Botany. — *The process of water-intake by discs of potato tuber tissue.* By DIRKJE E. REINDERS. (Communicated by Prof. J. C. SCHOUTE.)

(Communicated at the meeting of June 25, 1938.)

§ 1. *Introduction.*

In all experimental work done with potato tuber discs no special attention has been given to the increase of weight they show in water.

The phenomenon was noticed by BERRY and STEWARD (Annals of Bot. 48, 1934), who give in table I the fresh weight of the discs of different storage tissues at the beginning and also at the end of a stay in an aerated solution of 0.75 m aeq. K Br. We see an increase in weight in general, though very divergent for the different kinds of storage tissues. The experiments now to be described refer to the intake of water from pure water, without any salts.

In my preliminary experiments the increase in weight was found to be greater when the water is aerated than when it is not. This seemed to be an indication that it might be an active process depending on the respiration, as is the case with the intake of salts.

The aim of this investigation is to study this increase in weight. It can be supposed to be the resultant of: 1. An increase by intake of water. 2. A decrease by respiration.

§ 2. *Method.*

All experiments were carried out with the same potato-variety called "Friesche roode star". Potatoes of as far as possible the same size and a regular oblong form are used. The discs of 1 mm thickness and a diameter of 1.7 cm are cut perpendicular to the morphological longitudinal axis of the stolone with a handmicrotome according to F. C. STEWARD (Protoplasma 11, 1930).

The whole apparatus is chromed, so that the tissue does not come into contact with brass. After rinsing for a short time in running tap water, the discs are left for 24 hours in stagnant tap water. Next day the discs from one and the same potato are weighed, and are arranged in series of 10 discs each, with the same middle-weight. Weighing was done on a torsionbalance (HARTMANN–BRAUN), after they had been superficially blotted off for 15 sec. under a weight of 200 grams (see E. C. D. BAPTISTE, Annals of Bot. 49, 1935).

Glass beakers of 600 cc (Jena Geräteglas 20.) are filled with 500 cc distilled tap water (distilled over Jena glass).

In an opening of a paraffined cork plate over the glass beaker a cylinder of Jena-glass is fixed, over which, on the underside, coarse-meshed tulle is stretched. In this are the potato discs at a distance of 1 cm below the

watersurface. The water is aerated. After each observation the water (or, as the case may be, hetero-auxine solution) is renewed. The experiments are done in a room for constant temperature of 21° C.

For the determination of the dry weights the discs are dried in an oven of 96° C.

§ 3. *Experiments.*

The increase in weight is caused by intake of water, which is a vital process.

When potato discs are put into water and their weight is determined from time to time, this is found to increase. That the respiration of the discs in aerated water is fairly vigorous is seen in table I. The respiration gives a loss of dry matter in 8 days from 53—71 mg.

TABLE I. Water intake of 10 discs of 1 mm thickness and 1.7 cm diam. from 500 cc aerated distilled tap water in 8 days. temp. 21° C.

Experiment No.	Fresh weight in mg 10 discs together		Increase in mg	Increase in % of original fresh weight	Dry weight in mg 10 discs together		Loss of dry matter by respiration in mg	Loss of fresh weight through this in mg	Water intake in mg 10 discs together
	At beginning	After 8 days			At beginning	After 8 days			
			a					b	a+b
1	2538.0	3310.8	772.8	30.5	449	378	71	28.4	801.2
2	2580.9	3166.4	585.5	22.7	457	404	53	21.2	606.7
3	2548.2	3022.2	474.0	18.6	384	318	66	26.4	500.4
4	2733.7	3420.3	686.6	25.1	489	422	67	26.8	713.4

If we suppose that the material respired is glucose, and that this is completely broken down to CO_2 and water, the CO_2 will pass off into the air and the water will remain in the discs. A respiration of 60 mg glucose therefore means a reduction of the fresh weight of $60/180 \times \times (180 - 6 \times 18) = 24$ mg. That in spite of this the discs increase in weight can only be accounted for by their taking in water. This water intake is then the sum of the increase in fresh weight found + the amount of the decrease in fresh weight caused by respiration (last column table I).

The course of the increase in weight in aerated water is shown in fig. 1 curve *A*, whilst curve *B* represents the increase in weight in unaerated water, which is considerably less, and amounts only to about the half of the increase in aerated water. Experiments which last longer than a few days are hardly possible in unaerated water; the water becomes turbid and the discs flabby, in spite of the water being constantly renewed. In

aerated water, however, the discs can be kept alive for an indefinite time (tests of one month and longer). Curve C of fig. 1 indicates the increase

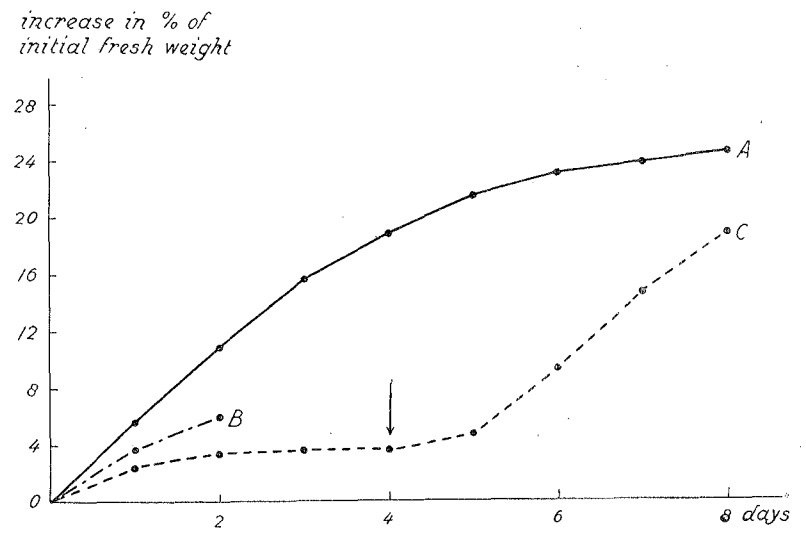

Fig. 1. Table II, exp. 11.

A. shows the increase in fresh weight in % of the initial fresh weight in aerated water.

B. shows the same in unaerated water.

C. shows the same in an environment free from oxygen, from ↓ aeration with air is again applied.

in weight in an environment free from oxygen (anaerobic jar of McINTOSH and FILDES). It is seen that here, as in most cases (see also table II) there is still a slight increase in weight in the first 24 hours, and that the weight then remains constant. The air was freed from oxygen by binding the oxygen by means of hydrogen (controlled with a reduced methylene blue solution) except in experiment 9 (table II), in which purified nitrogen gas continued to pass through for 3 days (the methylene blue solution not remaining quite reduced). The weight in that case continues slightly to increase, and does not come to a standstill as in the other experiments. It is possible to leave the discs both for 2 days and for 4 days in the environment without oxygen without any deleterious effects worth mentioning. If the discs are then put back into ordinary conditions, i.e. if they are again aerated with air (experiments 6—11 of table II), the weight again begins to increase. In the first 24 hours the increase is slight, but afterwards stronger and goes on with pretty well the same rapidity as the controls at the beginning. This is even the case with experiment 10, where we see a small loss in weight from the 3rd to the 4th day.

There is, however, microscopically a difference with regard to the occurrence of cell-divisions. Studying potato tuber discs after a stay in aerated water microscopically, I saw, in accordance with STEWARD

TABLE II. Water-intake of 3 series of 10 discs of the same potato, each in 500 cc distilled tap water.

Series A is aerated, series B is not aerated and 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.

Experiment No.		Increase in fresh weight in % of initial fresh weight after:							
		1	2	3	4	5	6	7	8 days
5	A control		10.3		19.2				
	B unaerated		3.8		8.1				
	C without O_2		2.0		1.6				
6	A control	4.8	9.1	12.8	15.5	18.0	20.6		
	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		
7	A control	5.1	9.6	13.7	17.0	19.6	21.7		
	B unaerated	3.6	5.1						
	C without O_2	2.3	1.8 ↓	2.7	6.9	12.3	16.7		
8	A control	4.1	8.4	12.2	15.8	18.6	20.6		
	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		
9	A control	6.0	11.4	15.2	18.8	21.7	23.7	24.6	
	B								
	C without O_2 (N_2 passing through)	2.1	2.8	3.6 ↓	6.3	11.0	16.3	20.3	
10	A control	6.5	12.7	18.0	23.3	26.8	29.0	30.0	31.0
	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	8.3
11	A control	5.6	10.8	15.5	18.8	21.4	23.0	23.8	24.5
	B unaerated	3.7	4.9						
	C without O_2	2.4	3.4	3.6	3.6 ↓	4.6	9.2	14.6	18.7

(Protoplasma 16, 1932), a fairly considerable starch depletion in the surface region, whilst in the most cases almost a regular cambium had developed, having formed 3—4 new cell-walls in one original cell. In less favourable cases only the cells in the neighbourhood of the phloeem groups showed regular cell-divisions, and elsewhere cell-divisions occurred more sporadically.

In unaerated water cell-divisions never occurred.

Discs which have been in an environment free from oxygen for 2 days, and then have been aerated with air for 4 days, when examined microscopically after the experiment, show somewhat fewer, but still almost as many cell-divisions as the controls which have constantly been aerated with air. In experiment 8 (table II) e.g. we find that at *B* "unaerated" (water intake 14.6 %) no cell-divisions have occurred, whilst at *C* "without O_2" (water intake 10.9 %) they have and almost as many as in *A*.

With discs, however, of which the stay in an environment without oxygen has lasted 4 days, after which they have then been aerated for 4 days with air, no further cell-divisions occur. After a stay of 4 days in the anaerobic jar they have therefore practically lost the power to make cell-divisions.

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

If, instead of at the beginning of the experiment, potato discs are put into an environment without oxygen later on, after having first been aerated for 4 days with air, they are no longer able to support this. There is a great loss in weight in the course of the first day, which is continued in the second 24 hours. If the discs are again put into an environment aerated with air, this decrease comes to a standstill, and there is a tendency to recover — that is, the weight again begins to increase, but only slightly.

In connection with the experiments in an environment without oxygen, a few experiments were also carried out in an environment rich in oxygen, by aerating with 100 % oxygen (extra pure), which had first been passed through two washing bottles with distilled tap water. The result is this: the increase in fresh weight of the series rich in O_2 is somewhat less than that of the controls (17.0 % resp. 19.4 % in 4 days), whilst the dry weight of the series rich in O_2 is also a trifle less. The differences, however, are so slight that aerating with 100 % oxygen may be said to have practically no effect.

Effect of temperature.

In addition to those at 21° C, experiments were also made at 1—2° C, at 10—11° C, at 25° C, and at 30° C, all aerated with air, which had previously been brought up to that temperature. At 30° C no result was obtainable; the discs died after a short time, viz. a few days.

In table III the result is shown. For each experiment a control series of 10 discs at 21° C and a series of 10 discs of the same potato at another temperature, e.g. 25° C were taken in all cases.

TABLE III.

	Increase in fresh weight in % of initial fresh weight after:			
	2 days	4 days	6 days	8 days
21° C (average of 5 experiments)	12.5	20.3	24.8	27.1
25° C (average of 5 experiments)	17.1	25.9	30.3	32.4
21° C (average of 3 experiments)	10.3	16.4	20.5	22.8
10—11° C (average of 3 experiments)	4.7	8.6	11.0	12.7
21° C (average of 4 experiments)	11.3	20.1	25.7	28.5
1—2° C (average of 4 experiments)	1.6	3.0	4.8	6.6

We therefore see here that the process is gone through more rapidly according as the temperature is higher. The relation between the increases at lower and higher temperatures does not remain constant, but varies in the sense that the increase at the higher temperature becomes in course of time relatively less great than that at the lower temperature; e.g. after 2 days the increase at 21° C is 7.0 times as great as that at 1—2° C, whilst after 8 days it is no more than 4.3 times as large.

Effect of hetero-auxine.

Hetero-auxine was found to have a remarkable effect, which, as we shall see, is double, viz. a favourable one both on the respiration and on the water intake.

Various concentrations were worked with. The concentrations 1 to 1000 and 1 to 10.000 were found to have an injurious effect, the discs being limp and dead after a short time.

The lower concentrations 1 to 10^5, 1 to 10^6, and 1 to 10^7 proved to exert such an influence that the increase in weight in the first two days is slighter than that of the controls, whilst in the next days a promotion of the increase in weight (i.e. of the water-intake) is seen. The concentration 1 to 10^5 has the strongest effect, 1 to 10^6 somewhat less, whilst the concentration 1 to 10^7 has only a very slight effect.

The type of this course is shown in fig. 2 for a hetero-auxine solution of 1 to 10^5.

If we do not renew the hetero-auxine solution after each observation we

find the same effect, but less pronounced. E.g. the increase in weight in % of initial fresh weight is in 8 days in dist. tap water 22.5 %; in hetero-auxine solution 1 to 10^6 26.5 %; in the same (not renewed) 23.8 %.

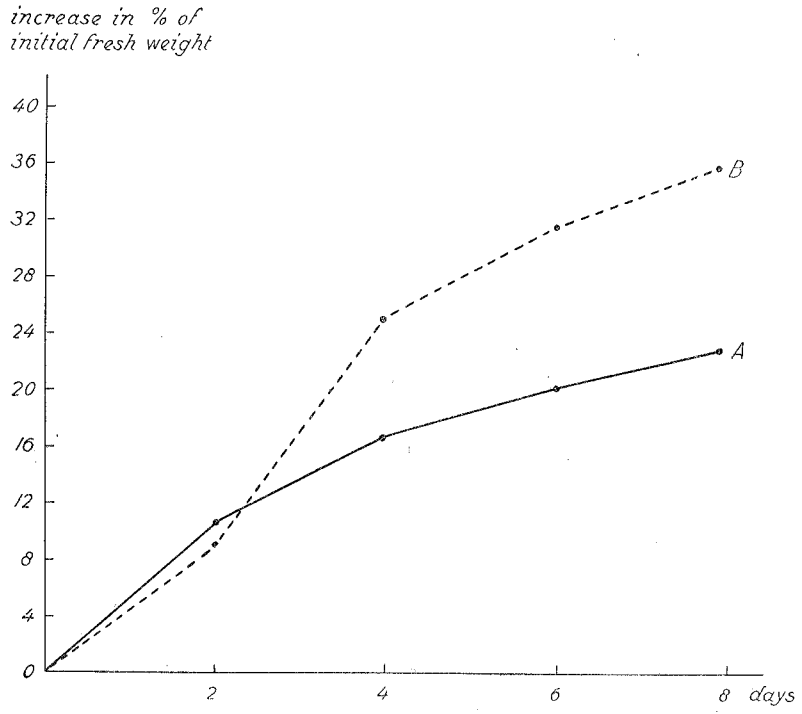

Fig. 2. Table IV, exp. 26.

A. shows the increase in fresh weight in % of the initial fresh weight of 10 discs in aerated dist. tap water.

B. the same of 10 discs of initially equal fresh weight in an aerated solution of hetero-auxine 1 to 10^5.

Some deviations of this normal course occur, in which there is no difference in the increase in weight in the first two days between the discs in hetero-auxine solution and those in water.

If we determine the dry weight at the end of the experiment, that is after a stay of 8 days in the hetero-auxine solution or in water, as the case may be, we see a great difference (table IV). The discs are found to have lost much more dry matter under the influence of the hetero-auxine. The concentration 1 to 10^5 gives an extra loss of 40—53 mg; 1 to 10^6 an extra loss of 9—16 mg, whilst 1 to 10^7 is found to have practically no effect whatever.

It can also be observed microscopically that more starch has disappeared out of the cells under the influence of the hetero-auxine. This is already noticeable after 8 days, but with an experiment which was continued for 5 weeks the cells of the discs which had been in hetero-auxine 1 to 10^6 were practically empty, there being only sporadically a few grains of

TABLE IV. Water intake of potato discs in water and in hetero-auxine solution in 8 days at 21° C.

In each exp. 10 discs in 500 cc aerated dist. tap water and 10 discs in 500 cc aerated hetero-auxine solution, all figures are for the 10 discs together.

Hetero-auxine concentration	Experiment No.	A: Initial fresh weight in mg, Control	B: Initial fresh weight in mg, Hetero-auxine	C: Control discs, final fresh weight in mg	D: Increase in mg	E: Increase in % of initial fresh weight	F: Hetero-auxine discs, final fresh weight in mg	G: Increase in mg	H: Increase in % of initial fresh weight	J =G—D: Hetero-auxine discs more increased than the controls in mg	K: Dry weight in mg, Control	L: Dry weight in mg, Hetero-auxine	M =K—L: Hetero-auxine discs, extra respirated in mg	P: Loss in fresh weight due to extra respiration in mg	R =J+P: Hetero-auxine discs, extra water taken up in mg
1 to 10^5	24	2763.9	2763.5	3607.8	843.9	30.5	3950.4	1186.9	42.9	343.0	411	364	47	18.8	361.8
	25	2538.0	2539.0	3310.8	772.8	30.5	3644.6	1105.6	43.6	332.8	378	338	40	16.0	348.8
	26	2580.9	2580.7	3166.4	585.5	22.7	3500.6	919.9	35.6	334.4	404	351	53	21.2	355.6
1 to 10^6	27	2717.9	2718.0	3489.4	771.5	28.4	3811.2	1093.2	40.2	321.7	360	351	9	3.6	325.3
	28	2664.2	2661.1	3203.8	539.6	20.3	3304.0	642.9	24.2	103.3	362	350	12	4.8	108.1
	29	2705.5	2705.1	3326.5	621.0	23.0	3542.8	837.7	31.0	216.7	468	452	16	6.4	223.1
	30	2641.4	2642.5	3280.9	639.5	24.2	3453.1	810.6	30.6	171.1	370	361	9	3.6	174.7
	31	2730.8	2719.1	3263.8	533.0	19.5	3507.8	788.7	29.0	255.7	358	342	16	6.4	262.1
1 to 10^7	32	2630.9	2630.9	3273.6	642.7	24.4	3319.0	688.1	26.1	45.4	397	400	—3	—1.1	44.3
	33	2563.4	2563.3	3111.9	548.5	21.5	3165.2	601.9	23.5	53.4	374	368	6	2.4	55.8

starch present. In the cells of the control discs there was still a great deal of starch present.

The reaction with FEHLING's experimental liquid on the evaporated hetero-auxine solution when exp. is finished, is negative; it is therefore not an effect on the permeability which makes the exosmosis of sugar more rapidly.

So I come to the conclusion that this extra-loss in dry weight will be due to a more vigorous respiration caused by hetero-auxine.

If then, we again suppose the respired substance to be glucose, that means that an extra respiration of 47 mg (Experiment 24, table IV) is the cause of a decrease of the fresh weight from $47/180 \times (180 - 6 \times \times 18) = 18.8$ mg. The water intake of the hetero-auxine discs is therefore not 343.0 mg more than that of the controls, but $343.0 + 18.8 = = 361.8$ mg more.

What the connection really is between the effect on the respiration and that on the water intake I tried to unravel by making the following experiments with hetero-auxine 1 to 10^6. Instead of giving the discs hetero-auxine solution during the whole 8 days of the experiment, hetero-auxine was only given for the first 2 days, after which this was replaced by dist. tap water.

With each such experiment, therefore, there are two control series.

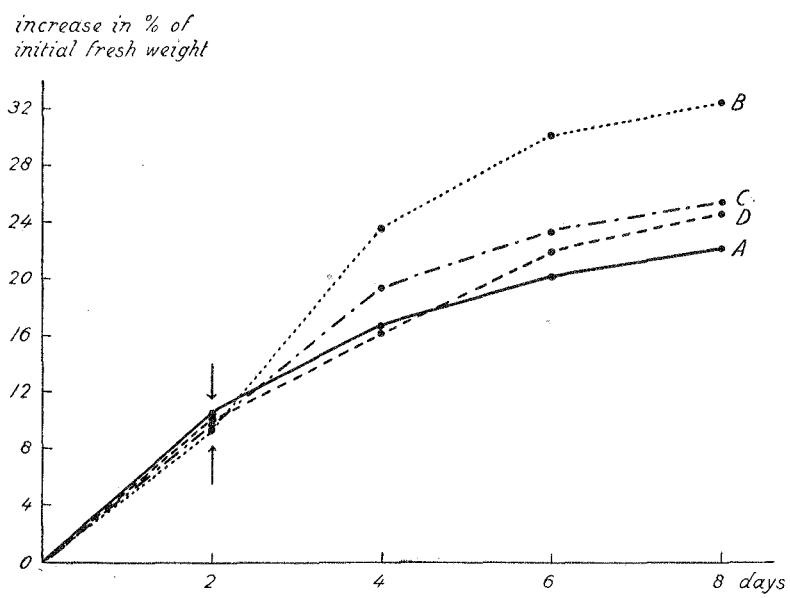

Fig. 3. Table V, exp. 38.
Water-intake of 4 series of 10 discs each, of the same potato from aerated dist. tap water and hetero-auxine 1 to 10^6 at 21° C.
A. dist. tap water.
B. hetero-auxine 1 to 10^6.
C. first 2 days hetero-auxine 1 to 10^6, from ↑ dist. tap water.
D. first 2 days dist. tap water, from ↓ hetero-auxine 1 to 10^6.

TABLE V. Water-intake of potato discs in aerated dist. tap water with or without the addition of hetero-auxine. At ↓ hetero-auxine 1 to 10^6 is replaced by water, or water is replaced by hetero-auxine 1 to 10^6.

Experiment No.		Initial fresh weight of 10 discs together in mg	Increase in % of initial fresh weight				Dry weight of 10 discs together in mg
			After 2 days	After 4 days	After 6 days	After 8 days	
34	A water	2641.4	10.1	17.0	21.8	24.2	370
	B hetero-auxine 1 to 10^6	2642.5	8.8	21.3	27.1	30.6	361
	C 2 days hetero-auxine 1 to 10^6, then water	2642.4	9.0 ↓	18.8	24.6	27.5	375
35	A water	2730.8	7.9	13.1	17.1	19.5	358
	B hetero-auxine 1 to 10^6	2719.1	7.9	19.6	25.7	29.0	342
	C 2 days hetero-auxine 1 to 10^6, then water	2721.7	7.9 ↓	16.3	20.8	23.7	363
36	A water	2612.3	10.3	18.5	23.8	26.2	366
	B						
	C 2 days water, then hetero-auxine 1 to 10^6	2613.3	10.2 ↓	17.0	23.6	27.0	353
37	A water	2717.9	10.1	20.9	25.8	28.4	360
	B hetero-auxine 1 to 10^6	2718.0	8.8	28.2	36.8	40.2	351
	C 2 days water, then hetero-auxine 1 to 10^6	2716.9	10.6 ↓	21.2	29.2	32.1	346
38	A water	2516.3	10.5	16.6	20.1	22.0	320
	B hetero-auxine 1 to 10^6	2517.7	9.3	23.4	29.9	32.2	310
	C 2 days hetero-auxine 1 to 10^6, then water	2516.4	9.6 ↓	19.2	23.3	25.3	321
	D 2 days water, then hetero-auxine 1 to 10^6	2519.1	10.1 ↓	15.9	21.8	24.5	312

viz. one which was always given dist. tap water, and the other always hetero-auxine solution (table V, experiments 34, 35, and 38).

We see that there is an after-effect (curve C, fig. 3). The fresh weight increases more than in dist. tap water, but less than when hetero-auxine solution is given during the entire experiment.

The dry weight, determined at the end of the experiment, is, however, practically equal to that of the series which was the whole time in dist. tap water (e.g. table V, exp. 34, 375 mg and 370 mg respectively, and also exp. 38, 321 mg and 320 mg resp.).

Perhaps we may expect that here, too, somewhat more dry matter would be respired than in dist. tap water, but the action of hetero-auxine lasted so short a time (only 2 days), that the differences are too small to fall outside the limit of error ($\pm$ 7 mg).

The converse experiment is this: dist. tap water is given for the first two days, and hetero-auxine for the next 6 days. Here, too, there are two controls, one which is always given dist. tap water and the other always hetero-auxine solution (table V, exp. 36, 37, and 38).

We now see (fig. 3, curve *D*), although to a slighter extent, the typical course of the curve, viz. the first 2 days (third and fourth day from the beginning of the experiment) no promotion of the water intake, whilst afterwards there *is* such a promotion. If we now determine the dry weight at the end of the experiment, we find that here, as was the case when hetero-auxine was given during the entire experiment, more dry matter has been respired than with the series in dist. tap water (e.g. table V, exp. 37, 346 mg and 360 mg resp.).

Recapitulating the results of these latter experiments, it may therefore be said that the influence which hetero-auxine exerts is most strongly manifested if it is given at the very beginning of the experiment. Discs which have first spent a few days in aerated dist. tap water are able to react to hetero-auxine in the same way, but no longer to the same extent. There is an after-effect on the water-intake, and not on the respiration under the influence of hetero-auxine given in a former period. The course of events may perhaps be imagined as follows: the extra energy which the discs will obtain by the more vigorous respiration under the influence of hetero-auxine will enable them to perform their vital functions in a more intensive fashion, so that we see a promotion of the water-intake in this case.

Besides these effects on the respiration and on the water-intake it is further to be noted that discs in hetero-auxine solution at the end of an experiment are somewhat thicker than the controls (there is so much more water in them), whilst microscopically the cells also give the impression of being somewhat larger. Cell-divisions in the layers under the cut surface occur to a much smaller extent than with the controls.

I desire to thank Prof. Dr. W. H. ARISZ heartily for his good advice.

Summary.

1. The increase in weight undergone by discs of potato in water is due to water-intake.

2. The intake of water is a vital process; no water is taken in in an environment without oxygen.

In an environment rich in oxygen no more water is taken in than with aeration with ordinary air.

3. The process depends on the temperature; it is gone through more rapidly at a higher temperature.

4. Hetero-auxine exerts a double influence, promoting both the respiration and the water-intake.

Laboratory for Plant Physiology.

Groningen, June 1938.

LITERATURE.

W. E. BERRY and F. C. STEWARD, 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 (1934).

F. C. STEWARD, 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 (1930).

E. C. D. BAPTISTE, The effect of some cations on the permeability of cells to water. Annals of Botany **49**, 345 (1935).

F. C. STEWARD, R. WRIGHT and W. E. BERRY, 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 upon surface: volume effect and salt accumulation. Protoplasma **16**, 576 (1932).