and 1 to 10^7. In these concentrations and under the given testconditions hetero-auxin apparently has no favourable effect on the cell-division; on the contrary. JOST (1935) states that hetero-auxin in the concentration 1 to 1000 and 1 to 10.000 favourably affects the cell-division, while it has a favourable effect on the cell-elongation in the concentration 1 to 10^8.

§ 3. The influence of infiltration of the hetero-auxin solution.

In this § a few experiments are discussed, in which the hetero-auxin solution is infiltrated in the tissue. Three series of 10 discs with the same average fresh weight are composed in the usual way. Series A is given distilled water. Series B is given hetero-auxin solution 1 to 10^6, added in the ordinary way. With series C the hetero-auxin solution, also of 1 to 10^6, is infiltrated. That is done in the usual manner (p 19). With all of these the hetero-auxin solution and the water of the control is renewed every other day, after each observation.

It is seen that there is no difference between series B and series C. In both cases the water-intake under the influence of the hetero-auxin is very much strengthened in comparison with the control, and equally so, no matter whether the hetero-auxin solution in the tissue is infiltrated or given in the ordinary manner. With one exp. e.g. the water-intake in 8 days of series B and C amounts to 29.6 % resp. 30.1 %, while that of the control series is 19.3 %; with another experiment the water-intake of B and C is 31.0 % resp. 28.8 %, while that of the control is only 23.0 % of the initial fresh weight.

§ 4. The influence of hetero-auxin, when the solution is not renewed during the experiment.

In the previous tests the discs, as is always done in this investigation, are supplied with a fresh hetero-auxin solution, or, as the case may be, water with the control, after every observation, that is, every second day.

In this § a few experiments are discussed, in which the hetero-auxin solution is not renewed. Hetero-auxin in a concentration of 1 to 10^6 is used. Series A is given distilled water, which is renewed in the normal manner. Series B is given hetero-auxin, also renewed in the usual way. Series C is likewise given hetero-auxin, but this is not renewed during the whole of the experiment. The discs of series C, therefore, remain during the entire experiment, 8 days, in the same solution. At the end of the test the dry weight is determined. In the season 1938—1939 a fourth series (D) is taken, the initial dry weight of which is determined, so that here the respiration

TABLE 17.

The influence of hetero-auxin, if the solution is not renewed during the course of the experiment.
Series A: distilled water.
Series B: hetero-auxin 1 to 10^6.
Series C: hetero-auxin 1 to 10^6, the solution is not renewed during during the course of the experiment.
Fresh weight and dry weight are stated in mg for 10 discs together.

exp. no.		initial fresh weight	water-intake as increase in % of initial fresh weight after:				dry weight mg		respiration
		mg	2	4	6	8 days	initial	final	mg
283 (31-5-'38)	A	2665.0	9.0	14.1	17.1	19.2	—	301	—
	B	2666.3	8.5	24.0	28.4	31.2	—	269	—
	C	2666.2	8.4	24.6	29.0	31.6	—	293	—
289 (2-7-'38)	A	2610.4	8.9	13.4	16.7	19.0	—	310	—
	B	2609.7	8.5	17.7	21.4	23.1	—	313	—
	C	2609.0	8.7	16.4	19.5	21.5	—	310	—
341 (14-1-'39)	A	2353.1	5.5	11.3	15.4	17.2	—	330	46
	B	2353.4	7.9	19.4	25.6	28.3	—	324	52
	C	2352.7	8.7	17.4	24.3	28.2	—	315	61
	D	2353.9	—	—	—	—	376	—	—
353 (28-1-'39)	A	2424.5	9.3	16.6	21.6	23.8	—	343	54
	B	2423.8	9.5	21.6	34.5	42.5	—	322	75
	C	2423.8	9.7	20.2	25.2	27.6	—	342	55
	D	2425.1	—	—	—	—	397	—	—
368 (17-2-'39)	A	2491.4	7.0	12.2	15.0	17.1	—	349	79
	B	2489.2	7.8	21.3	25.2	27.4	—	348	80
	C	2489.4	8.4	18.4	21.6	23.9	—	351	77
	D	2493.1	—	—	—	—	428	—	—
381 (4-3-'39)	A	2417.9	6.9	11.3	15.1	16.9	—	329	66
	B	2417.3	7.5	19.1	25.6	28.9	—	315	80
	C	2417.4	7.6	14.7	20.0	23.1	—	341	54
	D	2416.3	—	—	—	—	395	—	—

in mg of dry substance is known. All these tests are summarized in table 17.

It will be seen that the influence of hetero-auxin in the normal case, that is to say, when it is renewed after each observation (series B), is without exception favourable to the water-intake and usually fairly strong. The influence of a hetero-auxin solution which is only added once, at the beginning of the test, and which is not renewed (series C), also has a favourable effect on the water-intake, but to

a les extent than if the solution is renewed. In test 283 and in test 341 the influence of series C (hetero-auxin not renewed) is practically as strong as that in series B; in all the other cases the difference is greater.

The influence of hetero-auxin on the respiration is less regular. In most cases the hetero-auxin, when it is constantly renewed (series B), acts favourably on the respiration, in the usual manner. Furthermore there are two tests, nos. 289 and 368, in which the hetero-auxin evidently has not the slightest effect on the respiration, either in series B or in series C. The influence of hetero-auxin solution added once only, and which is not renewed (series C), is, however, very unequal. The respiration is like that of the control, as well as inhibited or promoted in comparison with that of the control. In the case of promotion it acts both more and less favourably than when the hetero-auxin solution is renewed regularly.

We see, therefore, that the influences which the hetero-auxin exerts on the water-intake and on the respiration, may occur fairly independently of each other. In cases in which the influence of the hetero-auxin on the respiration is lacking, there is none-the-less a distinct promotion of the water-intake, e.g. in tests 289 and 368. The same applies to series C in test 353, while in test 381 with series C the respiration is inhibited and the water-intake, on the other hand, is distinctly promoted.

§ 5. The influence of the moment at which, and the period during which, the hetero-auxin is applied.

In § 1 of this chapter we have seen that in a certain sense there is a relation between the strength of the influence of hetero-auxin on the respiration and the strength of the influence on the water-intake, the concentration 1 to 10^7 having practically no effect, either on the water-intake or on the respiration, the concentration 1 to 10^6 having a distinct influence, while the concentration 1 to 10^5 has a somewhat stronger effect on both processes, especially, however, on the respiration.

Whether there is a connection, however, in the sense that with the increased energy which the tissue will have acquired through the stronger respiration, the water-intake now also occurs to a greater extent, is not yet certain, and is indeed unlikely, according to the tests in the previous §. In these tests it happens that hetero-auxin in certain cases has the normal favourable effect on the water-intake, while the influence on the respiration may be lacking or even inhibitory.

This problem will be further illustrad teby the following experi-

ments. In these cases hetero-auxin solution is given, during a certain number of days. In one case at the beginning of the experiment, while afterwards, for the rest of the time, distilled water is applied. In the other case there is first a period of a few days in distilled water, while after this is hetero-auxin applied. The water-intake and the respiration of in both cases are now compared with those of the controls. The controls employed are one continuously in water, the other in hetero-auxin solution for the entire duration of the test. Hetero-auxin in only one concentration, of 1 to 10^6, is used. This is done in the following way.

A number of series of 10 discs of equal average fresh weight are selected in the usual manner. Series A is given distilled water, which is renewed in the usual way every other day. Series B is given hetero-auxin solution 1 to 10^6, and this is likewise renewed in the ordinary manner every second day. Series C is given a hetero-auxin solution of 1 to 10^6 during the first two days, after which this is replaced by distilled water. Also with the following renewals distilled water is applied. Series D is given distilled water for the first two days, after which hetero-auxin solution 1 to 10^6 is substituted. With the following renewals hetero-auxin solution is also invariably applied. The initial dry weight of series E, if present, is determined.

The results of these tests are summarized in table 18. A picture of the course of the water-intake in these tests is given by fig. 10, in which test 275 is represented graphically. It is seen that hetero-auxin, in the normal case, in which it is applied during the entire duration of the experiment (series B), invariably, without exception has a favourable effect on the water-intake. The strength of the influence may differ very greatly with different tests. On the average the strength of the influence in 1937—1938 and that in 1938—1939 are practically the same.

With series C, first two days hetero-auxin solution and then always water, the water-intake is in all cases, without exception, distinctly promoted in comparison with the control (A) in water. But in all cases less strong, usually much less strong, than the influence of series B. Only in two tests of 1939, tests 387 and 392, where the influence of hetero-auxin in the normal case (B) is weak, is the influence of series C during 6 days practically as strong as that of B; only in the last few days the difference manifests itself plainly. In the converse case, with series D, first two days distilled water and then invariably hetero-auxin solution, we see the normal typical course of the curve, although to a much weaker degree. Here, too, no promotion of the water-intake occurs in the two first days, during which hetero-auxin solution is received (the third and fourth days

TABLE 18.

Water-intake and respiration influenced by hetero-auxin, applied during various periods.

Series A: continuously distilled water.
Series B: continuously hetero-auxin 1 to 10^6.
Series C: during the first two days hetero-auxin 1 to 10^6, afterwards distilled water (at ↓).
Series D: during the first two days distilled water, afterwards hetero-auxin 1 to 10^6 (at ↑).
Series E: determination of initial dry weight.

Fresh weight and dry weight are stated in mg for 10 discs together. The water-intake is stated as increase of fresh weight in % of the initial fresh weight. The respiration is stated as loss of dry substance, in mg for 10 discs together.

exp. no.		initial fresh weight	water-intake as increase in % of initial fresh weight after:				dry weight mg		respiration
		mg	2	4	6	8 days	initial	final	mg
253 (17-5-'38)	A	2641.4	10.1	17.0	21.8	24.2	—	370	—
	B	2642.5	8.8	21.3	27.1	30 6	—	361	—
	C	2642.4	9.0↓	18.8	24.6	27.5	—	375	—
256 (18-5-'38)	A	2730.8	7.9	13.1	17.1	19.5	—	358	—
	B	2719.1	7.9	19.6	25.7	29.0	—	342	—
	C	2721.7	7.9↓	16.3	20.8	23.7	—	363	—
228 (9-4-'38)	A	2612.3	10.3	18.5	23.8	26.2	—	366	—
	B								
	D	2613.3	10.2↑	17.0	23.6	27.0	—	353	—
240 (26-4-'38)	A	2717.9	10.1	20.9	25.8	28.4	—	360	—
	B	2718.0	8.8	28.2	36.8	40.2	—	351	—
	D	2716.9	10.6↑	21.2	29.2	32,1	—	346	—
275 (8-6-'38)	A	2516.3	10.5	16.6	20.1	22.0	—	320	—
	B	2517.7	9.3	23.4	29.9	32.2	—	310	—
	C	2516.4	9.6↓	19.2	23.3	25.3	—	321	—
	D	2519.1	10.1↑	15.9	21.8	24.5	—	312	—
387 (14-3-'39)	A	2479.9	9.3	14.0	18.0	20.2	—	346	77
	B	2480.0	9.3	17.1	20.7	24.7	—	334	89
	C	2479.1	9.9↓	16.5	20.7	22.9	—	354	69
	D	2479.3	8.8↑	13.7	17.5	21.9	—	343	80
	E	2480.4	—	—	—	—	423	—	—
389 (18-3-'39)	A	2445.7	7.9	12.5	16.0	17.6	—	327	66
	B	2445.8	7.6	16.3	19.8	22.8	—	339	54
	C	2445.8	8.1↓	17.4	21.1	23.2	—	336	57
	D	2446.0	7.5↑	11.6	14.4	16.4	—	339	54
	E	2444.9	—	—	—	—	393	—	—
390 (21-3-'39)	A	2471.8	6.8	11.9	14.4	15.4	—	349	57
	B	2471.0	7.5	16.5	20.1	22.8	—	339	67
	C	2470.6	7.2↓	14.7	18.2	20.5	—	348	58
	D	2472.0	6.6↑	11.0	15.5	18.5	—	338	68
	E	2470.0	—	—	—	—	406	—	—
391 (25-3-'39)	A	2481.6	7.3	12.4	14.7	16.5	—	336	66
	B	2480.3	8.5	21.4	24.2	27.0	—	316	86
	C	2480.3	9.0↓	17.4	20.3	22.8	—	344	58
	D	2481.0	7.9↑	13.2	15.4	17.2	—	334	68
	E	2482.2	—	—	—	—	402	—	—
392 (28-3-'39)	A	2431.0	7.7	13.0	17.5	20.4	—	354	69
	B	2430.7	7.3	15.1	19.3	25.5	—	352	71
	C	2430.5	7.9↓	14.8	19.4	22.0	—	359	64
	D	2430.9	6.9↑	12.0	15.8	20.6	—	341	82
	E	2430.4	—	—	—	—	423	—	—

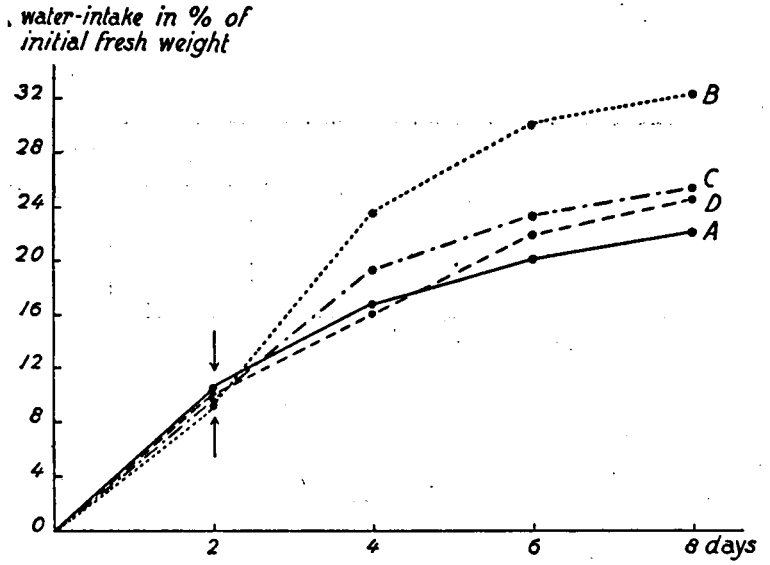


Fig. 10. Water-intake of 4 series of 10 discs each, of the same potato, from aerated distilled water and from aerated hetero-auxin solution. (exp. 275 of table 18).
A: distilled water.
B: hetero-auxin 1 to 10^6.
C: first two days hetero-auxin 1 to 10^6, from ↓ distilled water.
D: first two days distilled water, from ↑ hetero-auxin 1 to 10^6.

from the beginning of the experiment). After that the promotion becomes distinctly evident, but the influence remains comparatively feeble. In all the tests, without exception, the influence on the water-intake of series D, which has had hetero-auxin for 6 days, is slighter than that of series C, which has had hetero-auxin for two days only. But with series C those are the first two days of the experiment, while series D received hetero-auxin for the last 6 days. Evidently, therefore, it makes a great difference, during which period of the experiment the hetero-auxin is given. The influence of hetero-auxin on the water-intake evidently, therefore, makes itself felt most strongly when it is given at the beginning of the experiment.

In some tests of the season 1938—1939 the p_H and the resistance are also measured. These data are given in a separate table, table 16, and the results have been dealt with in § 2. From these it is found that the extra loss of dry substance, as it occurs with the discs in the hetero-auxin solution, is not due to an extra exosmosis, so that the

TABLE 19. (experiments of table 18).

Respiration of potato discs under the influence of hetero-auxin 1 to 10^6, applied during various periods. The respiration of series A is stated as loss of dry substance for 10 discs together. Duration of the experiment 8 days.

exp. no.	respiration mg	extra respiration in mg for 10 discs together		
	A invariably distilled water	B invariably hetero-auxin	C first 2 days hetero-auxin, then water	D first 2 days water, then hetero-auxin
253 (17-5-'38)	—	9	−5	—
256 (18-5-'38)	—	16	−5	—
228 (9-4-'38)	—	—	—	13
240 (26-4-'38)	—	9	—	14
275 (8-6-'38)	—	10	−1	8
387 (14-3-'39)	77	12	−8	3
389 (18-3-'39)	66	−12	−9	−12
390 (21-3-'39)	57	10	1	11
391 (25-3-'39)	66	20	−8	2
392 (28-3-'39)	69	2	−5	13

probability of an influence on the respiration is very great. The fall in the resistance at the beginning and the subsequent rise with the series C and D agree exactly, as is the case with the series A and B; no special remarks are called for.

With regard to the respiration, we find that hetero-auxin, in the normal case, in which it is applied during the entire duration of the test (series B), always acts favourably on the respiration in 1937—1938. (Table 19). In the season 1938—1939 (tests 387 to 392 inclusive) there are a few exceptions. In test 389 the hetero-auxin did not promote the respiration, but in contrast with this the loss of dry substance under the influence of the hetero-auxin is slighter than that of the control. And in test 392 no influence is noticeable; the loss of dry substance of series B is only 2 mg larger than that of the control in water (A). The influence of the hetero-auxin on the water-intake is also slight in this test.

Whether it has any connection with this is difficult to say, but I would point out that at the beginning of test 389 there is no difference in the resistance between the fresh water and the fresh hetero-auxin solution. This is the case at the beginning of the third two-days' period also; there the resistance of the fresh hetero-auxin solution is even somewhat greater than that of the fresh water. (Table 16). And in test 392 the resistance at the beginning of the third, and also

at the beginning of the fourth two-days' period, both of the fresh-water and of the fresh hetero-auxin solution, is abnormally low. Between the resistance of the water and that of the hetero-auxin solution there is but little difference, and at the beginning of the fourth period practically none at all. The p_H at the beginning of the fourth period is also uncommonly high, viz. 5.0, while the normal is 4.5.

The remarkable point about test 389 is that the respiration is inhibited by the hetero-auxin exceptionally, that is, the loss of dry substance under the influence of the hetero-auxin is slighter than that of the control. But never-the-less the water-intake is promoted in the normal way, even fairly strongly, and at any rate not exactly feebly, by the hetero-auxin. This indicates that the influences which hetero-auxin has on the water-intake and on the respiration must be fairly independent of each other; in any case there is no direct connection.

With series C, first two days hetero-auxin and afterwards water only, matters stand as follows (table 19). With the tests in 1937—1938, tests 253, 256, and 275, practically no influence is noticeable. The respiration is practically like that of the control. As a matter of fact, the dry weight at the end of the test with series C is in all cases a trifle greater than that of the control, viz. in tests 253 and 256 both 5 mg, and in test 275 it is 1 mg greater, so that so much less dry material has been respired. The tests in the season 1938—1939 are quite in agreement with this. Nowhere a promotion of the respiration is found. In all the tests the final dry weight of series C is either practically the same as that of the control (test 390), or it is greater, so that the respiration has been somewhat less. In tests 387 and 391 the respiration with series C was in both cases 8 mg less than that of the control, in test 389 the difference is 9 mg and in test 392 it is 5 mg.

The uniformity in these tests, respiration invariably slighter with series C than with the control, makes it very probable that this difference is a real one. The differences with the tests in 1937—1938, only three in number, are only 5 mg, and might still fall within the limits of variability. But in the season 1938—1939 it is confirmed, and the differences are also somewhat larger. In test 391 the inhibition with series C, e.g., is 8 mg, where hetero-auxin, if applied continuously (series B), gives such a strong promotion, viz. 20 mg extra loss of dry weight. We may therefore suppose that it is true that hetero-auxin, if given only during the first days of the experiment (series C), does not promote, but does really somewhat inhibit the respiration.

The remarkable thing is therefore the contrast between the effect on the water-intake and that on the respiration. The water-intake with series C is very distinctly promoted in comparison with that of the control, but more feeble than in series B, which is not surprising. But the respiration shows, instead of a promotion, an inhibition, though not a strong one, in comparison with that of the control. The effects on the water-intake and on the respiration thus behave fairly independently in this case also. This points to the connection not being so very direct.

With series D, first two days distilled water and then always hetero-auxin solution, we find the following (table 19). When the dry weight at the end of the test is determined, it is found that here, in most cases, much more dry substance is respired than with the control, series A, in water. Practically the same additional amount is respired as in series B, where the discs are give hetero-auxin during all the 8 days. The separate tests of the season 1938—1939 however, give, very irregular results. In some cases the extra respiration of series D is much smaller than with series B, e.g. tests 387 and 391. The respiration of series D scarcely differs at all in these two tests from that of series A. But in other cases the extra respiration of series D is stronger (test 240), and even much stronger (test 392) than that of series B.

But we must take into account that the variability of the dry weight with equivalent series with the same average initial dry weight may diverge as much as + and — 5 mg.(Chapter I, § 2). On these comparatively small differences this, of course, is a rather considerable amount. In any case these figures (tables 18 and 19) give a clear and convincing picture of the difference between the series C and D.

The differences between the series C and D also show that the influences which hetero-auxin exerts on the water-intake and on the respiration behave fairly independently of one another. With series C, first two days hetero-auxin solution and then water, the water-intake is fairly strongly promoted as compared with the control although more feeble than with series B, where hetero-auxin was applied during all the 8 days. But instead of a promotion of the respiration, a weak inhibition is to be noted, the loss of dry substance being in general somewhat slighter than that of the control. With series D, first two days water and then hetero-auxin, the influence on the water-intake, on the contrary, is only very feeble, weaker than with series C, while the respiration is strongly promoted. One gets the impression that this promotion, however irregular the figures may be, is about as strong as with series B, but perhaps a trifle less.

§ 6. The influence of hetero-auxin under anaerobic conditions.

In this § a few tests will be discussed, the object of which is to determine whether hetero-auxin exerts any influence in an environment without oxygen, and, if so, of what nature that influence is.

Hetero-auxin in only one concentration, of 1 to 10^6, is employed. A number of series of 10 discs with the same average initial fresh weight are selected. Series A is given distilled water, and is aerated in the ordinary manner with air. Series B is given hetero-auxin and is likewise aerated in the ordinary way with air. Series C is given distilled water, and remains in an environment without oxygen, namely in the Mc Intosh and Fildes' anaerobic jar (cf. chapter II, § 1). Series D is given hetero-auxin and also remains in an environment without oxygen. The test-period is 4 days. The observation is made, as in all other tests (as is the custom), every other day. The hetero-auxin solution and the distilled water are renewed once, viz. after the first observation, i.e. after 2 days.

A number of experiments are carried out in March/April 1939, fairly late in the spring, and these tests are repeated in November/December 1939. All these tests are summarized in table 20.

The second series of tests are therefore made shortly after the new crop. They are to be preferred to the first group, as these potatoes are in a better condition, and as with this series, as we shall see, no loss of weight occurs in an anaerobic environment in the second period and no extra exosmosis in comparison with the discs with air aeration.

With regard to the water-intake, in nearly all the tests the favourable effect of the hetero-auxin on the water-intake when aerated with air, is found to make itself evident after 4 days. The increase in the fresh weight of series B with hetero-auxin is dictinctly greater after 4 days than that of series A, in water. This is not the case in an environment without oxygen. There is no difference between the series C and D, the curves which show the course of the increase in fresh weight coinciding completely. In an anaerobic environment there is therefore no influence of the hetero-auxin on the water-intake. This is seen most plainly in fig. 11.

With both series in an anaerobic environment, series C and D, there is still a slight increase in weight in the first two-days' period in all the tests, as is also noted in chapter II. In the next two days, however, there is a difference between the tests in the spring and those in the autumn. With the tests in the autumn (potatoes in healthy condition), the fresh weight now remains constant, or there is even a slight further increase (test 434). With the experiments in

TABLE 20.

Water-intake and respiration of potato discs influenced by hetero-auxin in an anaerobic environment and with air- aeration.

Series A: distilled water, aerated with air.
Series B: hetero-auxin 1 to 10^6, aerated with air.
Series C: distilled water, in anaerobic environment.
Series D: hetero-auxin 1 to 10^6, in anaerobic environment.

Fresh weight and dry weight are stated in mg for 10 discs together. The respiration is stated as loss of dry substance in mg for 10 discs together.

exp. no.		initial fresh weight	water-intake as increase in % of initial fresh weight after:				dry weight mg		respiration
		mg	1	2	3	4 days	initial	final	mg
March–April 1939									
383 (8-3-'39)	A	2433.9	5.0	9.1	11.5	14.1	—	362	33
	B	2434.4	4.4	9.3	15.0	17.4	—	355	40
	C	2434.3	3.3	3.5	3.0	2.4	—	368	27
	D	2434.3	3.0	3.6	2.8	2.3	—	371	24
	E	2435.1	—	—	—	—	395	—	—
388 (15-3-'39)	A	2399.7	5.9	9.8	—	14.9	—	363	29
	B	2398.9	4.9	8.6	—	18.3	—	348	44
	C	2399.3	3.5	3.0	—	2.2	—	360	32
	D	2399.1	3.6	3.0	—	1.4	—	376	16
	E	2399.9	—	—	—	—	392	—	—
402 (14-4-'39)	A	2416.0	—	10.5	—	15.6	—	361	56
	B	2415.5	—	10.2	—	22.9	—	364	53
	C	2415.6	—	4.2	—	2.7	—	384	33
	D	2415.6	—	4.2	—	2.6	—	383	34
	E	2415.1	—	—	—	—	417	—	—
405 (20-4-'39)	A	2455.2	—	8.8	—	15.3	—	411	57
	B	2454.9	—	8.6	—	16.5	—	408	60
	C	2454.6	—	3.6	—	1.6	—	430	38
	D	2454.3	—	3.4	—	−1.2	—	418	50
	E	2455.9	—	—	—	—	468	—	—
407 (26-4-'39)	A	2493.9	—	9.9	—	15.8	—	393	42
	B	2493.8	—	9.1	—	16.1	—	387	48
	C	2493.1	—	3.7	—	2.8	—	406	29
	D	2493.4	—	4.3	—	3.4	—	395	40
	E	2494.9	—	—	—	—	435	—	—
Nov.–Dec. 1939									
426 (18-11-'39)	A	2293.4	—	7.5	—	11.2	—	322	41
	B	2293.0	—	9.1	—	18.3	—	312	51
	E	2292.2	—	—	—	—	363	—	—
434 (30-11-'39)	A	2434.0	—	8.0	—	14.8	—	425	59
	B	2434.2	—	10.1	—	22.0	—	419	65
	C	2434.6	—	3.7	—	5.3	—	453	31
	D	2434.6	—	4.1	—	5.0	—	463	21
	E	2435.3	—	—	—	—	484	—	—
439 (5-12-'39)	A	2364.5	—	11.3	—	18.0	—	362	41
	B	2364.0	—	11.3	—	22.2	—	348	55
	C	2365.6	—	3.2	—	3.2	—	379	24
	D	2365.6	—	2.8	—	3.0	—	369	34
	E	2366.4	—	—	—	—	403	—	—
441 (9-12-'39)	A	2296.7	—	9.9	—	17.8	—	399	41
	B	2296.5	—	9.1	—	18.5	—	387	53
	C	2296.6	—	2.6	—	2.5	—	404	36
	D	2296.5	—	2.6	—	2.9	—	399	41
	E	2295.0	—	—	—	—	440	—	—

spring, however, the weight in the second two-days' period does not remain altogether constant; there is a slight or somewhat stronger decline in the fresh weight, which, however, is exactly the same for the series C and D. This is probably due to the already advanced season, as a result of which the tissue of the potatoes was in a less good condition, and not so well able to stand a period of 4 days in an environment without oxygen. It is consequently possible that the figures for the dry weight in an anaerobic environment are more difficult to interpret. We must allow for the possibility of there having been an extra exosmosis.

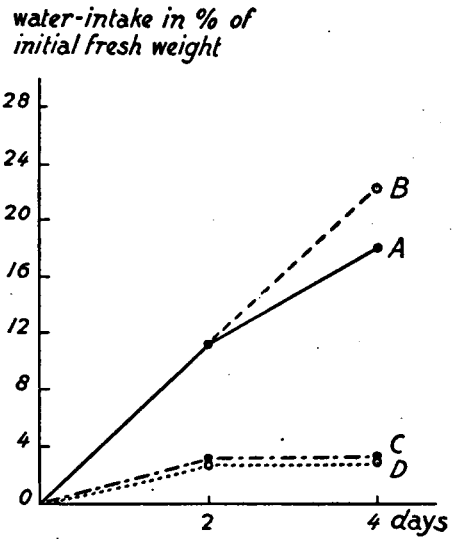


Fig. 11. The water-intake influenced by hetero-auxin in an anaerobic environment and with air-aeration. (exp. 439 of table 20).
A: distilled water, aerated with air.
B: hetero-auxin 1 to 10^6, aerated with air.
C: distilled water, in anaerobic environment.
D: hetero-auxin 1 to 10^6, in anaerobic environment.

Let us now first pay closer attention to the determination of the conductivity, as this was carried out in all these experiments. In all the tests the resistance of the old and of the new water, and also that of the old and of the new hetero-auxin solution is invariably determined at the beginning of the test, after 2 days, and at the end of the test, after 4 days (table 21).

If we now first pay attention to the figures of the determinations of the resistance of the tests in Nov./Dec., that is, with material in healthy condition, we find that a pronounced fall in the resistance has occurred in the first two-days' period with all three tests. With test 434 the fall in the resistance in an anaerobic environment is less marked than with aeration with air; in test 439 it is also somewhat less pronounced. In test 441 it is practically the same for all four series. In the second two-days' period, a fall in the resistance is also to be noted, but along the whole line it is less strong than in the first period. In test 434 the fall in the resistance is less marked in

TABLE 21.

Exosmosis under anaerobic conditions and with air-aeration, in water and in a hetero-auxin solution. Determinations of p_H and of electrical resistance of the experiments of table 20.

Series A: distilled water, aerated with air.
Series B: hetero-auxin 1 to 10^6, aerated with air.
Series C: distilled water, in anaerobic environment.
Series D: hetero-auxin 1 to 10^6, in anaerobic environment.

The electrical resistance is stated in Ω.

exp. no.		beginning of experiment		after 2 days				after 4 days	
		p_H	resistance	p_H old	p_H new	resistance old	resistance new	p_H old	resistance old
March-April 1939									
exp. 383	A	—	170 000	—	—	44 000	210 000	—	90 000
	B	—	130 000	—	—	40 000	160 000	—	62 000
	C	—	170 000	—	—	39 000	210 000	—	64 000
	D	—	130 000	—	—	40 000	160 000	—	64 000
exp. 388	A	—	140 000	—	—	50 000	150 000	—	100 000
	B	—	110 000	—	—	41 000	130 000	—	80 000
	C	—	140 000	—	—	35 000	150 000	—	60 000
	D	—	120 000	—	—	34 500	130 000	—	56 000
exp. 402	A	4.5	260 000	7.0	4.5	45 000	230 000	6.5	74 000
	B	4.5	200 000	7.0	4.5	45 000	180 000	6.5	56 000
	C	4.5	260 000	5.5	4.5	46 000	230 000	5.5	34 000
	D	4.5	200 000	5.5	4.5	48 000	180 000	5.5	34 000
exp. 405	A	4.5	270 000	7.0	4.5	39 000	260 000	6.5	67 000
	B	4.5	190 000	7.0	4.5	35 000	200 000	6.5	78 000
	C	4.5	270 000	6.0	4.5	42 000	260 000	5.5	34 000
	D	4.5	190 000	6.0	4.5	40 000	200 000	5.5	25 000
exp. 407	A	4.5	255 000	7.0	4.5	46 000	200 000	6.5	68 000
	B	4.5	200 000	7.0	4.5	42 000	160 000	6.5	70 000
	C	4.5	255 000	5.5	4.5	48 000	200 000	5.5	38 000
	D	4.5	200 000	6.0	4.5	50 000	160 000	6.0	44 000
Nov.-Dec. 1939									
exp. 434	A	4.5	320 000	7.0	4.5	46 000	250 000	6.5	85 000
	B	4.5	200 000	7.0	4.5	42 000	170 000	6.5	53 000
	C	4.5	320 000	5.5	4.5	75 000	250 000	5.5	100 000
	D	4.5	200 000	5.5	4.5	75 000	170 000	5.5	90 000
exp. 439	A	4.5	210 000	7.0	4.5	41 000	220 000	6.5	100 000
	B	4.5	160 000	7.0	4.5	40 000	160 000	7.0	60 000
	C	4.5	210 000	5.5	4.5	48 000	220 000	5.5	75 000
	D	4.5	160 000	5.5	4.5	48 000	160 000	5.5	70 000
exp. 441	A	4.5	240 000	6.5	4.5	47 000	210 000	5.5	85 000
	B	4.5	180 000	6.5	4.5	47 000	160 000	6.0	90 000
	C	4.5	240 000	5.5	4.5	45 000	210 000	5.5	80 000
	D	4.5	180 000	5.5	4.5	46 000	160 000	5.0	80 000

this period in both series in an anaerobic environment, C and D, than with aeration with air; in the two other tests little can be said of this, the fall in the resistance being practically equal in an anaerobic environment and with air-aeration. This shows, therefore, that there has been no stronger exosmosis in an anaerobic environment with these tests with potatoes in healthy condition than in the series with air-aeration, either with water (C) or with hetero-auxin (D.) But there is a fairly marked loss of dry substance in an anaerobic environment, although slighter than with air-aeration.

In chapter I, § 2, the probability has been demonstrated that the loss of dry substance, occurring in these tests with potato discs, is actually due to respiration. Since it is found here that there is no stronger exosmosis in an anaerobic environment than in the ordinary case of aeration with air, we are equally justified assuming respiration in this case also.

If we now turn to the tests made in the previous spring, in March/April, we find that here, too, a marked decline in the resistance occurs in the first two-days period, and also to practically the same extent in als 5 tests. In the second two-days' period, however, there is a distinct difference between series A and B on the one hand and series C and D in an environment without oxygen on the other. There is a fall in the resistance in both of them, but in A and B it is much less pronounced than in the first period. In C and D, however the decline is equally great or even greater than in the first period; only with exp. 383 and exp. 388 it is less strong than in the first period, but in that case at any rate stronger than in series A and B. This points to a stronger exosmosis in an anaerobic environment in these tests, at least in the second period. Probably this is attributable to the less favourable condition of the material. This is also indicated by the loss in fresh weight, which occurs in the second period. Both phenomena are, however, the same in series C and D, that is with as well as without hetero-auxin. It will therefore be wise, when discussing the results in an anaerobic environment of this series of tests not, for the moment, to speak of respiration, but only of loss of dry weight.

Turning to the loss of dry substance in the tests in table 20, we find the following. Between series A and B, which are aerated with air, there is a difference in all the tests in this sense, that series B (with hetero-auxin) has lost more dry substance by respiration than series A. This difference is somewhat more distinctly pronounced in the tests with the material in healthy condition, i.e. the autumn-tests, than with the tests in the spring. The average of all the 5 tests made in spring (tests 383 to 407 inclusive) is found to be a respiration of

TABLE 22 (experiments of table 20).

Respiration influenced by hetero-auxin, with air-aeration and in anaerobic environment. Duration of the experiment 4 days.
The respiration is stated as loss of dry substance, in mg for 10 discs together and in % of the initial dry weight.

exp. no.	air-aerated				anaerobic			
	distilled water		hetero-auxin 1 to 10^6		distilled water		hetero-auxin 1 to 10^6	
	A		B		C		D	
	mg	%	mg	%	mg	%	mg	%
March—April 1939								
exp. 383	33		40		27		24	
„ 388	29		44		32		16	
„ 402	56		53		33		34	
„ 405	57		60		38		50	
„ 407	42		48		29		40	
average of these 5 experiments	43	10.2	49	11.6	32	7.6	33	7.8
November–December 1939								
exp. 426	41		51		—		—	
„ 434	59		65		31		21	
„ 439	41		55		24		34	
„ 441	41		53		36		41	
average of these 3 experiments	47	10.6	58	13.1	30	6.8	32	7.2

43 mg in 4 days in series A and a respiration of 49 mg dry substance in 4 days in series B, both for 10 discs together. The average of all 3 tests made in the autumn (tests 434, 439 and 441) is found to be a respiration of 47 mg in series A, while that in series B averages 58 mg dry substance in this period, both for 10 discs together (table 22).

In an environment without oxygen it is less distinct. The figures for the separate tests are very irregular. It happens both that the respiration (loss of dry weight) of series C (water) is much greater than that of series D (hetero-auxin) and conversely: in some cases, also, there is hardly any difference. The average of all 5 tests made in the spring (tests 383 to 407 inclusive), is found to be a loss of dry weight of 32 mg in 4 days for series C, while this amounts to 33 mg in the same time for series D. This, therefore, seems to indicate that hetero-auxin in an environment without oxygen apparently has no

influence on the loss of dry substance. The average of all 3 tests made in the autumn is found to be a respiration of 30 mg in 4 days for series C, while the respiration of series D, i.e. with hetero-auxin, averages 32 mg in the same period, both for 10 discs together, (table 22).

However irregular the individual tests may be, the conclusion may, in my opinion, be drawn that hetero-auxin in an anaerobic environment apparently has no effect on the respiration.

The loss of dry substance in an anaerobic environment in the tests in the spring and in those in the autumn is found to be approximately the same, if expressed in mg; in both cases it amounts to approx. 30 mg for 10 discs together. Expressed in % of the initial dry weight, the loss of dry substance in an anaerobic environment in the 5 tests that are made in the spring amounts to an average of 7.6 % of the average initial dry weight, while, in the 3 tests made in the autumn, it comes to an average of 6.8% of the average initial dry weight. This is given in table 22, in which the respiration of the series with air-aeration is also given in %. For series A (water) the respiration for the spring-tests is 10.2 % of the average initial dry weight, and for the autumn tests 10.6 % of the average initial dry weight.

As we have seen, a stronger exosmosis occurs in the tests in the spring, according to the determinations of the conductivity, in an anaerobic environment in the second period than with aeration with air, which is not the case with the autumn tests. According to the proportion of the % loss of dry substance, anaerobically and with aeration, in the spring and in the autumn tests, it is probable that this stronger exosmosis is actually the cause of a somewhat greater loss of dry substance, but it is not considerable. Apparently the substances which diffuse out of the tissue only form a comparatively small part of the total amount of dry substance.

We have now seen that there actually does occur indeed no slight degree of respiration in an environment without oxygen, which, however, is considerably slighter than with good aeration. The influence of hetero-auxin on the respiration may now be defined somewhat more precisely. On the slight respiration occurring in the anaerobic environment hetero-auxin has no effect. Hetero-auxin apparently only has an influence on the aerobic part of the respiration as it occurs with good aeration.

Let us now turn our attention to the decline in the fresh weight in an anaerobic environment in the second period of the spring tests. This decline in the fresh weight in the second period is about 1.5 to 2 %, or approx. 40 mg per 10 discs, as the fresh weight of 10 discs together is approximately 2500 mg. Since the total loss of dry

substance is only ± 30 mg, even when there is no extra exosmosis to be taken into account (tests 434 to 441 inclusive, in table 22), this decline must be attributed practically entirely to the renewed loss of water.

Late in the spring of 1939, when the discs are found not to be able to stand a period of 4 days in an anaerobic environment so well, another attempt is made to carry out experiments of shorter duration, viz. 2 days. This is done in the first place with hetero-auxin 1 to 10^6. But it is found that no difference whatever is to be noticed in the dry weight after 2 days in the series A and B, that is, with aeration with air. The respiration of series B, with hetero-auxin, is as great (14 mg) as that of series A, with water (15 mg). Hetero-auxin in the concentration 1 to 10^5 is also used. But with this, too, no difference in dry weight is to be found after 2 days between the series A (water) and B (hetero-auxin) when aerated with air. Moreover this concentration in an anaerobic environment has a deleterious effect, the discs of series D, at the end of 2 days, being flabby, and showing a marked decline in the fresh weight. Hetero-auxin in the concentration 5 to 10^6 is also tried. But here, too, with aeration with air, no difference in dry weight is found to exist between series A and B after 2 days. And in an anaerobic environment this concentration also has a deleterious effect on the discs; a fairly marked decline in the fresh weight is found to have occurred at the end of the experiment, and the discs are flabby.

§ 7. The influence of auxin-a.

In June 1938 a few tests are made with auxin-a, of which 0.5 mg is supplied by the Org. Chem. Lab. at Utrecht [1]). Immediately on receipt of the auxin-a, an experiment is started, while the auxin-a, dissolved in 100 cm^3 distilled water, is kept in the refrigerator as stock-solution. Three series of 10 discs are taken; series A is given distilled water, series B is given auxin-a in the concentration 1 to 10^9, and series C auxin-a 1 to 10^8. The experiment lasts for 4 days, and the solutions are not renewed during this time. The auxin-a is not found to have had any effect. The water-intake of the series with auxin-a and that of the control are absolutely alike, the curves showing the course of the increase in fresh weight run perfectly parallel. At the end of the experiment the water-intake of series A is 13.3 %, of series B 13.1 % and of series C 13.9 % of the initial fresh weight.

1) I here desire to express my thanks to Prof. Dr F. KÖGL, Utrecht, for his kindness in placing the auxin-a at my disposal.

A few days later a second experiment is started with auxin-a in the concentration 0.5 to 10^6. This test also lasts 4 days; after 2 days the auxin-a solution is renewed, as is also the water of the control. Here, too, no influence of the auxin-a on the water-intake is to be found. The curves showing the course of the increase of the fresh weight of the series with auxin-a and the control series coincide entirely. At the end of the experiment the water-intake of series A is 17.7 % and that of series B is 17.5 % of the initial fresh weight.

These tests are carried out very late in the season (June), and it is better, for that reason, not to attach too much importance to the results of these tests. It has been unfortunately impossible to repeat the tests in the winter, as no more auxin-a has been available.

§ 8. Summary.

Hetero-auxin promotes the water-intake of potato discs. Not before two days have passed the promoting influence becomes evident, the strongest effect is to be found in the period from the second to the fourth day.

The concentration 1 to 10^5 has the greatest effect, the influence of the concentration 1 to 10^6 is a trifle weaker, while the effect of the concentration 1 to 10^7 is scarcely noticeable. Stronger concentrations, as 1 to 10.000 and 1 to 1000, are deleterious; the discs become flabby and are soon dying.

Hetero-auxin also exerts a promoting influence on the loss of dry substance. From determinations of the resistance of the external solution and from reacting on sugars in the evaporated external solution, it has appeared that this extra loss of dry weight under the influence of hetero-auxin is not caused by a stronger exosmosis. So probably a promoting influence of the hetero-auxin on the respiration is the cause. The concentration 1 to 10^5 has the greatest effect, the influence of the concentration 1 to 10^6 is considerably less, while the concentration 1 to 10^7 has practically no effect.

The promoting effect of the hetero-auxin, concentration 1 to 10^6, on the water-intake is as strong, when the tissue is infiltrated with the hetero-auxin solution as when it is applied in the usual way.

The favourable influence of the hetero-auxin, concentration 1 to 10^6, on the water-intake is less great, when the hetero-auxin solution is applied at the beginning of the experiment without being renewed afterwards, than when a fresh solution is applied every other day after each observation in the usual way. As to the influence on the respiration, there is no distinct effect in the case when the hetero-auxin solution is not renewed.

The influence on the water-intake of the hetero-auxin, concentration 1 to 10^6, asserts itself strongest when it is applied at the beginning of the experiment. The water-intake is promoted to a higher degree if the potato discs are in the hetero-auxin solution only during the first two days of the experiment, than if they are in the hetero-auxin solution during the following six days of the experiment, while in this case the discs are in distilled water during the first two days.

The respiration is not promoted, on the contrary somewhat inhibited, if hetero-auxin (1 to 10^6) is applied to the discs only during the first two days. When the discs remain in the hetero-auxin solution during the last six days of the experiment, the respiration is promoted about as much as when the hetero-auxin is applied during the whole experiment, that is eight days.

In an anaerobic environment no influence of the hetero-auxin is noticeable, either on the water-intake or on the respiration.

In preliminary experiments auxin-a proves to have no influence on the water-intake.

CHAPTER IV.

THE INFLUENCE OF VITAMIN B_1.

Researches of the last few years (BONNER 1937) have taught that the Vitamin B_1 or aneurin, a substance which is necessary for the growth of lower organisms such as yeast, is also an important factor for root-growth; for the cultivation of excised root tissue it is even indispensable. (WHITE 1937; ROBBINS and BARTLEY 1937; BONNER 1937; BONNER and ADDICOTT 1937). J. BONNER (1937, Science 85) has found, that the concentration 0,2 γ per cm^3 is optimal for the growth of excised pea roots.

The aim of this investigation is to see whether this substance, which has proved to be also an important growth-substance as hetero-auxin, may have an influence on the water absorption with potato discs. A pure chemical product of Merck is used for these experiments. A stock-solution of 10 mg per 100 cm^3 distilled water is kept in the refrigerator at 1° to 2° C, from which the various dilutions are prepared. Two series of 10 discs each are taken of the same potato, one of them in the solution of Vitamin B_1, the other as a control. The external solution is renewed every second day.

In the first place is studied the concentration, that BONNER has found optimal for the growth of excised pea roots, namely 0,2 γ per cm^3, that is 0,1 mg per 500 cm^3 of water. No difference with the control has been stated. In one case the water-intake of the series with aneurin is 20.1 % and that of the control 19.4 % of the initial fresh weight in 4 days; with another experiment the water-intake of the two series is respectively 23.2 % and 23.0 % in 8 days. The concentration 1 mg per 500 cm^3 has no influence either. With two exp.'s the water-intake of the series, to which Vitamin B_1 is added, is 20.6 % and 20.6 % of the initial fesh weight in 4 days, while that of the control is 19.4 % and 20.4 % respectively. With another experiment the water-intake of the series with Vitamin B_1 and the control respectively amount to 25.6 % and 25.8 % of the initial fresh weight in 8 days. Also still stronger concentrations, 5 mg and 10 mg per 500 cm^3, don't have any influence on the water absorption of potato discs. With 5 mg Vitamin B_1 per 500 cm^3 the water-intake is e.g. 20.7 % of the initial fresh weight in 4 days and the water-intake of the control is 20.4 %. With 10 mg per 500 cm^3 the water-intake amounts e.g. to 24.6 % in 8 days, while that of the control is 26.3 %.

So it is clear that the Vitamin B_1 exerts no influence when it is applied quite at the beginning of the experiment. Now it is possible that the tissue at the beginning of the experiment contains a sufficient quantity of the Vitamin B_1 and it may be that after some time a lack makes itself felt. Therefore the vitamin-solution (concentr. 0.5 mg per 500 cm^3) is applied after eight days. During the first 8 days the discs remain in aerated distilled water, just as the control, and the water absorption in that period is 29.6 % of the initial fresh weight for both series. During the following 4 days no difference with the control occurs, the water-intake of the Vitamin B_1 series being 31.7 % and that of the control 32.3 % at the end of the experiment. The phenomenon that the water absorption decreases after some time, is not caused by lack of Vitamin B_1 either.

These experiments are made in Jan./Febr. 1938. In June two experiments are repeated, with which the dry weight is determined at the end of the experiment. The water absorption is not influenced here either. As to the loss of dry substance, with one exp. the series with the vitamin solution (0.1 mg per 500 cm^3) has lost 5 mg less than the control, while with another experiment it is 6 mg more, the differences falling within the limits of the variability.

So according to these experiments it does not seem probable, that Vitamin B_1 exerts any influence on the respiration of potato discs in aerated distilled water. HELLINGA (1940) has not found any

influence of aneurin on the oxygen-consumption of potato discs either.

The discussion of the results is given together with that of the other investigated storage tissues in chapter VI (p. 121).

A list of the literature cited, is given at the end of Part II.

PART II. EXPERIMENTS WITH VARIOUS STORAGE TISSUES, COMPARED WITH THE POTATO.

CHAPTER V.

EXPERIMENTAL PART.

§ 1. Introduction.

In the preceding part the water-intake, which is shown by potato discs in water, has been investigated. In addition to this a number of experiments are also carried out with discs of other storage tissues. In the first place in order to obtain an idea as to whether the great increase in weight shown by potato discs in water is a general phenomenon or not. In each case the tissue is infiltrated with water in order to find out whether, and to what extent, the increase in fresh weight might be due to the substitution of water for the air from the intercellular spaces. Also it is endeavoured to get an impression of the respiration of various tissues by determining the loss of dry substance. At the same time the exosmosis is controlled by determining the electrical resistance of the external solution every other day.

Except with horse-radish, the influence of hetero-auxin with all the objects is determined. This is done with the intention, if possible, of throwing more light on the nature of the influence which hetero-auxin exerts on potato discs. Use is made only of hetero-auxin in the concentration 1 to 10^6, which is given during the entire duration of the experiment and is renewed every second day.

Use is made of: the roots of the mangold (a form of *Beta vulgaris L.*), the roots of the turnip (a form of *Brassica napus L.*), and horse-radish (a form of *Raphanus sativus L.*); with the roots of the carrot (*Daucus carota L.*), and also with the root-tubers of the dahlia (*Dahlia variabilis Desf.*) and with the rhizome-tubers of the Jerusalem artichoke (*Helianthus tuberosus L.*).

The method followed in these tests is exactly like that described for potato discs (part I). Discs of a thickness of 1 mm and with a diam. of 1.7 cm are employed, of which in each case two or more series of 10 discs are taken from the same tuber. Each series is put into 500 cm^3 aerated distilled water or hetero-auxin solution, while

this is renewed every other day. In all the experiments aeration is given; no tests in an environment without oxygen are made with any of these tissues. The temperature is invariably 21° C.

§ 2. Experiments with mangold.

a. *General part.*

The material for these tests is supplied by the kindness of Dr J. G. OORTWIJN BOTJES, Oostwold (Groningen); variety Barres of Strynö.

The discs, 1 mm thick, 1.7 cm in diameter, are cut out of the root-portion of the mangold, perpendicularly to the longitudinal axis of the tuber. The top, the hypocotyle grown together with the root-portion, is avoided. The discs therefore consist chiefly of the xylemparenchyma of the root, while a cambium-ring is striked once or twice. The diam. of the parenchyma-cells is e.g. 150—250 μ, usually approx. 200 μ, rather smaller around the vascular groups, usually approx. 150 μ. With another specimen the diam. of the parenchyma cells is 50—100 μ, in the free parenchyma usually 70—100 μ, and around the vascular groups 50—80 μ. Whether the tissue contains starch, remains somewhat doubtful; the impression is that here and there small groups of very small starch granules (diam. 1—2 μ) occur, e.g. in the vicinity of the vascular groups.

Simultaneously with the water-intake, the respiration of dry substance is investigated here also. First the variability which exists in the dry weight between two series of 10 discs with the same average fresh weight from the same tuber is determined. The variability in dry weight between two equivalent series is seen to be very slight, not diverging more than 3 mg. For the various pairs of equivalent series is found: 180 and 182 mg; 171 and 174 mg; 106 and 105 mg; 132 and 135 mg dry substance.

Preliminary experiments, in which both mangolds and sugar-beets are used, show that the beet-tissue absorbs a fairly considerable amount of water, at first fairly strongly and later less so. Sugar-beets in general display a slighter water-intake than mangolds; they also cannot stand a lengthy stay of 8 days in aerated water; after a few days they have become a dirty brown and are somewhat flabby, while the increase in weight changes in most cases into a loss of weight, which may even be fairly marked in the last few days of the test.

In older experiments with mangolds the water-intake and the respiration of discs from various parts of the beet (left and right half respectively) is compared. The differences are not particularly large, the water-intake diverging 1 to 1.5 % of the initial fresh weight

and the respiration 0.2 % to 4.1 % of the initial dry weight. However, the differences are slighter if the series are formed from the same part of the beet, the water-intake of two equivalent series in that case diverging maximally 0.2 % of the inital fresh weight and the respiration diverging minimally 1.3 % and maximally 2.9 % of the initial dry weight.

The water-intake with different beets varies, however, fairly greatly. The minimum reached in 8 days is 8 %, the maximum is 20 % of the initial fresh weight. The same is the case with the respiration; the respiration with the different specimens differing from a good 20 % of the initial dry weight to 9.5 %.

With some experiments the p_H and the electrical resistance of the water are also measured; an example is given in table 23. Only in the first two days' period the p_H increases a little, from 4.5 to 5.0 or 5.5, so not to so great an extent by far as is the case with the potato. The electrical resistance in the first period falls somewhat too, but also not nearly so much as with the potato tissue.

With one exp. e.g. after two days the p_H of one series has increased to 5.0 and the resistance has fallen to 190.000 Ω, with the other series the p_H has reached 5.5. and the resistance has fallen somewhat more, namely to 160.000 Ω, while the resistance of the water at the beginning of the experiment is 210.000 Ω. This is an indication, that apparently substances, making the water more alcaline, are given off, as is the case with the potato tissue.

After the first days have passed the p_H only changes very little, the electrical resistance generally shows a slight increase, later on becoming stronger, compared with that of the fresh water, which indicates that substances are taken in from the water. But this is not accompanied by a decrease of the p_H, as with the exosmosis in the first days a rising of the p_H goes together.

So except the slight decrease of the electrical resistance at the beginning, no exosmosis occurs. With the same right, as is done with the potato discs, we may say here, that the loss of dry substance is caused almost entirely by respiration.

In order to determine, if this increase in fresh weight, in other words the water-intake, really is an intake of water by the living cells of the tissue and not a substitution of water for the air from the intercellular spaces, the following is done.

Series of 10 discs with the same average fresh weight are composed in the usual way; the air being withdrawn from the discs in one series in the same way as is described for potato discs (part I, page 19). The infiltration takes place very quickly and completely, the outward appearance of the discs changes at once from white (containing much

TABLE 23.

Exosmosis and absorption of various storage tissue discs in water and in a solution of hetero-auxin, concentration 1 to 10^6. The external solution is renewed every other day. The p_H and the electrical resistance of the external solution (old and new) are measured. The electrical resistance is stated in Ω. Of each object one example is chosen.

	initial		after 2 days				after 4 days				after 6 days				after 8 days	
	p_H	electrical resistance	p_H		electrical resistance		p_H		electrical resistance		p_H		electrical resistance		p_H	electrical resistance
			old	new	old	new	old	new	old	new	old	new	old	new	old	old
mangold (exp. 414)																
A hetero-auxin	4.5	145 000	5.5	4.5	100 000	160 000	5.0	4.5	275 000	140 000	4.5	4.5	400 000	130 000	4.5	400 000
B water	4.5	200 000	5.0	4.5	150 000	220 000	4.5	4.5	320 000	210 000	4.5	4.5	450 000	170 000	4.5	400 000
turnip (exp. 386)																
A hetero-auxin	4.5	180 000	5.5	4.5	190 000	160 000	5.0	4.5	420 000	240 000	5.0	4.5	470 000	160 000	4.5	430 000
B water	4.5	230 000	5.0	4.5	200 000	190 000	5.0	4.5	300 000	330 000	5.0	4.5	540 000	200 000	4.5	530 000
carrot (exp. 417)																
A hetero-auxin	4.5	230 000	7.0	4.5	42 000	230 000	6.5	4.5	80 000	180 000	5.5	4.5	260 000	190 000	4.5	340 000
B water	4.5	360 000	6.5	4.5	100 000	360 000	5.0	4.5	220 000	250 000	5.0	4.5	350 000	250 000	4.5	410 000
dahlia (exp. 428)																
A hetero-auxin	4.5	160 000	6.0	4.5	70 000	200 000	5.5	4.5	75 000	120 000	4.5	4.5	480 000	150 000	4.5	360 000
B water	4.5	210 000	5.5	4.5	110 000	250 000	5.0	4.5	130 000	160 000	4.5	4.5	370 000	190 000	4.5	360 000
Jerusalem artichoke (exp. 431)																
A hetero-auxin	4.5	140 000	7.0	4.5	60 000	180 000	7.0	4.5	50 000	160 000	6.0	4.5	170 000	145 000	4.5	460 000
B water	4.5	180 000	6.0	4.5	150 000	230 000	5.0	4.5	230 000	190 000	5.0	4.5	340 000	200 000	4.5	380 000

air) to colourless and transparent. After this about $^3/_4$ hrs. later, both series, the infiltrated and the control, are weighed again. Both have increased in weight, the infiltrated discs distinctly more than the controls (table 24). This is also clearly shown in figure 12. With one exp. the difference between A and B is slight; with another there is no difference, the control series and the infiltrated series weighing equally much, though with the infiltrated discs the air has indeed been clearly substituted by water, the discs having become completely transparent. To explain this, it is supposed that the mangolds behave different as to infiltration. The one will stand an infiltration e.g. without further consequences, so only the air being replaced by

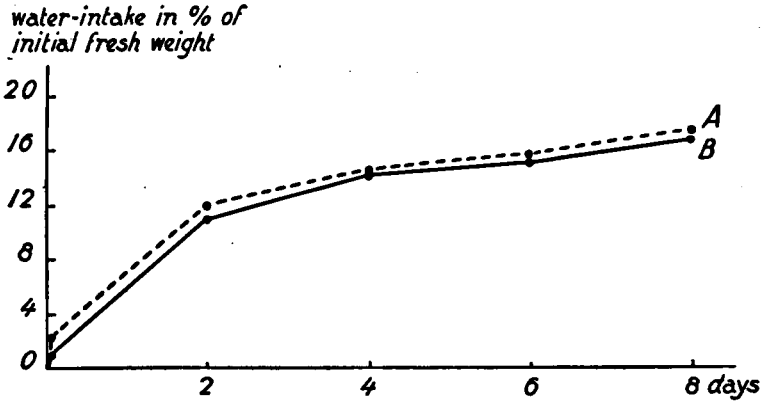


Fig. 12. The water-intake of mangold discs after the air has been removed from the tissue by infiltration. (exp. 304, first half).
A: infiltrated.
B: control.

water, while another will be damaged by it; so that exosmosis occurs, causing a loss of weight, which possibly abolishes the increase of weight caused by the infiltration. An experiment, not mentioned here, gives an indication in this direction. The mangold, used for this experiment, is visibly in an unfavourable condition and is already somewhat flabby (not quite turgescent). Infiltration causes in this case a loss of weight of —1.14 % respectively —0.24 %, opposed to an increase of weight of +0.41 % respectively + 0.12 % with the control during the time of infiltration. We see, however, that all series, the infiltrated as well as the control, show normal increase in weight during the following days. So the infiltrated discs take in water in the ordinary way and to the same extent as the

control discs do, in spite of the fact that the air in their intercellular spaces is replaced by water.

So this water-intake cannot be due to the substitution of water for the air in the intercellular spaces.

It is also seen, that with all experiments the loss of dry weight of the infiltrated discs is somewhat greater than with the controls, though the differences are slight. This will probably be caused by a somewhat stronger exosmosis of the dying parts, because the infiltrated condition will be somewhat deleterious to the tissue after some time.

This somewhat greater loss of dry weight of course also causes a somewhat lower fresh weight. Therefore the phenomenon, the water-intake of the infiltrated discs being somewhat less great than that of the controls in the last days of the experiment, entirely or for the greater part is only apparent. This phenomenon, the difference in fresh weight, obtained by infiltration one series of discs at the beginning of the experiment, later on becoming smaller or being leveled up — in some cases the control discs even become heavier than the infiltrated ones —, may be caused by:

1) The water-intake of the control discs is somewhat larger because besides the usual water-intake, the air from the intercellular spaces is replaced by water in the course of the experiment.

2) The water-intake of the infiltrated discs becomes less great than that of the controls in course of time because infiltration will be somewhat deleterious to the tissue after some time.

3) The water-intake of both series in itself may be the same, but because the loss of dry substance with the infiltrated discs is somewhat greater, the fresh weight of the infiltrated series will be found somewhat less.

As the last possibility (3) already can partly explain the leveling up of the difference in fresh weight between the control discs and the infiltrated ones, the causes (1) and (2) will not play an important rôle.

b. *The influence of hetero-auxin.*

On the occasion of the influence which hetero-auxin exerts on the water-intake and on the respiration of potato discs also here is controlled, whether, and if so, what influence hetero-auxin will have on the tissue of the mangold. Only one concentration is used, namely 1 to 10^6. The experiments are summarized in table 25.

With the experiments (4 exp.'s), made late in the season (May 1939), we see that hetero-auxin has a slight accelerating influence on the water-intake in most cases. Properly speaking in two exp.'s there is little or no difference with the control. With one exp. the

water-intake of the hetero-auxin series is somewhat superior to that of the control with the series taken from the root-part of the mangold and with the series taken from the hypocotyle-part of the tuber the water-intake of the hetero-auxin series is somewhat inferior to that of the control. Both differences, however, don't have any real significance.

Together with the water-intake also the p_H and the resistance of the external solution are determined, embodied in table 23. It is seen, that there is a slight increase of the p_H in the first days, as a rule somewhat more with the hetero-auxin series than with the control, and at the same time a slight decrease of the electrical resistance. In the second two days' period this already changes in an increase of the resistance in most cases, with the hetero-auxin series as well as with the control. Not always, but indeed in many cases, the increase of the resistance with the hetero-auxin solution is greater than with the control. This is no wonder, in so far, because the hetero-auxin itself will be taken in. Taking this into consideration, the increase of the resistance with the hetero-auxin solution is often still greater than with the control. Besides the increase, which the p_H shows in the first few days, the p_H remains practically equal to that of the fresh water or hetero-auxin solution in the following two days' periods.

No sugars are found with Fehling's test-method in the evaporated external solution at the end of the experiment, either with the hetero-auxin series or with the control.

As to the respiration, also no real differences are found either. In most cases the loss of dry substance with the hetero-auxin series is somewhat less than with the control, an odd time it is somewhat more, but the differences scarcely have any real significance. So hetero-auxin does not exert here, as is the case with the potato discs, an accelerating influence on the respiration of dry substance.

In November/December 1939, at the beginning of the winter-season, a short time after the new crop of the mangolds, these experiments are repeated (6 exp.'s). The course of the p_H and that of the electrical resistance of the external solution behave just as with the experiments made in May. But hetero-auxin does not have any promoting influence on the water-intake, not even a slight one as is found in the month of May. On the contrary, a distinct inhibition with respect to the control is found. The water-intake of the hetero-auxin series is inferior to that of the control in nearly all experiments; in most cases fairly much, sometimes little, in other cases there is practically no difference between the control and the hetero-auxin series.

As to the respiration of dry substance, there are no real differences found. In some cases the hetero-auxin discs have respirated some mg less than the controls, in other cases it is just the reverse, or there is practically no difference at all.

Apparently the influence of hetero-auxin on mangold is quite different from that on the potato tissue. Hetero-auxin now has a promoting influence, now an inhibitory influence or none at all on the water-intake. Neither hetero-auxin exerts an influence on the respiration of dry substance with the tissue of the mangold. But in most cases the differences between the hetero-auxin series and the control series, especially with regard to the water-intake, are too great to be only due to variability. It is remarkable that the hetero-auxin just promotes the water-intake in May, while in autumn an inhibition is shown. That is the more remarkable, because with the potato discs the promoting influence of the hetero-auxin is greatest at the beginning of the winter-season, while the promotion of the water-intake in spring seems to become less strong according to some experiments.

§ 3. Experiments with turnip.

a. *General part.*

The material for these experiments is drawn from trade, therefore the origin may be fairly different. With the tuber of the turnip we have to do with the secundary xylem, almost exclusively developed parenchymatically. The shape of the parenchyma cells is irregularly isodiametrical, diam. 50—100 μ, mostly 80—100 μ. Starch occurs, but little and in very small granules, diam. 2—4 μ, some 5—8 μ. Many scattered phloem-groups occur, but only a few tracheal elements. WEISS (1880, 1883) has studied the development of these conductive elements in the xylem-parenchyma of the fleshy roots of Brassica and Raphanus.

The discs, 1 mm thick and 1.7 cm in diameter, are cut at right angles to the long axis of the tuber, from the root-portion. The top, the hypocotyle grown together with the root-portion, is avoided.

In preliminary experiments the variability of the water-intake is observed, however, without determination of the respiration at the same time. The variability of the water-intake between 2 equivalent series is very slight, not diverging more than 0.2 à 0.4 % of the initial fresh weight.

Just as is done with the other objects, it is studied here if the increase in fresh weight of the turnip discs is really caused by an intake of water by the cells themselves or if it might be due to a

substitution of water for the air from the intercellular spaces. Four (4) equivalent series of 10 discs are taken from the same tuber, one of these (A) is infiltrated immediately after composing the series. The water-intake of the series A and B, the last as a control, is noticed during the next 8 days. Of the series C and D the initial dry weight is determined. So we get an impression of the variability of the dry weight of two series of equal fresh weight. The loss of dry substance of the series A and B is calculated with regard to the initial dry weight as an average of the series C and D. The variability in dry weight between C and D is very slight, only 1 to 3 mg. After the infiltration the tissue of turnip becomes fairly transparent, due to the substitution of water for the air. This takes place quickly and rather completely, e.g. better than with the horse-radish. The quantity of air substituted by water is very great, as we see in table 24. More clearly this is shown in fig. 13. After about $^3/_4$ hours, the duration

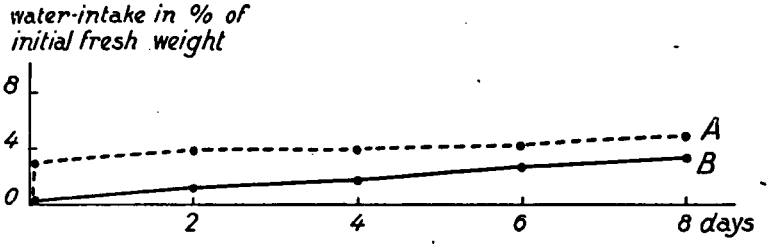


Fig. 13. The water-intake of turnip discs after the air has been removed from the tissue by infiltration. (average of the exp.'s of table 24).
A: infiltrated.
B: control.

of the infiltration, series A has much more increased in weight than the control-series B, which also has increased in weight slightly by the normal water-intake. In the next days series A and B behave identically, the increase in weight taking place to practically the same extent. However, the increase with turnip discs is very slight, so that it does not be so evident as e.g. with mangold, that the infiltrated discs really take in water to the same extent as the controls. Later on the increase of the infiltrated series becomes less, by which the difference between the infiltrated discs and the controls diminishes at the end of the experiment. As to the cause of this a gradual substitution of water for the air from the intercellular spaces may take place with the control discs. But it may just as well happen that infiltration becomes somewhat deleterious after some time, by which

the water-intake of the infiltrated discs becomes inferior to that of the controls.

Only in one case the infiltrated series is infiltrated again at the end of the experiment and then weighed. A slight decrease in weight occurs, probably caused by exosmosis provoked by the treatment of infiltration. In none of the tests the control series is infiltrated at the end of the experiment. But if this infiltration should take place, an increase in weight might occur to an extent as with the infiltration of series A at the beginning of the experiment, this would be a good evidence that the water-intake is not to be accounted for by a substitution of water for the air from the intercellular spaces. But if, at the end of the experiment the effect of infiltration with the control series B might be less great than with the series A at the beginning this would prove nothing. If, namely, the tissue should be not in so fresh a condition as at the beginning of the experiment, it is quite possible that an infiltration may be not endured so well, damage and consequently an abnormal exosmosis may occur. However, in table 24, we see that the loss of dry substance of the infiltrated series and that of the control does not differ very much. This is an indication that infiltration is apparently endured quite well after some time, at least it does not give rise to a considerably stronger exosmosis.

From determinations of the conductivity of the external solution (table 23) it follows that the loss of dry substance of turnip discs will really be caused by the respiration of the tissue.

b. The influence of hetero-auxin.

The result of the experiments in which the influence of hetero-auxin on the water-intake and on the respiration of turnip discs is determined, is to be seen in table 25.

Hetero-auxin proves to have a strong promoting influence on the water-intake of turnip discs, the increase in fresh weight being much greater than with the control discs. This is shown clearly in fig. 14. This influence asserts itself chiefly during the first four days, during the latter half of the experiment the difference between the hetero-auxin series and the control does not increase much more, the curves practically run parallel. Yet the hetero-auxin solution as well as the water of the control is invariably renewed every other day.

The discs of the hetero-auxin series are also perceptibly somewhat thicker than the controls at the end of the experiment. As a rule this thickness manifests itself as slight knobby elevations, just on the spots where phloem-groups are found. Microscopically no particular details are observed. The cut cells of the external surface of

the discs form a brownish mass (colouring red with phloroglucin — hydrochloric — acid). With one of the examined discs this mass on the slight elevations is thickened, while nothing particular with the phloem-groups under it is observed. With another disc of the same series the mass on the spot of a knobby elevation has not thickened at all, but instead of this the phloem-tissue under it has expanded.

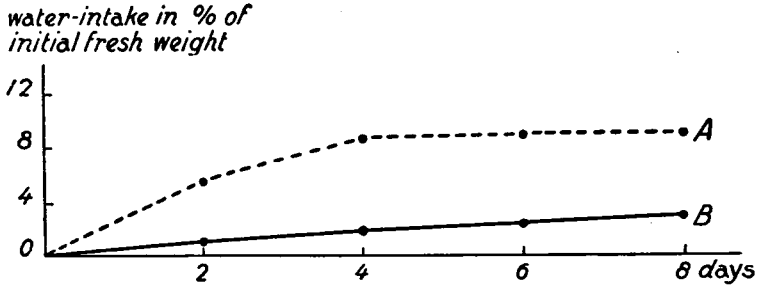


Fig. 14. The influence of hetero-auxin on the water-intake of turnip discs. (average of the exp.'s of table 25).
A: hetero-auxin, concentration 1 to 10^6.
B: control.

Apparently a stronger growth has occurred here, though no distinct details are to be seen at the tissue.

With one experiment the p_H and the electrical resistance of the external solution, old and new, are determined every second day. This is established in table 23. We see that the p_H rises somewhat, though it is slight; in the last second days'period the p_H remains unchanged. The electrical resistance decreases a little in the first two days'period, though it is very slight; with the hetero-auxin series this is not the case at all. In the following two days'periods the electrical resistance increases with regard to that of the fresh solution, with the hetero-auxin series as well as with the control, both to a considerable degree.

In the first place we can infer from this circumstance, that the loss of dry substance of the discs in water is really caused by respiration as it is not due to exosmosis. And further it becomes evident that the increase of the electrical resistance is practically equally strong in both series, with the hetero-auxin series the increase is even somewhat stronger than with the control in most periods. So the greater loss of dry substance under the influence of hetero-auxin cannot be accounted for by a possible detrimental action and consequently a stronger exosmosis. This gives rise to the supposition that

the influence which hetero-auxin exerts is really an influence on the respiration. This conception is fortified by the fact that no sugars are found with Fehling's solution in the evaporated external solution at the end of each two days'period, either with the hetero-auxin series or with the control. The loss of dry substance, so the respiration, of the hetero-auxin series is greater than that of the control in all experiments. With two exp.'s the difference is very distinct and doubtless. With two other exp.'s, however, the difference is very slight, falling within the limits of the variability of the initial dry weights of two equivalent series.

Nevertheless in my opinion we may infer from these results, that the hetero-auxin exerts a promoting influence on the respiration of turnip discs. So hetero-auxin exerts a similar influence as with potato tissue, i.e. a promoting one on the water-intake as well as on the respiration. Relatively this influence with turnip discs is far greater than with potato discs, as the water-intake as well as the respiration of turnip are only slight compared with those of the potato.

§ 4. Experiments with horse-radish.

a. *General part.*

The material for these experiments is drawn from trade, therefore the origin may be fairly different. With the tuber of the horse-radish we have to do with the almost exclusively parenchymatically developed secundary xylem. The discs, 1 mm thick and 1.7 cm in diameter, are cut perpendicularly to the longitudinal axis of the tuber; the top, the hypocotyle grown together with the root-portion, is avoided as much as possible.

The shape of the parenchyma cells is irregularly isodiametrical, diam. 50—100 μ, mostly 60—80 μ. The cells contain a fairly considerable amount of starch occurring in small granules, diam. 10—15 μ. Only very few woody elements occur. Groups of stretched cells are found, it gives the impression to be phloem-tissue (WEISS, 1880, 1883). In these cells little or no starch occurs.

In preliminary experiments the water-intake of horse-radish discs is observed during a longer time. In an experiment during 15 days, the increase in fresh weight goes on to the 8th day inclusive (7.6%), then the weight remains constant during a few days, followed by a decrease till 5.8 %. In another experiment the fresh weight increases till 8.2 % is reached in 16 days; during the following 9 days the weight practically does not change any more, on the 25th day after the beginning of the experiment the total increase is still 8.2 %.

The variability of the initial dry weight is very small. With an experiment, not further mentioned here, 3 equivalent series are taken from the same tuber. The fresh weights, for 10 discs together, are respectively 2409.7 mg, 2409.8 mg, and 2409.4 mg; the dry weights, also for 10 discs together, amounted to 174 mg, 177 mg and 173 mg respectively.

The variability of the water-intake and that of the loss of dry substance are not determined with the horse-radish. The water-intake and the loss of dry substance of a series taken from the left half of a tuber compared with the water-intake and the loss of dry substance of discs taken from the right half of the same tuber, is determined. The water-intake with the series from the different halves of the horse-radish differs a good 1 %, while the loss of dry substance differs 8 mg or 4.6 % expressed in % of the initial dry weight.

Just as with the other tissues also with the horse-radish it is studied if the increase in fresh weight is really due to water-intake or may be only a substitution of water for the air from the intercellular spaces. Equivalent series of 10 discs are taken, one of these being infiltrated at the beginning. The tissue of the horse-radish normally looks whitish, as it contains so much air. After infiltration it turns transparent, but in most cases this does not succeed completely; whitish air-containing spots being left. With the weighing just after the infiltration, it turns out that a considerable quantity of air is replaced by water. This is plainly shown in table 24, the increase in weight of the infiltrated series being considerably larger than that of the control. Of course the control has increased in weight also by the usual water-intake. The difference between the two is a measure for the quantity of air replaced by water. This is shown in figure 15. During the following days the two series continue to

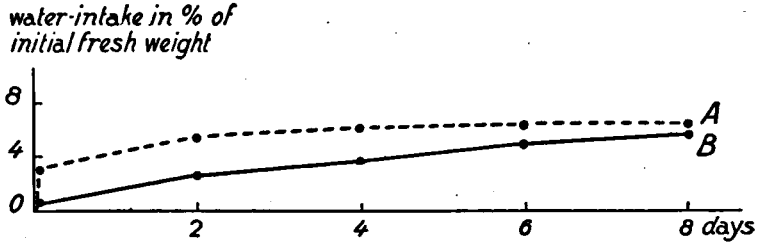


Fig. 15. The water-intake of horse-radish discs after the air has been removed from the tissue by infiltration. (average of the exp.'s of table 24).
A: infiltrated.
B: control.

take in water, the increase in weight being practically equally strong. The curves indicating the water-intake run about parallel in the first 4 days, later on the increase of the infiltrated series becomes less great than that of the control. The reason of this circumstance is probably due to the phenomenon that the infiltration, however, is apparently somewhat detrimental to the tissue. After some time the discs show foul and brownish spots and are not quite fresh any more.

In table 24 we see furthermore that the loss of dry substance with the infiltrated discs is greater than with the control discs, in most cases even much greater. Probably this is caused by a stronger exosmosis from the dying parts, due to a deleterious effect of the infiltration. It is to be regretted, that no determinations of the electrical resistance of the external solution are made with the horse-radish, so that exosmosis is not really determined in this way.

The greater loss of dry substance with the infiltrated discs than that with the control discs is the cause that also the fresh weight is found somewhat less great than would be the case with a smaller loss of dry substance. Partly this accounts for the phenomenon that in the last few days of the experiment the increase of weight of the infiltrated series is no longer so great as that of the control series.

§ 5. Experiments with carrot.

a. *General part.*

The material for these tests is drawn from trade, therefore the origin may be fairly different. The discs, 1 mm thick and 1.7 cm in diameter, are cut at right angles to the long axis of the root. The discs are taken from the inner cylinder of secundary xylem, which has developed almost entirely parenchymatically. The phloem is avoided. In the centre the parenchyma consists of about isodiametrical cells (diam. 80—120 μ), in which some tracheal elements are found. In the more radially arranged secundary xylem the tracheal elements are found on radial rows, the diameter of the parenchyma cells of this part being somewhat smaller (60—80 μ). Very little starch is found in the cells, but it does not fail entirely, sporadically a few granules occur, diameter 2—4 μ. TURNER (1938) also states that the cells of carrot contain little or no starch. The main carbohydrate reserve of carrot roots is represented by saccharose and glucose (BRAUNER, BRAUNER and HASMAN 1940).

It is seen that series of discs with an equal initial fresh weight have also an equal initial dry weight. The difference is very slight, maximally 5 mg. The water-intake of two equivalent series of 10

discs is practically quite the same not diverging more than 0.5 %; so is the respiration, not diverging more than 1.2 %. With the various specimens the water-intake may differ fairly widely. The water-intake of the discs of the control series with the various experiments varies from approx. 4 % to 9 %. The respiration with the series of different specimens varies from minimally 4.3 % of the initial dry weight to maximally 18.0 % of the initial dry weight.

The respiration with the two experiments which give a picture of the small mutual variability between equivalent series, show small amounts in comparison to the following experiments, especially one of them. Also the water-intake with these two experiments is very small. Probably this is due to the unfavourable time of the year, the advanced season (month of May). The other experiments are made in the winter-season.

With some experiments also the p_H and the electrical resistance of the old and of the new external solution are determined. An example is given in table 23. It turns out that the p_H increases somewhat in the first days, later on the increase becomes less; in the last two days'period the p_H practically remains constant. The electrical resistance decreases in the first few days. So some exosmosis takes place, substances which raise the p_H will be lost. In the last half of the experiment this decrease of the electrical resistance changes into an increase, which indicates that substances are taken in from the water. In the last two days'period this increase is even fairly strong. This increase of the electrical resistance causes no perceptible changing of the p_H.

Also with the experiments made in November 1939 at the beginning of the winter-season, determinations of p_H and of the electrical resistance in the external solution are made. The same result is found, as to the p_H as well as to the electrical resistance, which shows a decrease in comparison with that of the fresh solution in the first half of the experiment. This decrease is already less great in the 2nd two days' period than in the first. In the third two days'-period the decrease changes into an increase of the resistance, which becomes much stronger in the last two days.

Just as with the potato and with the other objects these figures give the impression, that, of not entirely, yet for the greater part, the loss of dry substance with carrot discs is caused by respiration.

In table 24 the experiments are summarized in which one series of carrot discs is infiltrated with water. Two equivalent series of 10 discs are taken, one of them being infiltrated immediately after composing the series. Due to the infiltration the tissue becomes transparent, because water has penetrated into the intercellular

TABLE 24.

The water-intake of various storage tissues after the air has been removed from the discs by infiltration.
Series A: infiltrated.
Series B: control.
Fresh weight and dry weight are stated in mg for 10 discs together. The respiration is stated as loss of dry substance in % of the initial dry weight.

	number of experiments	initial fresh weight	water-intake as increase in % of initial fresh weight after:					initial dry weight	respiration
		mg	½ hour	2	4	6	8 days	mg	%
mangold	4								
A infiltrated		2601.5	1.9	12.8	15.6	16.1	17.1	—	19.0
B control		2601.2	1.2	11.8	15.1	15.9	16.9	—	16.2
C		2602.0	—	—	—	—	—	189	—
turnip	4								
A infiltrated		2322.3	2.9	3.8	3.9	4.2	4.9	—	8.1
B control		2322.3	0.2	1.2	1.8	2.6	3.4	—	6.6
C		2322.5	—	—	—	—	—	149	—
horse-radish	4								
A infiltrated		2523.5	3.1	5.4	6.1	6.3	6.3	—	14.9
B control		2523.5	0.6	2.6	3.7	4.9	5.7	—	8.7
C		2523.4	—	—	—	—	—	196	—
carrot	5								
A infiltrated		2626.6	1.3	4.2	5.1	5.6	5.9	—	16.6
B control		2625.9	0.7	3.8	5.0	5.7	6.3	—	15.0
C		2626.1	—	—	—	—	—	219	—
dahlia	5								
A infiltrated		2068.6	−1.0	−0.5	−0.5	0.5	0.9	—	10.0
B control		2068.4	−1.5	−0.3	−0.2	1.0	1.3	—	9.1
C		2068.4	—	—	—	—	—	124	—
Jerusalem artichoke	3								
A infiltrated		2293.2	1.2	3.6	4.8	5.3	6.0	—	5.1
B control		2292.7	−0.8	2.0	3.1	3.7	4.2	—	4.6
C		2294.2	—	—	—	—	—	260	—

spaces. This penetrating goes on fairly quickly and almost completely. But in some cases the radial rows of tracheal elements give the impression that the intercellular spaces here are not entirely infiltrated. After the infiltration both series, infiltrated and control, are weighed again. The weight of the infiltrated discs has increased more than that of the controls. The quantity of air replaced by water

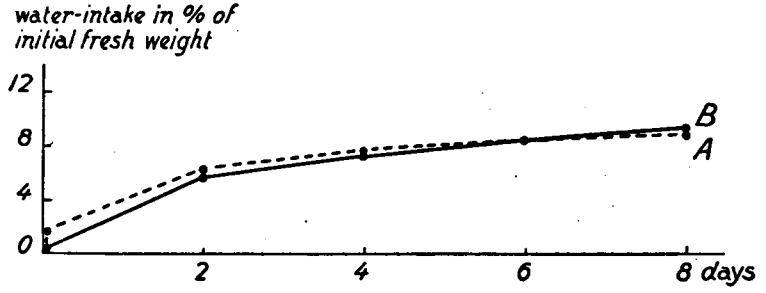


Fig. 16. The water-intake of carrot discs after the air has been removed from the tissue by infiltration. (exp. 311).
A: infiltrated.
B: control.

in this way is even rather considerable (but less great as with turnip tissue). A measure for this is the difference in weight between the control discs and the infiltrated ones, the control having increased also somewhat by the normal water-intake. In the following days the two series go on taking in water, both to about the same amount (see figure 16, in which exp. 311 is given as an example). At least in the first days; in the last part of the experiment the increase of the infiltrated series becomes somewhat less great than that of the control. Most probably a deleterious effect of the infiltration may be the cause, the tissue enduring such a treatment less well after some time. It is to be regretted, that exosmosis is not stated by determinations of the electrical resistance in the external solution. Also it remains possible for the control series to show a small extra increase of weight, if the air in the intercellular spaces really may be replaced by water in course of time, either only partly or entirely, as the case may be.

These experiments show distinctly that the entire increase of weight of the discs in water cannot be accounted for by the substitution of water for the air from the intercellular spaces. Practically the infiltrated discs take in water to nearly the same amount as the control discs. The increase of weight of both therefore may be due to the intake of water by the cells of the tissue themselves.

The loss of dry substance with both series, infiltrated and control, is practically the same in most cases. Only in an odd case the loss of dry substance of the infiltrated series is greater than that of the control. However, only with one exp. the difference is of some importance. This may probably be caused by a stronger exosmosis as a consequence of a somewhat deleterious effect of the infiltration,

b. *The influence of hetero-auxin.*

The experiments showing the influence of hetero-auxin are summarized in table 25. As to the water-intake, the influence of the hetero-auxin is not clear. It is rather different with the various experiments. In most cases the increase in fresh weight with the hetero-auxin series is somewhat greater than with the controls, but the differences are only very slight. In a few cases, e.g. with two exp.'s, but especially with one exp., the water-intake of the discs in the hetero-auxin solution is indeed considerably greater than that of the controls. In another exp., however, the increase of weight of the discs in the hetero-auxin solution is smaller than that of the controls, but the difference is hardly of any importance.

Nevertheless, the course of the curves indicating the increase in fresh weight gives the impression that hetero-auxin does accelerate the water-intake just as with the potato tissue. However, the magnitude of the influence is very different with the various specimens of the carrot. In most cases the influence is very slight, even so slight that the differences between both series fall within the limits of variability. The average of all experiments demonstrates a slight acceleration; this is graphically shown in figure 17.

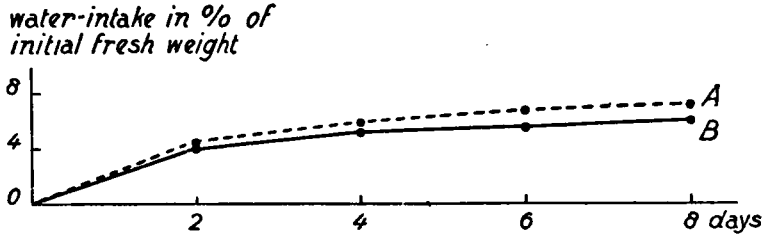


Fig. 17. The influence of hetero-auxin on the water-intake of carrot discs. (average of the exp.'s of table 25).
A: hetero-auxin, concentration 1 to 10^6.
B: control.

However, the water-intake of the carrot tissue is only small compared with that of the potato tissue. The increase in fresh weight in 8 days with carrot is only a few %, as a rule 5 to 7 %, while with potato it amounts to 20 to 25 % of the initial fresh weight. If the influence of the hetero-auxin relatively is equally strong, it is no wonder that the differences between the hetero-auxin series and the control are absolutely so small.

In some of these experiments both the p_H and the electrical resistance of the old and of the new external solution is determined,

an example is given in table 23. We see that the course of the p_H and that of the electrical resistance is practically the same for both series, the hetero-auxin series as well as the control. As to the p_H, if there is any difference, the increase with the hetero-auxin series is slightly more than with the control. The electrical resistance decreases strongly in the first few days with both series, which apparently proves that substances exosmize out of the discs. This is the case during the first 4 days, in an odd case during the first 6 days. In the two last two days' periods, in an odd case only during the last two days, there is instead of a decrease just an increase of the electrical resistance to be seen. So now substances are apparently absorbed from the external solution. This is the case with both series, hetero-auxin series as well as the control, to about the same degree.

With Fehling's method no sugars are found in the evaporated external solution, either with the hetero-auxin series or with the control, at the end of each two days'period.

So the slightly greater loss of dry substances under the influence of the hetero-auxin (table 25) is not caused by a stronger exosmosis. This is an indirect evidence that the influence which hetero-auxin exerts is really an influence on the respiration.

With the figures of the determinations of the electrical resistance it is striking that the course, the decrease at the beginning and the increase later on, is indeed about equal in both series; that is that the initial difference of the electrical resistance between the fresh water and the fresh hetero-auxin solution is maintained. This differs somewhat from most of the other objects, where the electrical resistance of the water and that of the hetero-auxin solution in some cases become about equal at the end of a period, especially in the last days of the experiment. In that case we may suppose that the substance hetero-auxin itself is taken in, while the exosmosis at first and later on the absorption have practically been equally great. Might this maintainance of the difference in electrical resistance between the hetero-auxin series and the control in the case of carrot indicate that the substance hetero-auxin itself is little or not at all taken in by the carrot tissue?

In table 25 it is shown, that the influence of hetero-auxin on the respiration as well as on the water-intake is slight. In nearly all experiments the respiration of the discs in the hetero-auxin solution is indeed greater than that of the control discs, though the differences are slight. As a rule the difference is only a few mg, falling within the limits of the variability. It is striking, however, that just with one exp., where the influence of the hetero-auxin on the water-

intake is strongest, also the respiration of the hetero-auxin series is accelerated to the greatest extent.

The impression, which these experiments give as a whole, is that hetero-auxin does exert a similar influence as with the potato tissue; that means accelerating on the water-intake as well as on the respiration. In most cases, however, the influence of the hetero-auxin, or the sensibility of the tissue to hetero-auxin, is very slight, so that it only scarcely or not at all becomes evident. In an odd case, however, the influence is distinct and in such a case we see that the nature of the influence is entirely the same as with the potato tissue.

§ 6. Experiments with dahlia.

a. *General part.*

The material for these experiments are the root-tubers of plants of various varieties from the botanical garden at Groningen. The discs, 1 mm thick and 1.7 cm in diameter, are cut perpendicularly to the longitudinal axis of the tuber. The discs therefore consist of the medullary tissue, surrounded by the secundary xylem almost entirely parenchymatically developed. The surface occupied by the medullary tissue is of a various size. The parenchyma cells of the medullary tissue are about of an isodiametrical shape, diameter 70—100 μ. The parenchyma cells in the secundary xylem are stretched in a radial direction, length 100—120 μ, width $\pm$ 30 μ.

The water-intake of the dahlia discs deviates somewhat from the manner which we have met with the tissues discussed so far. For in the first few days the increase of weight is very slight or nought (zero), sometimes there is even a slight decrease in weight. Then this changes into an increase of weight. The curve, indicating the course of the water-intake, then shows a peculiar sudden rise, at least if the water-intake is of some importance. In many cases, however, the water-intake remains very small, so that the curve has a fairly horizontal course.

In two experiments the variability of the water-intake between two equivalent series of 10 discs with an equal average initial fresh weight of the same tuber is studied. This variability does not prove to be great, it scarcely diverges 1 %.

With one exp. also the conductivity and the p_H of the external solution are determined. It becomes evident that the p_H somewhat increases during the first few days, later on the p_H remains equal to that of the fresh water. It appears that the electrical resistance shows an increase in all periods with regard to that of the fresh water. In the first few days this increase is slight, becoming invariably

greater in the following days. The course of the p_H and that of the increase of the electrical resistance is the same for two equivalent series. Also with some experiments, in which the influence of hetero-auxin is studied, determinations of the electrical resistance in the external solution are made (an example is given in table 23). In the first two days'period, and in most cases also in the second two days'period, a more or less great decrease of the electrical resistance occurs, while in the following periods an increase of the electrical resistance is found. These figures do not exclude with certainty, that the loss of dry substance occurring during the experiment is not for a very slight part due to exosmosis. However, the conclusion is justified that the loss of dry substance is caused by respiration for the greatest part by far.

The variability of the initial dry weight of two equivalent series with the same initial fresh weight is very slight, the dry weights are approx. the same. With 3 experiments two equivalent series are taken from the same tuber. The figures, fresh weight as well as dry weight invariably for 10 discs together, are as follows. 1) Series A: fresh weight 1946. 9 mg, dry weight 120 mg; series B: fresh weight 1946. 5 mg, dry weight 119 mg. 2) Series A: fresh weight 1874. 5 mg, dry weight 115 mg; series B: fresh weight 1875.1 mg, dry weight 115 mg. 3) Series A: fresh weight 1980. 7 mg, dry weight 85 mg; series B: fresh weight 1989. 5 mg, dry weight 82 mg.

The variability of the respiration is determined with one exp. This variability appears to be very slight, the loss of dry substance is exactly the same for both series.

Just as is done with the other objects also with dahlia is studied whether infiltration may have an influence on the water-intake or not. Three equivalent series of 10 discs are taken from the same tuber. One series (A) is infiltrated immediately at the beginning. With this infiltration the air from the intercellular spaces is replaced by water. With the dahlia discs this succeeds very quickly and invariably almost completely. The discs become quite transparent. Sometimes the intercellular spaces between the radially arranged tracheal elements seem not to be quite well infiltrated. These experiments are summarized in table 24. After the infiltration the discs of both series are weighed again. Remarkable it is that here in contrast to the discs of other tissues, a loss of weight has occurred. This decrease in fresh weight may sometimes be even fairly great. Between the infiltrated series and the control the difference is such that the infiltrated discs are heavier than the control discs. This is due to the substitution of water for the air from the intercellular spaces. Only with two exp.'s there is practically no difference.

What is the reason of this, is difficult to say. It is not very probable that it is due to e.g. a considerable stronger exosmosis and consequently a greater loss of weight caused by infiltration. For at the end of the experiment it appears that the loss of dry substance of the infiltrated discs is nearly as great as that of the control discs; in these two experiments as well as in all others. In the following days the curve indicating the water-intake of the infiltrated discs runs practically parallel to that of the control. In most cases, however, the control makes up for the arrears, sometimes even to such an extent that the total increase of weight of the control discs is somewhat greater than that of the infiltrated ones at the end of the experiment.

This may be due to various causes. It is possible, however, that with the control the air from the intercellular spaces is replaced by water, giving an extra increase of weight regarding the already infiltrated discs. A second possibility is that the infiltration is somewhat deleterious to the tissue after some time. The physiological processes may take place at a lower level, per consequence the water-intake of the infiltrated discs will be somewhat smaller.

The separate experiments are somewhat irregular. However, if we take the average the case is very surveyable. In the first two days the control makes up for the arrears and after that the curves of both series, the infiltrated series and the control, coincide almost entirely. This is also clearly shown in figure 18, in which exp. 323 is stated

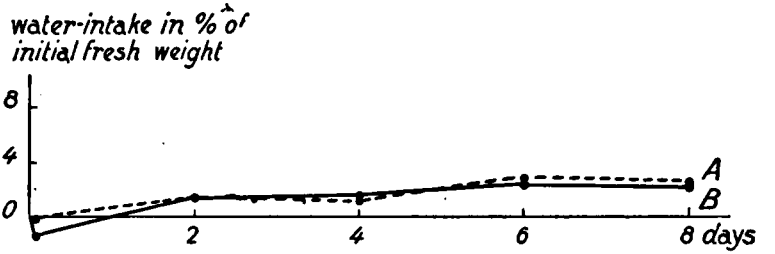


Fig. 18. The water-intake of dahlia discs after the air has been removed from the tissue by infiltration. (exp. 323).
A: infiltrated.
B: control.

graphically. So it really makes the impression that the air from the intercellular spaces is replaced by water with the control discs. In consequence of this the increase in fresh weight may be somewhat greater at the beginning of the experiment. However, it does not

account for the further water-intake, which exists independently of that hypothetical infiltration.

b. *The influence of hetero-auxin.*

The experiments in which the influence of hetero-auxin on the water-intake and on the respiration of dahlia discs is observed have been summarized in table 25. Here with dahlia, only with one exp. the water-intake of the discs in the hetero-auxin solution is somewhat greater than that of the control discs in water. With all other experiments the hetero-auxin series as to the water-intake is inferior

TABLE 25.

The influence of hetero-auxin on the water-intake and on the respiration of various storage tissue discs.
Series A: hetero-auxin, concentration 1 to 10^6.
Series B: control.
Fresh weight and dry weight are stated in mg for 10 discs together. The respiration is stated as loss of dry substance in % of the initial dry weight.

	number of experiments	initial fresh weight	water-intake as increase in % of initial fresh weight after:				initial dry weight	respiration
		mg	2	4	6	8 days	mg	%
mangold	10							
A hetero-auxin		2424.6	7.4	10.3	11.8	12.8	—	14.1
B control		2424.6	8.1	11.6	12.9	13.5	—	14.9
C		2424.5	—	—	—	—	186	—
turnip	4							
A hetero-auxin		2305.1	5.5	8.7	9.0	9.1	—	10.4
B control		2305.1	1.0	1.8	2.4	3.0	—	6.2
C		2304.4	—	—	—	—	154	—
carrot	7							
A hetero-auxin		2597.5	4.2	5.8	6.6	7.1	—	14.6
B control		2597.2	4.0	5.1	5.5	6.0	—	12.7
C		2597.0	—	—	—	—	181	—
dahlia	6							
A hetero-auxin		2063.1	−0.1	1.6	3.1	4.4	—	29.2
B control		2062.9	0.0	2.8	4.7	5.3	—	15.5
C		2064.1	—	—	—	—	171	—
Jerusalem artichoke	3							
A hetero-auxin		2269.7	16.9	48.0	53.2	54.0	—	24.7
B control		2269.7	3.6	5.4	5.8	6.2	—	6.2
C		2270.3	—	—	—	—	273	—

to that of the control; with two exp.'s there is no actual difference.

As it has already been mentioned, in some of these experiments also the p_H and the electrical resistance of the external solution have been studied (table 23). The increase of the p_H with the hetero-auxin series is seen to be about the same as with the control in the first few days as well as later on. However, as a rule the increase of the p_H with the hetero-auxin series is somewhat greater. As a rule also the decrease and later on the increase of the electrical resistance with the hetero-auxin series occur just as with the control. With two exp.'s the decrease of the electrical resistance with the hetero-auxin series is greater than with the control in the two first two days'periods. The increase of the electrical resistance in the last part of the experiment is sometimes greater with the hetero-auxin series than with the control. In other cases the increase of the hetero-auxin series is about the same as with the control.

If we pay attention to the loss of dry substance (table 25), it becomes evident that with all experiments without any exception the discs in the hetero-auxin solution invariably have lost more, even fairly much more, dry substance than the discs of the control series.

The respiration itself differs very much with the various experiments. With one exp. e.g. the respiration of the control is 1.6 % of the initial dry weight, with another exp. this amounts to 26.4 % of the initial dry weight.

With all experiments, except two where the difference does not surpass 5 % (10 mg), the loss of dry substance of the discs in the hetero-auxin solution has been about 15 % (30 mg) more than that of the control discs. It is not allowed to infer that this loss is caused entirely by an influence of the hetero-auxin on the respiration with dahlia discs. It is striking that only with the experiments, where the exosmosis with the hetero-auxin series has been about equal to the exosmosis with the control, the acceleration of the loss of dry substance is fairly small with the hetero-auxin series. The difference with the control here only amounts to about 5 %, which, however, is real. So we have to realize the possibility that the very strong influence, which hetero-auxin exerts on the loss of dry substance with dahlia discs, may be caused for a smaller or greater part by a stronger exosmosis with the discs in the hetero-auxin solution.

However, no sugars are found with Fehling's solution in the evaporated external solution, either with the hetero-auxin series or with the control, at the end of each two days'period.

Though it may not be inferred with certainty that hetero-auxin exerts an accelerating influence on the respiration of dahlia discs, it

does not seem probable to me that the entire extra loss of dry substance can be accounted for by an extra exosmosis.

We have to consider that such a great difference in dry weight will manifest itself in the fresh weight. It is difficult to say with what number of mg of fresh weight a dry weight of 30 mg corresponds precisely. However, let us suppose that the substrate for the respiration with dahlia is fructose ($C_6H_{12}O_6$; M.G. = 180), admitting this being completely broken down to CO_2 and H_2O. According to: $C_6H_{12}O_6 + 6\,O_2 = 6\,CO_2 + 6\,H_2O$. The CO_2 will pass into the air and we may suppose that the water remains in the discs. A respiration of one gramme-molecule glucose means thus a diminution in the fresh weight of $6 \times 12 = 72$ g. The reduction of the fresh weight by respiration will thus be equal to 72/180 or 2/5 × the loss of dry substance. So an extra loss of dry substance of about 30 mg means a diminution in the fresh weight about $2/5 \times 30 = 12$ mg. As the average fresh weight of 10 dahlia discs together is approx. 2000 mg, that is thus approx. 0.6 %. So the water-intake of the hetero-auxin series is always about 0.6 % greater than the figures for the increase in fresh weight indicate. The extra loss of dry substance therefore with the discs in the hetero-auxin solution has no influence worth mentioning on the increase in fresh weight.

From these experiments it does not become evident if hetero-auxin exerts an influence on the water-intake or not. In some experiments the curves indicating the water-intake of both series coincide. In other cases, however, there is some difference, in most cases the water-intake of the hetero-auxin series being somewhat less than that of the control. However, the various experiments are rather different and the course of the curves in each experiment is also fairly irregular. If we take the average, which is established in figure 19, the curve indicating the water-intake of the discs in the hetero-

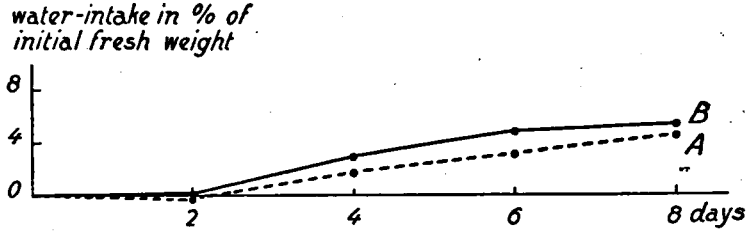


Fig. 19. The influence of hetero-auxin on the water-intake of dahlia discs (average of the exp.'s of table 25).
A: hetero-auxin, concentration 1 to 10^6.
B: control.

auxin solution is somewhat inferior to that of the control discs. The difference is slight and is almost, though not entirely, leveled up by the 0.6 % discussed above.

So with a great probability we may infer that hetero-auxin does not influence the water-intake of dahlia discs. At any rate it is clear, that hetero-auxin does certainly not promote this water-intake.

§ 7. Experiments with Jerusalem artichoke.

a. *General part.*

The material for these experiments is drawn from the botanical garden at Groningen. All tubers have been taken from the same plant. So the material is genetically homogen. Also the circumstances under which the tubers have grown have been entirely the same. The discs, 1 mm thick and 1.7 cm in diameter, are cut at right angles to the long axis of the tuber. The discs therefore consist principally of the medullary tissue, which occupies here a much greater surface than with dahlia. Around this a small ring of secundary xylem, almost entirely developed parenchymatically, is found. The shape of the parenchyma cells in the medullary tissue is about isodiametrical, diameter 60—100 μ, as a rule 70—80 μ. The parenchyma cells in the secundary xylem are stretched somewhat more in a radial direction, length 70—110 μ, width 60—80 μ.

In preliminary experiments the water-intake is observed, without determining the loss of dry substance. It is seen that the water-intake is very slight, the increase in fresh weight being only 3 to 5 % in 8 days. The course is quite normal, the increase being greatest in the first days, later on the increase is only slight.

The variability of the water-intake of two equivalent series of the same tuber is not determined. With an experiment not further mentioned here, the variability of the initial dry weight of two equivalent series of the same tuber with the same average initial fresh weight is determined. This variability does not differ more than 5 mg. The figures, fresh weight as well as dry weight invariably for 10 discs together, are as follows. 1) Series A: fresh weight 2351.2 mg, dry weight 280 mg; series B: fresh weight 2350.3 mg, dry weight 275 mg. 2) Series A: fresh weight 2294.0 mg, dry weight 289 mg; series B: fresh weight 2294.9 mg, dry weight 294 mg.

As with the other objects, the influence which infiltration may have on the water-intake is also studied here. Three equivalent series of 10 discs are taken from the same tuber. One of these (A) is infiltrated immediately at the beginning. Judging from the growing transparent of the tissue, the rapidity of the infiltration is fairly

slow, slower than with most of the other tissues. Infiltration does not seem to succeed completely, the tissue does not become perfectly transparent. However, this need not be an absolute criterion.

The quantity of air replaced by water is very considerable. This is proved when both series are weighed again after the infiltration of series A. The infiltrated discs are much heavier than the controls (table 24). Further is appears that the control discs show a decrease of the fresh weight in regard to the initial fresh weight, about three quarters of an hour before. This decrease is even fairly great. The infiltrated discs do not show such a decrease; due to the substitution of water for the air from the intercellular spaces the result is a slight increase in fresh weight.

With one experiment the control does not show this decrease of the fresh weight at the beginning, while the total water-intake at the end of this experiment is the greatest of the three experiments. Two factors seem to cooperate, first an increase in fresh weight caused by the intake of water and secondly a factor inhibiting this. This factor (x) only manifests itself at the very beginning of the experiment, and is evidently smaller with this exp. than with the two others.

Consequently it turns out that the course of the curve indicating the water-intake with the Jerusalem artichoke is essentially the same as with dahlia. Just as with dahlia there is a decrease of the fresh weight at the beginning and then begins the increase. However, with the Jerusalem artichoke this decrease occurs only at the very beginning. If we determine the water-intake every second day, the first determination made after two days have passed, we don't perceive a decrease at all and we find quite a normal course of the curve. With dahlia this decrease manifests itself during a longer period, the increase in fresh weight being almost nought or still negative after 2 days, sometimes even after 4 days; only after some time the distinct increase begins.

If we pay attention to the course of the water-intake of both series, the infiltrated and the control, during the experiment, it becomes evident that this has taken place to the same degree. The course of the curves is perfectly parallel (figure 20). The difference in weight, caused by infiltration of one series at the beginning, manifests itself during the whole experiment. With the Jerusalem artichoke therefore no substitution of water for the air from the intercellular spaces takes place in course of time. So the increase in weight during the experiment will be really caused by an intake of water by the cells of the tissue themselves.

In order to get an impression of the exosmosis, with some experi-

ments the conductivity of the external solution is determined; an example is given in table 23. Here it is proved that only in the first two days'period a not very great decrease of the electrical resistance occurs. In the following period the electrical resistance changes only

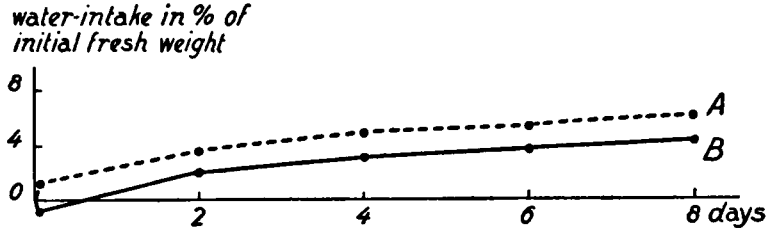


Fig. 20. The water-intake of Jerusalem artichoke discs after the air has been removed from the tissue by infiltration. (average of the exp.'s of table 24).
A: infiltrated.
B: control.

slightly and already inclines to an increase, which becomes clearly evident in the 3rd period and remains so in the last second days' period.

In my opinion it is allowed by this to ascribe the loss of dry substance during the experiment to respiration, just as is done for the other objects.

The respiration of the control series varies from 3.5 % to 5.8 % of the initial dry weight. The respiration of the infiltrated discs with two experiments is seen to be somewhat smaller than that of the control discs. With one exp., however, the loss of dry substance of the infiltrated discs is much greater than that of the controls. However, we have to set little value on this, since for the composing of the series of this experiment two small tubers are used, and in that case an equal average fresh weight is no guarantee of an equal average dry weight (see part I, chapter I, where this is studied for great and small potatoes).

Infiltration, though perhaps not quite complete, apparently has no deleterious effect on the tissue, as the loss of dry substance of the infiltrated discs and that of the control discs takes place to the same amount, while also the curves indicating the water-intake run perfectly parallel.

b. *The influence of hetero-auxin.*

The experiments in which the influence of hetero-auxin on the discs of Jerusalem artichoke is studied, have been summarized in

table 25. With one exp. the three series of 10 discs each are taken from the same tuber. With the two other exp.'s each series has 7 discs from one tuber, completed with 3 discs from another one. It was impossible to take the experiments otherwise, the material being limited.

With these experiments the conductivity of the old and of the new external solution was determined; so was the p_H at the same time. An example of these data is found in table 23. At first an increase of the p_H occurs with both series, which is greater with the hetero-auxin series than with the control. In the two last two days'-periods the p_H of the control series changes only very slightly with respect to that of the fresh water. The p_H of the hetero-auxin series still increases in the third period, though the increase is slighter than in the first days; in the last two days practically no increase is observed.

At the beginning of the experiment a fairly strong decrease of the electrical resistance occurs with the hetero-auxin series just as with the discs in water, as is already described. In the second two days'period this decrease with the hetero-auxin series is about equally great as in the first period, in contrast with the control, in which the electrical resistance remains practically unchanged in this period. With the hetero-auxin series the decrease of the electrical resistance changes into a slight increase in the 3rd two days'period, while the increase with the control series is already considerable. In the last two days a considerable increase is to be observed with both series, the hetero-auxin series as well as the control series.

So we see that the decrease of the electrical resistance with the hetero-auxin series has been greater than with the control in the first days, while this decrease has continued during a longer time. This may indicate that more substances are lost by exosmosis. Per consequence the loss of dry substance may be influenced somewhat by this, but it is difficult to say to what extent. It is to be regretted that no reaction with Fehling's method is applied on the evaporated external solution, in order to indicate possible sugars. For this reason it is advisable not to speak of respiration with the discs of the hetero-auxin series, but only of a loss of dry substance.

In table 25 we see that hetero-auxin exerts a very strong influence on the Jerusalem artichoke discs, on the water-intake as well as on the loss of dry substance. The influence is accelerating on both processes, to an exceedingly great extent, as has not occurred with any of the other objects. The influence on the water-intake is shown best in figure 21. The water-intake in 8 days of the discs in the hetero-auxin solution excells by nearly 50 % that of the controls,

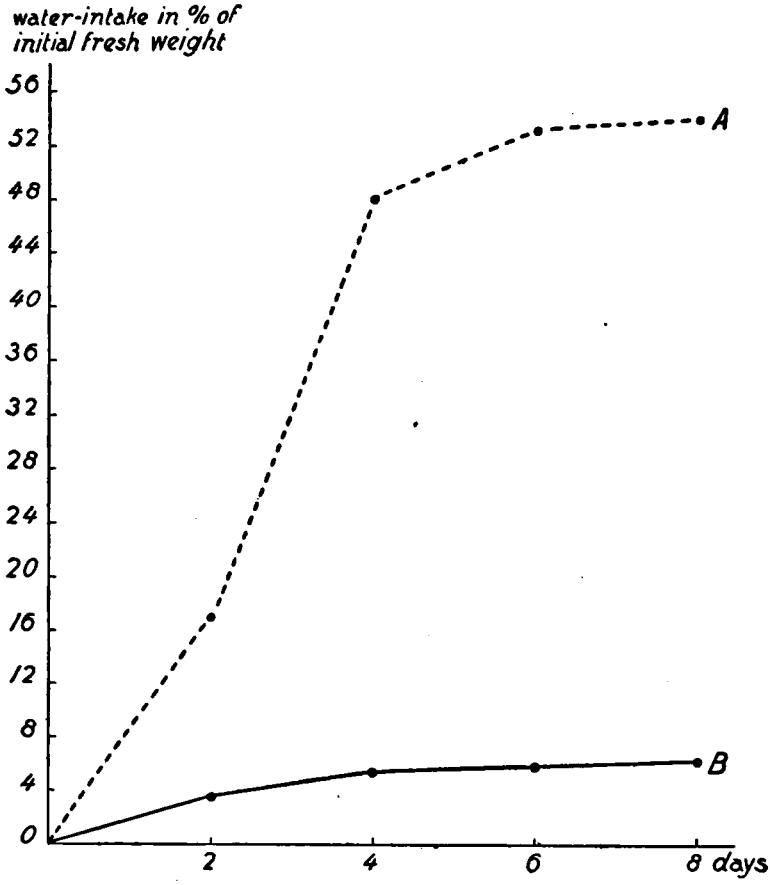


Fig. 21. The influence of hetero-auxin on the water-intake of Jerusalem artichoke discs. (average of the exp.'s of table 25).
A: hetero-auxin, concentration 1 to 10^6.
B: control.

of which the total water-intake is only a few %. The discs in the hetero-auxin solution have grown perceptibly thicker.

It is remarkable that with one exp. the extra great intake of water of the control is accompanied by an extra great respiration. For the water-intake of the control series with this exp. is 14.4 % in 8 days, while 2 to 5 % is normal. The respiration, 30 mg for 10 discs together in 8 days (or 11.1 % of the initial dry weight) is also very great, as

a rule this does not excell 14 to 16 mg for 10 discs together (or 5 to 6 % of the initial dry weight). This really seems to be an indication of a direct relation between water-intake and respiration. In the next chapter this will be discussed in more details.

So the loss of dry substance has appeared to be greatly accelerated by hetero-auxin. But also the exosmosis is somewhat stronger with the discs in the hetero-auxin solution than with the discs in water. Though it seems very improbable that the entire extra loss of dry substance with the hetero-auxin series will be caused by exosmosis, it is not allowed to infer with certainty a promoting influence of the hetero-auxin on the respiration of the Jerusalem artichoke discs. In my opinion, analogous to the other objects e.g. potato and turnip, it is probable that for a considerable part the extra loss of dry substance is caused by an influence of the hetero-auxin on the respiration, indeed.

Researches about the possible influence of hetero-auxin on the CO_2-production or on the oxygen-consumption could throw light on this question.

§ 8. Summary.

The principal data, obtained with the various storage tissues, are summarized in table 26, in which the average values of the water-intake and those of the respiration in aerated distilled water are stated.

In this table it is shown that the water-intake is a general phenomenon, not only specific to the potato discs. Discs of various storage tissues take in water in the same way, though all of them to a much smaller degree than the potato does.

It has turned out that infiltration cannot be the cause of this water-intake. When we infiltrate the tissue artificially, a smaller or a greater quantity of air is replaced by water according to the kind of tissue. With all objects the quantity of air replaced by water is greater than with the potato tissue. With the greater part of the investigated tissues this is clearly visible as the outward appearance of the tissue changes, the discs becoming colourless and transparent. Yet, the water-intake goes on undisturbedly. The curve indicating the water-intake of the infiltrated discs practically runs parallel with that of the control discs, though the control in most cases makes up for the arrears, at least partly. In the next chapter (p. 121) this will be further discussed.

The water-intake of the mangold discs approximates that of the potato discs most, while the water-intake of turnip is the slightest. The curve indicating the water-intake with the dahlia and the Jeru-

TABLE 26.

Water-intake and respiration by various storage tissues in aerated water during a period of 8 days. The water-intake is stated as increase of fresh weight in % of the initial fresh weight. The respiration is stated as loss of dry substance in mg for 10 discs together and in % of the initial dry weight. ( ) means that for the calculation of the respiration only this number of experiments has been available.

	number of experiments	water-intake %	respiration mg	respiration %
mangold	5	10.6	19.6	12.8
	2	16.9	31.0	16.2
	10	13.5	32.0	14.9
average		13.7	27.5	14.6
turnip	2	2.4	—	—
	3	3.7	9.0	6.6
	4	3.0	9.0	6.2
average		3.0	9.0	6.4
horse-radish	1	6.8	22.0	14.0
	4 (3)	5.7	17.0	8.7
average		6.3	20.0	11.4
carrot	2	2.9	14.0	8.2
	5	6.3	32.0	15.0
	7	6.0	24.0	12.7
average		5.1	23.0	12.0
dahlia	2 (1)	4.4	10.0	8.2
	5	1.3	11.0	9.1
	6	5.3	26.0	15.5
average		3.7	16.0	10.9
Jerusalem artichoke	4	3.6	—	—
	3	4.2	12.0	4.6
	3	6.2	17.0	6.2
average		4.7	15.0	5.4
potato	3	19.1	70.0	16.8
	4	19.0	76.0	17.1
	8	20.6	66.0	15.2
	5	23.4	68.0	15.6
average		20.5	70.0	16.2

salem artichoke deviates from the course of the curve with the other tissues. For with dahlia and also with Jerusalem artichoke there is a decrease of the fresh weight at the beginning of the experiment, this initial decrease being followed by the usual increase. With dahlia this decrease extends to a longer period of time as is the case with Jerusalem artichoke.

During the stay in the aerated water the discs of all investigated objects loose a smaller or a greater quantity of dry substance according to the kind of tissue. This loss of dry substance is greatest with the potato, only with the mangold it is approximated, expressed in % of the initial dry weight. Determinations of the conductivity of the external solution have taught, that with all of these storage tissues as well as with the potato discs there is some exosmosis in the first few days. Later on this changes into an increase of the electrical resistance of the external solution, indicating that the absorption has become prominent. So the loss of dry substance is principally caused by the respiration. This inference is strengthened by the fact that no sugars are found with Fehling's solution in the evaporated water at the end of each two days'period.

As a rule the tissues with the greatest water-intake show also the greatest respiration. First comes the potato with a water-intake of 20.5 % of the initial fresh weight in 8 days and a respiration of 16.2 % of the initial dry weight in the same period. The relation between water-intake and respiration is, however, not the same with all tissues. With the dahlia e.g. the water-intake in relation to the respiration is much smaller than with the Jerusalem artichoke. The magnitudes to which the water-intake and the respiration are related to — the water-intake related to the fresh weight and the respiration related to the dry weight —, however, are not to be compared.

The influence of hetero-auxin.

The influence, which hetero-auxin exerts on the potato tissue, i.e. a promoting influence on the water-intake as well as on the respiration, is also found with various of the other storage tissues.

The principal data are summarized in table 27, in which the average values for the water-intake and those for the respiration of the discs of various storage tissues in an aerated solution of hetero-auxin, concentration 1 to 10^6, compared with those in aerated distilled water, are given. In this table we see the following.

With the mangold the influence of hetero-auxin is not distinct, either on the water-intake or on the respiration.

With the turnip hetero-auxin exerts a promoting influence on the water-intake as well as on the respiration. The extra loss of dry

TABLE 27.

Water-intake and respiration by discs of various storage tissues influenced by hetero-auxin, concentration 1 to 10^6.
The water-intake is stated as increase of fresh weight in % of the initial fresh weight. The respiration is stated as loss of dry substance in % of the initial dry weight.

	number of experiments	water-intake		respiration	
		control %	hetero-auxin %	control %	hetero-auxin %
mangold	10	13.5	12.8	14.9	14.1
turnip	4	3.0	9.1	6.2	10.4
carrot	7	6.0	7.1	12.7	14.6
dahlia	6	5.3	4.4	15.5	29.2
Jerusalem artichoke	3	6.2	54.0	6.2	24.7
potato	5	18.8	32.8	15.2	20.5

substance under the influence of hetero-auxin is not caused by a stronger exosmosis; so the hetero-auxin promotes the respiration. The influence on the two processes is relatively stronger with the turnip than with the potato.

With the carrot the hetero-auxin has only a very slight, scarcely perceptible influence, which is, however, a promoting one on the water-intake as well as on the respiration. It has appeared that it is indeed an influence on the respiration as the extra loss of dry substance is not caused by a greater exosmosis.

With the dahlia the influence of hetero-auxin on the water-intake is not clear. As to a possible influence, this influence seems to be somewhat inhibiting instead of being promoting. However, hetero-auxin exerts a distinctly promoting influence on the loss of dry substance, even to a fairly great extent. But we must take into consideration that this extra loss of dry substance for a smaller or a greater part may be caused by exosmosis.

With the Jerusalem artichoke hetero-auxin exerts a strongly promoting influence on the water-intake as well as on the loss of dry substance. In both processes the influence is very much greater than with the potato discs. With the Jerusalem artichoke as well as with the potato the influence on the water-intake is greatest in the period between the second and the fourth day. But we have to consider seriously that the influence of the hetero-auxin on the loss of pry substance may partly be caused by exosmosis.

With none of the investigated tissues sugars are found with

Fehling's test-method in the evaporated external solution, either with the hetero-auxin series or with the control at the end of each two days'period.

In contrast to the potato discs, not with any of the storage tissues investigated, cell-divisions in the cell-layer under the cut surface occur during the eight days'stay in aerated distilled water. This is not the case with discs in a hetero-auxin solution either.

CHAPTER VI.

DISCUSSION OF THE RESULTS IN RELATION TO LITERATURE.

§ 1. The nature of the process of the water-intake.

In Chapter I, § 2, pag. 9, is indicated that the increase in weight which potato discs undergo in water, is due to an intake of water by the cells of the tissue themselves. This has also been made probable for each of the tissues treated in chapter V of this paper. This is done by infiltrating the discs with water, by which action they gain in weight as a proof that the air from the intercellular spaces is replaced by water. During the following days, however, the course of the water-intake is fundamentally equal to that of the control discs. In the first few days, with some of the objects, e.g. the Jerusalem artichoke, practically during the entire duration of the experiment, the difference in weight with the control caused by infiltration remains. In most cases the control does, however, partly make up for the arrears in the last half of the experiment. This has e.g. proved to be the case with the turnip, with the horse-radish and with the carrot. It is possible that the water-intake of the infiltrated discs becomes less than that of the control ones on account of infiltration being somewhat injurious to the tissue after some time. There are phenomena pointing to this; in some cases e.g. with the horse-radish the discs visibly come to a less good condition. Here also the loss of dry substance of the infiltrated discs is much greater than that of the controls, which points to a stronger exosmosis. Owing to this extra loss of dry substance also the fresh weight of the infiltrated discs will be somewhat less. This already partly accounts for the difference of fresh weight with the control becoming smaller in course of time. It is also possible that the water-intake of the control discs is somewhat larger, because, besides the ordinary water-intake yet the air from the intercellular spaces is partly or entirely replaced by water during the experiment. At any rate it

has become evident that infiltration is not the cause of the water-intake (compare VAN DER PAAUW 1935) with which we have to do in this investigation.

As has already been mentioned, the experiments are made with pure distilled water, so that the question arises if the water-intake may be due to an electro-osmotic watermovement, which as is known is strongest in pure water (HÖBER 1926, KRUYT 1929). Nevertheless it is impossible that this should be the cause; for with the experiments about the influence of salts on this process (Chapter II, § 5) it has appeared that, without considering the slight increasing or reducing influence which the various cations exert on the water-capacity of the tissue, the water-intake from these salt-solutions fundamentally takes place to the same degree as from pure water. This agrees with the results of L. and M. BRAUNER (1940) who also state a considerable water-intake from diluted salt solutions as well as from distilled water; though with the salt solution the electro-osmotic component will be opposed to the normal water-intake of the cells in contrast with the distilled water, in which case the electro-osmotic waterflow is added to the normal water-intake.

BÜNNING (1939) points to the fact that the magnitude of the electrical potential at the plasma boundary layers is dependent on the intensity of the respiration. He supposes that the water-intake might be regulated electro-osmotically by this as in my investigations (REINDERS 1938) the water-intake has proved to be closely connected with a sufficient respiration. However, this will be very improbable, due to the results of the investigation of the water-intake from diluted salt solutions, which have taught that electro-osmosis does not play any perceptible part.

In Chapter II, § 1 it is found that in an anaerobic environment no water-intake occurs. That means that the water-intake is dependent on a sufficient supply of oxygen of the tissue, that it is connected with the respiration of the tissue, just as various investigators have established for the intake of other substances in the course of the last years.

So STEWARD (1932; 1939) was the first to state that the intake of bromine by potato discs is dependent on a sufficient oxygen supply; afterwards this has also been confirmed for Rb(STEWARD and HARRISON, 1939). HOAGLAND and BROYER (1936) have found that the intake of salts by root-tissue is dependent on a sufficient oxygen supply too, just as COLLANDER and HOLSTRÖM (1937) stated this for the uptake of sulphonic-acid dyes by the tissue of the vascularbundle strands. ARISZ and OUDMAN (1938) have found that also the intake of asparagine by the tissue of *Vallisneria* leaves is

connected with a sufficient supply of oxygen and that it is greatly impeded by a withdrawal of oxygen.

In a former publication (REINDERS 1940) I proposed the question whether the intake of water is an active process itself or the consequence of other active processes. Processes perhaps, by which osmotically active substances arise, the water being absorbed osmotically. It is known that with a strong respiration of potato tissue, as after wounding, the disappearence of starch is accompanied by an increase of the amount of soluble sugars (HOPKINS 1927; STEWARD, WRIGHT and BERRY 1932). It seems quite possible that such an osmotic suction, in consequence of the formation of osmotically active substances with processes linked to vital activity, will be the cause of the water-intake. This representation of the course of the process seems to be quite well in accordance with the results of BRAUNER, BRAUNER and HASMAN (1940). As to the water-intake a confirmation of my results is found, though the results are not easy to compare. It is to be regretted that the authors applied only a very short rinsing in running tap water (15 minutes) instead of the generally used treatment before hand of 24 hours. Therefore the tissue, probably having not reached maximal turgescence at the starting of the experiment, will show a passive water-intake at the beginning, which may be quite different with the various experiments. At the same time the authors have controlled the concentration of soluble sugars (glucose and saccharose) with the potato discs. It has turned out that during the four days' stay in aerated water the amount of sugar in the tissue increases steadily, especially after the first day, the increase of glucose being rather considerable. Under anaerobic conditions both sugar-fractions reduce their concentration steadily, while no water-intake is to be observed, in accordance with my results (REINDERS 1938, 1940).

Now let us consider what is known in literature about the question whether the intake of water is or is not an active process.

BENNETT CLARK, GREENWOOD and BARKER (1936) have made an investigation whether the protoplast of a plant-cell only functions as a passive semi-permeable membrane, so if the water-intake is only a passive phenomenon, in other words: if the osmotic suction pressure of the cell sap is the single power to absorb water. For this purpose they compare the osmotic value of the cell sap as determined by the incipient-plasmolytic method with that, which they determine by the cryoscopic method. With some objects, viz.with tissues no longer growing actively as the petiole of *Caladium* and of *Rheum*, they have found that the osmotic value determined by both methods turned out to be the same indeed. However, with other

objects, viz. potential growing tissues as e.g. mangold they have found that the so-called „osmotic value" of the cell sap as determined by the plasmolytic method is markedly greater than the osmotic pressure of the cell sap as determined by the cryoscopic method. From this they have inferred that the potential growing tissues have a positive water secretion from the external solution into the vacuole. The water-absorbing power (A_t) of the cells of those tissues is greater than that which corresponds with the osmotic value of the cell sap (P_t). $A_t = P_t + x$. The x is thus ascribed to the activity of the living protoplast.

The value of these results, however, we have to call in question as BUHMANN (1935) has pointed out that with incipient-plasmolytical investigations the adhesion of the plasm to the cell wall may be the cause that too high values for the concentration of the cell sap are to be found, as the phase of incipient plasmolysis begins with retardation.

Through the comparison of the osmotic pressure of the vacuolar sap and that of the protoplasmic sap, found by determining the concentration of their respective pressure saps, with that, found by the incipient-plasmolytic method with cells of the leaves of the cotton-plant, also MASON and PHILLIS (1939) come to the inference that the protoplasm drives water into the vacuole with a considerable active secretion pressure. However, we have to take into account that it is not sure that they have worked with real vacuolar sap. The concentration of this sap may be stronger, for with that cautious pressing we get the vacuolar sap filtrated through the protoplasm, by which process substances are very likely to stay behind. So the concentration of the pressure sap will be weaker than that of the real cell sap.

BROYER and HOAGLAND (1940) state that there is no evidence by any method of sap expression that a mineral solute can be absorbed against a concentration gradient except when conditions of aerobic metabolism prevail.

STILES and JØRGENSEN (1917) have stated that the tissue of potato discs and that of carrot discs in distilled water show a considerable swelling till an equilibrium is reached.

Yet the course of the process here is as it is not be compared with that. STILES and JØRGENSEN have used the discs immediately after cutting them, so without a previous washing. They find a rapid water-intake, especially at the beginning; 80 to 90 % of the total water-intake is absorbed in the first two days. Then the increase is only slight, an equilibrium is practically reached, which remains for a long time. A difference with my experiments is that very strong

intake in the beginning. The reason is that they work with freshly cut tissue which is not saturated with water, as is the case when we apply a treatment before hand of e.g. a washing during 24 hours in tap water. Therefore, it is not allowed to compare the water-intake with the experiments of STILES and JØRGENSEN with the figures found in this investigation. Another difference is the temperature at which the experiments are made: 13° C in the experiments of STILES and JØRGENSEN and 21° C in the present investigation. Furthermore STILES and JØRGENSEN have used unaerated water, while with the present investigation the water is continuously aerated. This last is probably the reason why with their experiments an equilibrium is soon reached, while with the present investigation the intake goes on for a longer time. But let us try to make a comparison between the values of fig. 1 of STILES and JØRGENSEN (pag. 419) and those obtained in the present investigation. With their experiments the increase of weight during the first 24 hours amounts to approx. 13 % of the initial fresh weight. The total increase in approx. 9 days amounts to about 19 %. So the increase in unaerated water during the last 8 days has been approx. 6 % of the initial fresh weight, that is a good 5 % of the fresh weight which is reached 24 hours after the beginning of the experiment, so after the cutting of the discs. In the present investigation a water-intake of approx. 12 % in aerated water in 8 days at a temperature of 11° C is found (Chapter II, § 4).

The possibility must also be considered that here we have to do with a water-intake by the swelling of the protoplasm colloids themselves. DE HAAN (1933) has found with *Allium* that during the deplasmolysis the condition of the swelling of the protoplasm is changed continuously, the condition of the swelling namely invariably increases, at which process the permeability for water increases.

Only one thing is sure, viz. that the intake of water is obviously linked to the vital activity of the protoplasm, while a sufficient oxygen supply is necessary. The most obvious course of the process is, that by the vital processes various osmotically active substances are formed through which the water may be absorbed osmotically. This need not be an osmotic absorption by the vacuole contents; in my opinion the osmotically active substances produced by such vital processes in and between the protoplasm-particles themselves, dissolved in the so-called free water of the protoplasm, may just as well be the cause of an osmotic absorption of water. However, STEWARD, STOUT and PRESTON (1940) suggest that water-absorption is brought about by metabolic processes in a manner not readily explained by any simple osmotic theory. Anyhow, we may expect

a certain correlation between the strength of the respiration and the growth.

That after some days the water-intake becomes less strong, is explicable as the tissue gets saturated with water after some time, on which occasion the wall pressure will play a part. Probably also the growth at the surface of the discs, the formation of a corklayer, may be the cause that less water is let through or that the cell wall is less extensible. We have also to consider that the water liberated by respiration increases the water-richness of the tissue, which is the cause that less water will be taken in from the external solution. It is possible that during the experiment the intensity of the respiration remains undiminished. Further it is possible that the respiration becomes weaker after some time, as is stated for potato discs in Chapter I, § 4. In consequence of that also the processes linked to it, as the intake of water, will take place to a smaller degree. It is not possible to say something positive about this question; with none of the objects used in this investigation the respiration is studied in the course of time, except with the potato. With the potato it has been found that the respiration remains constant during the first 4 days (page 25), while after 8 days a diminution is stated. STEWARD, WRIGHT and BERRY (1932) have found that the respiration of potato discs remains constant during a good 6 days. It is also about this time after the beginning of the experiment that the water-intake becomes less strong.

§ 2. The relation between the amount of the water-intake and the strength of the respiration.

With potato discs it has become evident that the water-intake is connected to a sufficient supply of oxygen; under anaerobic conditions no water is taken in. The very slight intake in an anaerobic environment during the very first day is probably due to the fact that the tissue has still some oxygen at its disposal. The inconsiderable energy which is liberated with the respiration under anaerobic conditions is apparently not able to make the tissue take in water. The intake of water therefore is connected with the spending of energy; also HENDERSON (1934) points to this.

There are phenomena which plead in favour of a close connection between respiration and water-intake, namely the coincidence of a strong respiration with a large water-intake and the coincidence of a weak respiration with a small water-intake, as we have met this in some cases with the various series of the same object (table 26, pag. 118). With the horse-radish e.g. the respiration is found to be 14.0 % of the initial dry weight and the water-intake amounts to

6.8 % of the initial fresh weight as the averages of one series of experiments; while with another series of experiments the respiration is 8.7 % accompanied by a water-intake of 5.7 %. Also with the Jerusalem artichoke the weakest average respiration coincides with the weakest average water-intake. The same is the case with the carrot and properly speaking also with the dahlia though less marked. With the potato discs, however, this regularity is not to be observed (see also page 23).

If we make a comparison between the various tissues as to the strength of the water-intake and that of the respiration (table 26, page 118), it is striking that in general the tissues with the largest water-intake (potato, mangold) have also the strongest respiration. COLLANDER (1939) also remarks that the objects he used, which show only a slight salt-accumulation, have also a slight respiration in contrast to the strong respiration of the objects which strongly accumulate salts in the experiments of STEWARD and his collaborators.

BERRY and STEWARD (1934) have found that no simple relation exists between the strength of the respiration and the bromide absorption of the various storage tissues. Only the tissues which are still capable of renewed growth, possess the capacity to accumulate the bromide ion. Living storage tissues incapable of renewed growth respire, but cannot accumulate salts. The Jerusalem artichoke e.g. shows a very large Br-intake — later on confirmed by STEWARD, BERRY and BROYER (1936) — together with a very weak respiration, while dahlia has a very small Br-intake and a somewhat stronger respiration than the Jerusalem artichoke. The potato practically has an equally strong respiration as the dahlia and a fairly strong Br-intake. The respiration which BERRY and STEWARD state here for potato is much smaller than they found formerly (1932). Furthermore the respiration of turnip and that of carrot are practically equally strong, while turnip shows a very large Br-intake and carrot has only taken in Br to a small amount. ASPREY (1937) states that NH_4 is taken in to about the same amount by the Jerusalem artichoke and by the potato, while the Jerusalem artichoke takes in Cl to a distinctly greater amount than potato does.

The figures for the respiration and those for the water-intake of various storage tissues found by BERRY and STEWARD (1934), as they are to be inferred from the difference between the initial and the final fresh weight of a stay in an aerated potassium bromide solution, are stated in table 28. In these figures some agreement is found with the results of the experiments of the present investigation, viz. the potato shows a large water-intake, while that of the Jerusalem

TABLE 28.

Data concerning the water-intake and the respiration of table I of Berry and Steward (1934). Temperature 23.2° C. The water-intake is stated as increase of the fresh weight in g per 40 discs of 1.6 mm thickness and 3.4 cm diam. during a 91 hours' stay in an aerated solution of 0.75 m aeq. KBr.

	water-intake	mg CO_2 per g tissue per hour
carrot	6.07	0.1967
mangold	10.20	0.1537
turnip	8.44	0.1972
Jerusalem artichoke	0.88	0.0889
dahlia	1.46	0.1313
potato	8.20	0.1335

artichoke and the dahlia is very slight in both cases. The water-intake of the mangold is somewhat stronger than that of the potato with the experiments of BERRY and STEWARD, just quite the reverse as in the present investigation; but we have to consider that the varieties of the potato as well as of the mangold will have been different. With the carrot a different tissue is used, viz. BERRY and STEWARD have taken discs tangentially cut from the bark, while in the present investigation transversally cut discs from the xylem-parenchyma are used. The turnip has probably been a quite different variety.

If we pay attention to the relation between the water-intake and the respiration (table 26) it turns out that this is not the same with the various tissues. So the water-intake of the potato is stronger in relation to the respiration than is the case with the mangold tissue. When we compare the dahlia with the Jerusalem artichoke, dahlia has a very slight water-intake in relation to the respiration.

So there is a relation between the water-intake and the respiration in so far that the energy of the aerobic respiration creates the conditions — in what manner it is difficult to say with certainty — in consequence of which water is taken in. Also the tissues with the largest water-intake have as a rule the strongest respiration. However, the relation is restricted to this. It seems to me most probable that the water-intake is only a secondary phenomenon, a consequence of other metabolic processes, by which osmotically acting substances arise. When the respiration is more intensive, probably more osmotically active substances may be formed, so that also more water may be taken in. The extra energy occurring with a stronger respiration, however, will not in all cases be the cause that more osmotically active substances arise through which a greater amount of water could be taken in. There is no reason to expect this, a part of the energy obtained by respiration will of course be used for one or

more of the many other processes which take place in a living tissue. So an indirect relation between water-intake and respiration seems to me the most obvious. STEWARD, STOUT and PRESTON (1940) also have found an indirect relation.

§ 3. The occurrence of visible growth-phenomena.

With potato discs in aerated distilled water we have seen that cell-divisions occur along the cut surface, in favourable cases regularly along the entire cut surface, in less favourable cases only in the neighbourhood of the phloem-groups (HABERLANDT). With none of the other investigated storage tissues cell-divisions occur during the eight-days' stay in aerated distilled water. These observations agree quite well with those of BERRY and STEWARD (1934), who say that with the fleshy storage tissues there is yet a great difference as to the morphological nature, nature of the storage substance and as to physiological activity. Tissues such as the pulp of an apple are not able to further growth, they don't regenerate either at a cut surface. Discs of many other tissues, however, are able to renewed growth, viz. discs of stem-organs e.g. the potato to a great extent and also the Jerusalem artichoke as well as discs of root-organs e.g. carrot, mangold, dahlia and others. The occurrence of cell-divisions takes place most actively in the tissue laying most closely to an existing phellogen or vascular-bundle cambium. The divisions occur in moist air and in most cases they are not completed during a stay of short duration in aerated water. Only in some cases, as with the potato tissue, cell-divisions are also formed in aerated water. KNY (1889) records that with potato tissue in moist air cell-divisions are already visible after two days, while with dahlia tissue this is not the case until after a lapse of seven days. TURNER (1938) also states that with carrot discs in water growth is never observed under the cut surface.

§ 4. The influence of hetero-auxin.

In the present investigation we have seen that hetero-auxin promotes the water-intake and also the respiration of potato discs. This promoting influence on the water-intake does not become evident until after two days. The influence on the respiration differs as to the duration of the action.

With discs of other storage tissues hetero-auxin exerts a similar influence. With the turnip and with the Jerusalem artichoke hetero-auxin also acts in a promoting way on the water-intake as well as on the respiration. With both comparatively even much stronger

than with the potato, especially with the Jerusalem artichoke, with which, however, we have to take into account that the extra loss of dry substance may partly be caused by exosmosis. With the carrot there is only a very slight, scarcely perceptible influence of the hetero-auxin; in so far as we can speak of an influence, however, it is a promoting one on both processes, on the water-intake as well as on the respiration. With the mangold the influence is not clear. With dahlia there is a promotion of the respiration, even to a fairly great degree, though we have to take into account that this extra loss of dry substance may partly be caused by exosmosis. The water-intake of dahlia discs is not promoted; in so far as we can speak of an influence this is even rather somewhat inhibiting instead of conducive.

As to the manner in which the hetero-auxin exerts its influence within the cell (SÖDING 1938; WENT 1939) it has become evident Chapter III, § 6) that this action certainly is exercised through processes which are connected with the aerobic metabolism. In an anaerobic environment no water-intake takes place nor is brought about by the hetero-auxin either. Hetero-auxin also has no influence on the loss of dry substance caused by the anaerobic respiration. BRECHT (1936) already states that the action of growth-substance on the curvation of *Avena*-coleoptiles is influenced by the oxygen of the air. For in an anaerobic environment no curvation is to be seen when the growth-substance is applied to one side. The experiments of SWEENEY and THIMANN (1938) have proved that the oxygen supply plays an important part with the working of hetero-auxin on the protoplasmic streaming in stronger concentrations. W. S. STEWART (1938) finds no influence of the hetero-auxin on the extensibility of dead cell walls, in contradiction, however, to the results of ROBBINS and JACKSON (1937).

An influence of pure auxin on the respiration has never been established. (KÖGL, HAAGEN SMIT and VAN HULSSEN 1936; VAN HULSSEN 1936; BONNER 1936). As to the hetero-auxin, ROBERTSON PRATT (1938) has stated that the oxygen consumption of wheat-seedlings, of which the seeds are treated beforehand with a hetero-auxin solution during the soaking period, is considerably larger than that of the controls. This influence does not become evident until a rapid growth has set in, that is about two days after the beginning of the germination; immediately after the soaking period there is no difference in oxygen consumption with the controls. HELLINGA (1940) on the other hand, who investigates the influence of various substances on the oxygen consumption of potato discs with the Warburg-manometer-technique, does not find an increase of the

respiration under the influence of hetero-auxin. The duration of the experiments of HELLINGA is only a small number of hours, so that, as HELLINGA also remarks, the possibility remains that the influence of hetero-auxin can assert itself only after a lapse of a longer time. In the present investigation the influence of the hetero-auxin on the water-intake does not become noticeable till two days have expired.

With the experiments of SWEENEY and THIMANN (1938) it has turned out that, besides with the oxygen supply, the influence of hetero-auxin on the protoplasmic streaming is also connected with carbohydrates consuming processes. That the action of hetero-auxin within the cell is connected with the available amount of carbohydrates has further been shown by SCHNEIDER (1938).

Various investigators have stated a disappearance of starch under the influence of hetero-auxin. (ALEXANDER 1938; BORTHWICK, HAMNER and PARKER 1936; DOSTÁL 1936, 1938; DOSTÁL and HOSEK 1937; KRAUS, BROWN and HAMNER 1936; MITCHELL and STUART 1938—1939; N. W. STUART 1938—1939). MITCHELL and MARTIN (1938) only state a transfer of materials within the plant to the place treated, while MITCHELL and HAMNER (1938) and also CZAJA (1938) have found that the plants treated with growth-substance show a greater increase of dry substance than untreated plants do. ZIKA (1939), too, who obtains a greatly increased yield and starch grains of more than normal size by treating the seed-potatoes with hetero-auxin, must be mentioned here, just as CHOLODNY and GORBOVSKY (1939) who find in most cases a temporary intensification of photosynthesis by hetero-auxin in very low concentrations.

DOSTÁL and HOSEK (1937) have observed a rapid break-down of the reserve-starch under the influence of hetero-auxin in the basic parts of the stem of *Circaea intermedia*, independent of any growth phenomenon. So this agrees with what has been observed with potato discs in the present investigation (microphotograph, page 60). In this investigation it has also appeared that hetero-auxin has no influence on visible growth phenomena; the number of cell-divisions of potato tissue is indeed somewhat smaller with the discs treated with hetero-auxin than with the control discs. Neither is this the case when hetero-auxin is given to the other objects with which no cell-divisions occur in aerated water. TURNER (1938) communicates also that the cambium of carrot discs does not become active, even if hetero-auxin is added. In the present investigation it has turned out that hetero-auxin has no influence on the occurrence of cell-divisions with any of the examinated objects. Furthermore, DOSTÁL and HOSEK have stated that an accumulation of reducing sugars takes place at the cost of this accelerated starch hydrolysis. That

means thus an increase of the amount of osmotically active substances. It is now an obvious fact to suppose that this causes the greater water-intake under the influence of hetero-auxin. In accordance to this the stronger concentrations of hetero-auxin, which have the greatest influence on the respiration, also cause a stronger promotion of the water-intake. The weak concentration 1 to 10^7 scarcely exerts any influence on either process.

It is, however, also possible that hetero-auxin moreover increases the plastic extensibility of the cell walls, e.g. by increasing the swelling of the intermicellaric substance (RUGE 1937, 1938*a*, 1938*b*). RUGE has found that the increase of the plastic and the elastic extensibility of the membranes by hetero-auxin is a process which is independent of life. A suchlike influence may just as well be a cause of the promoting influence of hetero-auxin on the water-intake.

When hetero-auxin is only applied during a short time, viz. only during the first two days as is done with potato discs, we may imagine that a beginning of the conducive influence on the break-down of carbohydrates takes place, as a consequence of which some more osmotically active substances may be formed. A slight increase of the plastic extensibility of the cell walls may be added to this. The result is a somewhat weaker promotion of the water-intake than in the case when hetero-auxin is given during the entire experiment. The break-down of the carbohydrates has evidently not proceeded far enough to cause a greater loss of dry substance; why this is even somewhat inhibited, is not clear. When the hetero-auxin is applied at a later point of time, viz. after a lapse of two days as is done with potato discs, there is indeed a very strong promoting influence on the respiration, while the water-intake is only promoted to a slight degree. In this case hetero-auxin has indeed a promoting influence, even a strong one, on the break-down of carbohydrates, while the water-intake is only promoted very slightly. We may suppose, that in this case an increase of the amount of osmotically active substances will occur in the same way as in the case when hetero-auxin is applied during the entire experiment. In my opinion the most probable explanation for the small conducive action on the water-intake in this case is, that the growth at the surface of the disc, the cork formation, has brought about already alterations in the first few days, by which the cell wall is less easily extensible now or is less permeable for water. Various authors (e.g. WENT; VAN SANTEN 1940) have found that aging cells become less sensible to growth-substance. RUGE (1938*a*) points out that with the aging of the cells alterations take place in the composition of the intermicellaric substance,

through which circumstance the sensibility to growth-substance decreases.

With the turnip e.g. hetero-auxin is strongly conducive to the respiration as well as to the water-intake, while with the carrot the influence is very slight on both processes. So there is to a certain extent a close relation between the influence of hetero-auxin on the water-intake and that on the respiration. However, not always a stronger influence on the respiration will give rise to comparatively equally more osmotically active substances. Thus hetero-auxin in the concentration 1 to 10^5 has a much stronger influence on the respiration of potato discs than the concentration 1 to 10^6 has; while the influence on the water-intake, however, is somewhat greater, but not strikingly so. Thus it is also to be explained that hetero-auxin is conducive to the respiration of certain objects, while the water-intake is not or only slightly influenced. This is e.g. the case with dahlia. Probably the influence of the hetero-auxin on the respiration takes place in such a manner that it does not cause an increase of the water-intake, but that it only promotes the loss of dry substance.

So the influence of the hetero-auxin on vital processes in close connection with the respiration is primary. If, as a consequence of this more osmotically active substances occur, a promotion of the water-intake may be the result. At the same time we have to consider an influence which hetero-auxin may have on the extensibility of the cell wall, which influence may be quite different with the various tissues. Together with other factors this influence may also determine the degree of the water-intake.

§ 5. Exosmosis into and absorption from the external solution.

With the determination of the electrical resistance of the external solution, as is carried out with the various storage tissues in the present investigation, we have seen that the course is principally the same with all of them. For in the beginning of the experiment the conductivity of the external solution increases, which means that exosmosis takes place. Later on, mostly after the lapse of a few days, this changes into an increase of the electrical resistance showing that the absorption becomes prominent now. This course of events is also stated by various investigators, e.g. STILES (1927) and also ASPREY (1937). With the experiments of these two authors the change of exosmosis into the increase of the electrical resistance, so to the prevalence of the absorption, occurs somewhat sooner than with my experiments; viz. in most cases already after the lapse of one to three

days, while in the present investigation this does not become perceptible until in the third two-days' period, at the earliest in the second two-days' period. Stiles states further that the exosmosis of potato tissue is larger than that of most other tissues, such as carrot, Jerusalem artichoke and others. Also ASPREY finds that the exosmosis of the Jerusalem artichoke is smaller than that of potato discs. This agrees with the results of the present investigation, namely the exosmosis of the majority of the objects examined is smaller than that of potato discs in the first few days. Also INGOLD (1931) who studies the exosmosis only during a short period (some hours) finds that the exosmosis of potato discs is larger than that of mangold and carrot.

With all the investigated tissues we have also observed that the p_H of the external solution in which the discs are, shows an increase in the first few days; with most of the other tissues to a somewhat slighter degree than with the potato, while later on the p_H changes only little with regard to that of the fresh water. Also BRAUNER, BRAUNER and HASMAN (1940) have stated that the p_H of distilled water rises after immersing of potato discs. This alkalization of the medium they ascribe to ammonia given off by the tissue. LEMMON (1936) also has stated that the p_H of the solution in which potato discs are, changes in the direction of the neutrality point; under conditions of high initial alkalinity the p_H decreases and the reverse. PETRIE (1938) finds that the p_H of solutions in which carrot discs are immersed (the solutions being initially more alkaline or more acid), drift with time towards the value 5.2 to 5.4.

It is possible, that the exosmosis in the first few days of the experiment may have a perceptible influence on the loss of dry substance, viz. in so far that this may be somewhat greater than the loss that corresponds with the respiration. In an anaerobic environment and with air-aeration, however, the exosmosis is quite the same or somewhat greater with the anaerobic series (Chapter II, § 1); while the loss of dry substance in the case of air-aeration is much larger than under anaerobic conditions, which points to the fact that it is actual respiration. Also the fact that a considerable loss of dry substance does take place in the following periods, in which instead of exosmosis just an absorption occurs, as is shown for potato discs on page 16, is a proof that this loss of dry substance is really caused by respiration.

It does not seem very probable that the exosmosis has an important influence on the loss of dry substance in the first few days. A very small quantity of material, such as 0.5 mg hetero-auxin in 500 cm^3 of water, so the concentration 1 to 10^6, already shows a considerable

decrease of the electrical resistance as is e.g. shown in table 16, pag. 62.

With all the objects Fehling's test-method is used as a reagent on the evaporated external solution at the end of each period. This reaction, however, yields in every case a negative result, at the end of the first two-days' period as well as later on. So exosmosis of sugar does not take place, a substance which may possibly have only a slight influence on the conductivity. This therefore strengthens the supposition that the loss of dry substance is really caused by respiration.

With the influence of hetero-auxin on the discs of the Jerusalem artichoke and also on dahlia discs the exosmosis of the hetero-auxin series has proved to be greater than that of the control series. So the extra loss of dry substance with these objects, occurring under the influence of hetero-auxin, will not be caused by an influence on the respiration only, but will partly be exosmosis. However, as a very small amount of materials causes already a considerable decrease of the electrical resistance of the external solution, the share of the exosmosis in the loss of dry substance may be supposed to be slight. The reaction with Fehling's solution on the evaporated external solution at the end of each two-days' period yields a negative result, so exosmosis of sugars cannot be proved.

With the other objects it has appeared that the exosmosis under the influence of hetero-auxin is not enlarged than with the control. So we may conclude that the promoting influence of the hetero-auxin on the loss of dry substance is really an influence on the respiration.

It is, however, not quite certain. The possibility remains that e.g. substances, which strongly influence the conductivity of the water, are absorbed, while under the influence of the hetero-auxin substances that influence the conductivity only very slightly, are lost. The fact that they are no sugars, as is proved by the reaction with Fehling's solution, however, makes this possibility very improbable.

Investigations of the respiration by determining the oxygen consumption or the carbon-dioxide production could throw light on this question.

These investigations are carried out in the Botanical Laboratory of the State University at Groningen. To the Director, Professor Dr W. H. Arisz, I wish to express my hearty thanks for his kind advice during the progress of the work and for his valuable criticism.

LITERATURE.

ALEXANDER, T. R., 1938. Carbohydrates of bean plants after treatment with indole-3-acetic acid. Plant Physiol. **13**, 845.

ARISZ, W. H. and OUDMAN, J., 1938. Effect of decreased oxygen pressure on absorption and transport of asparagine and caffeine in Vallisneria leaves. Chronica Botanica **IV**, 16.

ARISZ, W. H. and OUDMAN, J., 1938. Absorption and transport of asparagine in leaves of Vallisneria. Proc. Kon. Akad. v. Wetensch., Amsterdam **41**, 810.

ARTSCHWAGER, E., 1924. Studies on the potato tuber. Journ. of Agricult. Res. **27**, 809.

ASPREY, G. F., 1933. Studies on antagonism. I. The effect of the presence of salts of monovalent, divalent and trivalent kations on the intake of Ca and NH_4 by potato tuber tissue. Proc. Roy. Soc. London, Serie B, vol. **112**, 451.

II. The effect of previous immersion of potato tuber tissue in solutions of monovalent, divalent and trivalent kations on the subsequent absorption of the NH_4 ion. Proc. Roy. Soc. London, Serie B, vol. **113**, 71.

ASPREY, G. F., 1935. On the relationship between exosmosis and salt absorption by potato tuber tissue previously treated with various salt solutions. Protoplasma **24**, 497.

ASPREY, G. F., 1937. Some observations on the absorption and exosmosis of electrolytes by storage organs with particular reference to potato and artichoke tubers. Protoplasma **27**, 153.

BAPTISTE, E. C. D., 1935. The Effect of Some Cations on the Permeability of Cells to Water. Annals of Bot. **49**, 345.

BENNET-CLARK, T. A., GREENWOOD, A. D. and BARKER, J. W., 1936. Water relations and osmotic pressures of plant cells. New Phytologist **35**, 277.

BERRY, W. E. and STEWARD, F. C., 1934. The Absorption and Accumulation of Solutes by Living Plant Cells. VI. The Absorption of Potassium Bromide from Dilute Solution by Tissue from Various Plant Storage Organs. Annals of Bot. **48**, 395.

BONNER, J., 1936. The growth and respiration of the Avena coleoptile. Journ. of Gen. Physiol. **20**, 1.

BONNER, J., 1937. The role of vitamins in plant development. Botanical Rev. **3**, 616.

BONNER, J., 1937. Vitamin B_1 a growth factor for higher plants. Science **85**, 183.

BONNER, J. and ADDICOTT, F., 1937. Cultivation in vitro of excised pea roots. Bot. Caz. **99**, 144.

BONNER, J. and GREENE, J., 1938—1939. Vitamin B_1 and the growth of green plants. Bot. Gaz. **100**, 226.

BONNER, J. and GREENE, J., 1939. Further Experiments on the Relation of Vitamin B_1 to the Growth of Green Plants. Bot. Gaz. **101**, 491.

BORTHWICK, H. A., HAMNER, K. C. and PARKER, M. W., 1936—1937. Histological and microchemical studies of the reactions of tomato plants to indoleacetic acid. Bot. Gaz. **98**, 491.

BOSWELL, J. G. and WHITING, G. C., 1938. A study of the polyphenol oxidase system in potato tubers. Annals of Bot. N.S. **2**, 847.

BOSWELL, J. G. and WHITING, G. C., 1940. Annals of Bot. N.S. **4**, 257.

BOYSEN JENSEN, P., 1938. Growth Regulators in the Higher Plants. Ann. Rev. of Bioch. **7**, 513.

BRAUNER, L. und M., 1938. Untersuchungen über den photoelektrischen Effekt in Membranen II. Rev. Fac. Sci. Univ. Istanbul **III**, 3.

BRAUNER, L. and BRAUNER, M., 1940. Further studies of the influence of light upon the water intake and output of living plant cells. New Phytologist **39**, 104.

BRAUNER, L., BRAUNER, M. and HASMAN, M., 1940. The Relations between Water-Intake and Oxybiosis in living Plant-Tissues. Rev. Fac. Sci. Univ. Istanbul **V**, 266.

BRECHT, F., 1936. Der Einfluss von Wuchsstoff- und Säurepasten auf das Wachstum von Avena- und Helianthus-Keimlingen und seine Abhängigkeit vom Sauerstoffgehalt der Luft. Jahrb. wiss. Bot. **82**, 581.

BROYER, T. C. and HOAGLAND, D. R., 1940. Methods of sap expression from plant tissues with special reference to studies on salt accumulation by excised barley roots. Amer. Journ. of Bot. **27**, 501.

BUHMANN, A., 1935. Kritische Untersuchungen über vergleichende plasmolytische und kryoskopische Bestimmungen des osmotischen Wertes bei Pflanzen. Protoplasma **23**, 579.

BÜNNING, E., 1939. Die Physiologie des Wachstums und der Bewegungen. Lehrbuch der Pflanzenphysiologie II. Berlin.

CHOLODNY, N. G. and GORBOVSKY, A. G., 1939. Effect of indole-3-acetic acid on photosynthesis. Science, N.S. **90**, no. 2324.

COLLANDER, R., 1939. Permeabilitätsstudien an Characeen. III. Die Aufnahme und Abgabe von Kationen. Protoplasma **33**, 215.

COLLANDER, R. und HOLMSTRÖM, A., 1937. Die Aufnahme von Sulfosäurefarbstoffen seitens pflanzlicher Zellen. Ein Beispiel der adenoiden Tätigkeit der Protoplasten. Acta Soc. pro Fauna et Flora Fenn. **60**, 129.

C AJA, A. TH., 1938. Der Einfluss der Streckungswuchsstoffe auf die Massenverteilung in der Pflanze. Planta **28**, 354.

DAM, L. VAN, 1935. A method for determining the amount of oxygen dissolved in 1 c.c. of water. Journ. of Exp. Biol. **12**, 80.

DOSTÁL, R., 1936. Korrelationswirkung der Speicherorgane und Wuchsstoff. Ber. d. deutsch. bot. Ges. **54**, 418.

DOSTÁL, R., 1938. Les corrélations et les phytohormones. Etudes et recherches sur phytohormones, p. 101.

DOSTÁL, R. und HOSEK, M., 1936—1937. Über den Einfluss von Heteroauxin auf die Morphogenese bei Circaea (Das Sachssche Phänomen). Flora N.F. **31**, 263.

FUNKE, H., 1939. Über den Nachweis kleiner Wuchsstoffmengen. Jahrb. wiss. Bot. **88**, 373.

HAAN, I. DE, 1933. Protoplasmaquellung und Wasserpermeabilität. Rec. trav. bot. néerl. **30**, 234.

HAAN, I. DE, 1935. Ionenwirkung und Wasserpermeabilität. Ein Beitrag zur Koazervattheorie der Plasmagrenzschichten. Protoplasma **24**, 186.

HABERLANDT, G., 1913. Zur Physiologie der Zelltheilung. Sitzber. preuss. Akad. d. Wiss. Berlin, 1e Halbb., 318.

HABERLANDT, G., 1930. Über Zellteilungshormone. Scientia.

HAYWARD, H. E., 1938. The structure of economic plants. New York.

HELLINGA, J. J. A., 1940. On the Effect of Substances, produced by Fungi on the Respiration of the Tissue of Potato Tubers. I and II. Proc. Kon Akad. v. Wetensch., Amsterdam **43**, 249.

HENDERSON, L., 1934. Relation between root respiration and absorption. Plant Physiol. **9**, 283.

HOAGLAND, D. R. and BROYER, T. C., 1936. General nature of the process

of salt accumulation with description of experimental methods. Plant Physiol. **11**, 471.

HOAGLAND, D. R. and BROYER, T. C., 1940. Hydrogen-ion effects and the accumulation of salt by barley roots as influenced by metabolism. Amer. Journ. of Bot. **27**, 173.

HÖBER, R., 1926. Physikalische Chemie der Zelle und der Gewebe. Leipzig.

HOPKINS, E. F., 1927. Variation in sugar content in potato tubers caused by wounding and its possible relation to respiration. Bot. Gaz. **84**, 75.

HULSSEN, C. J. VAN, 1936. Ademhaling, gisting en groei. Een onderzoek over de werking van auxinen en van biotine. Diss. Utrecht.

INGOLD, C. T., 1931. On the Effect of Previous Treatment with Salt Solutions on Subsequent Outward Diffusion of Electrolytes from Plant Tissue. Annals of Bot. **45**, 709.

JÄRVENKYLÄ, Y. T., 1937. Über den Einfluss des Lichtes auf die Permeabilität pflanzlicher Protoplasten. Ann. Bot. Soc. Zool.-Bot. Fenn, Vanamo **9**, Nr 3, 1.

JOST, L., 1935. Wuchsstoff und Zellteilung. Ber. d. deutsch. bot. Ges. **53**, 733.

KLEIN, G., 1931. Handbuch der Pflanzenanalyse I. Wien.

KNY, L., 1889. Ueber die Bildung des Wundperiderms an Knollen in ihrer Abhängigkeit von äusseren Einflüssen. Ber. d. deutsch. bot. Ges. **7**, 154.

KÖGL, F., HAAGEN SMIT, A. J. and HULSSEN, C. J. VAN, 1936. Über den Einfluss unbekannter äusseren Faktoren bei Versuchen mit Avena sativa. Z. physiol. Chem. **241**, 17.

KRAMER, P. J., 1940. Causes of decreased absorption of water by plants in poorly aerated media. Amer. Journ. of Bot. **27**, 216.

KRAUS, E. J., BROWN, N. A. and HAMNER, K. C., 1936—1937. Histological reactions of bean plants to indoleacetic acid. Bot. Gaz. **98**, 370.

KRUYT, H. R., 1929. Inleiding tot de physische chemie. Amsterdam.

LEMMON, P., 1936. Respiration of potato tissue in relation to hydrogen-ion concentration of a surrounding solution. Amer. Journ. of Bot. **23**, 296.

LEPESCHKIN, W. W., 1940. Einfluss des Lichtes auf die Exosmose und Speicherung von Salzen im Kartoffelknollengewebe. Protoplasma **34**, 55.

LUCKÉ, B. and MC CUTCHEON, M., 1932. The living cell as an osmotic system and its permeability to water. Physiol. Rev. **12**, 68.

MASON, T. G. and PHILLIS, E., 1939. Experiments on the Extraction of Sap from the Vacuole of the Leaf of the Cotton Plant and their Bearing on the Osmotic Theory of Water Absorption by the Cell. Annals of Bot. N.S. **3**, 531.

MITCHELL, J. W. and HAMNER, C. L., 1938. Stimulating effect of beta (3) indoleacetic acid on synthesis of solid matter by bean plants. Bot. Gaz. **99**, 569.

MITCHEL, J. W. and MARTIN, W. E., 1938. Effect of indoleacetic acid on growth and chemical composition of etiolated bean plants. Bot. Gaz. **99**, 171.

MITCHELL, J. W. and STUART, N. W., 1938—1939. Growth and metabolism of bean cuttings subsequent to rooting with indoleacetic acid. Bot. Gaz. **100**, 627.

NOVY, F. G., 1925. Microbic respiration IV. The so-called aerobic growth of anaerobes: potato respiration. Journ. of Infect. Diseases. **36**, 343.

PAAUW, F. VAN DER, 1935. The Entrance of Water into Cut Leafy Shoots under Conditions which prevent Transpiration. Rec. d. trav. bot. néerl. **32**, 293.

PETRIE, A. H. K., 1938. The intake of ions by carrot tissue at different hydrogen-ion concentrations. New Phytologist **37**, 211.

PRATT, ROBERTSON, 1938. Influence of indole-3-acetic acid on the respiration and growth of intact wheat seedlings. Amer. Journ. of Bot. **25**, 389.

PRINGSHEIM, E. G., 1935. Untersuchungen über den Respirationsquotienten verschiedener Pflanzenteile. Jahrb. wiss. Bot. **81**, 579.

RASSENLIJST, 16e. Instituut voor Plantenveredeling, Wageningen, 1940.

REINDERS, D. E., 1938. The process of water-intake by discs of potato tuber tissue. Proc. Kon. Akad. v. Wetensch., Amsterdam **41**, 820.

REINDERS, D. E., 1940. Onderzoekingen over de opname van water door parenchymatisch weefsel, in het bijzonder door dat van den aardappelknol. Dissertatie Groningen.

ROBBINS, W. J. and BARTLEY, M., 1937. Vitamin B_1 and the growth of excised tomato roots. Science **85**, 246.

ROBBINS, W. J. and JACKSON, J. R., 1937. Effect of 3-indole acetic acid on cell walls of stem and root. Amer. Journ. of Bot. **24**, 83.

RUGE, U., 1937. Untersuchungen über den Einfluss des Hetero-Auxins auf das Streckungswachstum des Hypokotyls von Helianthus annuus. Zeitschr. f. Bot. **31**, 1.

RUGE, U., 1938a. Untersuchungen über die Aenderungen der osmotischen Zustandsgrössen und der Membraneigenschaften des Hypokotyls von Helianthus annuus beim normalen Streckungswachstum. Planta **27**, 352.

RUGE, U., 1938b. Über das Appositionswachstum decapitierter Hypokotyle von Helianthus annuus. Ber. d. deutsch. bot. Ges. **56**, 165.

SANTEN, A. M. A. VAN, 1940. Groei, groeistof en pH. Dissertatie Utrecht.

SCHNEIDER, C. L., 1938. The interdependence of auxin and sugar for growth. Amer. Journ. of Bot. **25**, 258.

SKOOG, F., BROYER, T. C. and GROSSENBACHER, K. A., 1938. Effects of auxin on rates, periodicity, and osmotic relations in exudation. Amer. Journ. of Bot. **25**, 749.

SÖDING, H., 1938. Die Rolle des Auxins in der höheren Pflanze. Zeitschr. f. Bot. **32**, 497.

STEWARD, F. C., 1930. Diffusion of certain solutes through membranes of living plant cells and its bearing upon certain problems of solute movement in the plant. Protoplasma **11**, 521.

STEWARD, F. C., 1932. The absorption and accumulation of solutes by living plant cells. I. Experimental conditions which determine salt absorption by storage tissue. Protoplasma **15**, 29.
II. A technique for the study of respiration and salt absorption in storage tissue under controlled environmental conditions. Protoplasma **15**, 497.

STEWARD, F. C., 1933. The absorption and accumulation of solutes by living plant cells. V. Observations upon the effects of time, oxygen and salt concentration upon absorption and respiration by storage tissue. Protoplasma **18**, 208.

STEWARD, F. C., 1937. Salt accumulation by plants. The rôle of growth and metabolism. Trans. Faraday Soc. **33**, 1006.

STEWARD, F. C. and BERRY, W. E., 1934. The absorption and accumulation of solutes by living plant cells. VII. The time factor in the respiration and salt absorption of Jerusalem artichoke tissue (Helianthus tuberosus), with observations on ionic interchange. Journ. of Exp. Biol. **11**, 103.

STEWARD, F. C., BERRY, W. E. and BROYER, T. C., 1936. The Absorption and Accumulation of Solutes by Living Plant Cells. VIII. The effect of oxygen upon respiration and salt accumulation. Annals of Bot. **50**, 345.

STEWARD, F. C. and HARRISON, J. A., 1939. The Absorption and Accumulation of Salts by Living Plant Cells. IX. The Absorption of Rubidium bromide by Potato Discs. Annals of Bot. N.S. **3**, 427.

STEWARD, F. C. and PRESTON, G., 1940. Metabolic Processes of Potato Discs under Conditions Conducive to Salt Accumulation. Plant Physiol. **15**, 23.

STEWARD, F. C., STOUT, P. R. and PRESTON, C., 1940. The Balance Sheet of Metabolites for Potato Discs Showing the Effect of Salts and Dissolved Oxygen on Metabolism at 23° C. Plant Physiol. **15**, 409.

STEWARD, F. C., WRIGHT, R. and BERRY, W. E., 1932. The absorption and accumulation of solutes by living plant cells. III. The respiration of cut discs of potato tuber in air and immersed in water, with observations on surface: volume effects and salt accumulation. Protoplasma **16**, 576.

STEWART, W. S., 1938. Extensibility of cell wall material in indole-3-acetic acid. Amer. Journ. of Bot. **25**, 325.

STILES, W., 1924. Permeability. London.

STILES, W., 1927. The exosmosis of dissolved substance from storage tissue into water. Protoplasma **2**, 577.

STILES, W. and JØRGENSEN, I., 1917. Studies in Permeability. V. The Swelling of Plant Tissue in Water and its Relation to Temperature and various Dissolved Substances. Annals of Bot. **31**, 415.

STUART, N. W., 1938—1939. Nitrogen and carbohydrate metabolism of kidney bean cuttings as affected by treatment with indole-acetic acid. Bot. Gaz. **100**, 298.

SWEENEY, B. M. and THIMANN, K. V., 1938. The effect of auxins on protoplasmic streaming II. Journ. of Gen. Physiol. **21**, 439.

THIMANN, K. V. and SWEENEY, B. M., 1937. The effect of auxins upon protoplasmic streaming. Journ. of Gen. Physiol. **21**, 123.

TURNER, J. S., 1938. The respiratory metabolism of carrot tissue. New Phytologist **37**, 232.

WEISS, J. E., 1880. Anatomie und Physiologie fleischig verdickter Wurzeln. Flora **63**, 97.

WEISS, J. E., 1883. Das markständige Gefässbündelsystem einiger Dikotyledonen in seiner Beziehung zu den Blattspuren. Hab. Schrift.

WENT, F. W., 1939. Growth Hormones in the Higher Plants. Ann. Rev. of Bioch. **8**, 521.

WENT, F. W., 1939. Analyses and Integration of various auxin effects. I an II. Proc. Kon. Akad. v. Wetensch., Amsterdam **42**, 581, 731.

WENT, F. W. and THIMANN, K. V., 1937. Phytohormones. New York.

WHITE, Ph. R., 1937. Vitamin B_1 in the nutrition of excised tomato roots. Plant Physiol. **12**, 803.

ZIKA, M., 1939. Über die Beeinflüssung der Stärkekorngrösse bei Solanum tuberosum durch β-indolylessigsäure. Planta **30**, 151.