POLYSACCHARIDE CHANGES IN THE CELL WALLS OF WATER ABSORBING POTATO TUBER TISSUE IN RELATION TO AUXIN ACTION

BY

A. CARLIER [1] AND K. BUFFEL

(Carnoy-Institute, University of Louvain)

(received July 1st, 1955)

CONTENTS

INTRODUCTION

Since the researches of STILES and JÖRGENSEN in 1917 water absorption by cut out plant reserve tissues has continuously aroused interest. Indeed, it was soon realized that this phenomenon is not of a simple osmotic nature. The fundamental work of REINDERS at Groningen, published from 1938 to 1942, in which potato tuber tissue was the experimental material, established clearly the relation of water intake to the metabolism of the parenchyma cells, for oxygen uptake proved to be a strictly indispensable condition.

Moreover, from the view point of auxin physiology, it was of great importance that Reinders found a distinct activating influence of growth substances upon the water absorption. Afterwards much attention has been paid to this remarkable fact, as it seemed possible that more light would be thrown by this way upon the very mechanism of auxin action itself. A short review of some important auxin researches on potato tissue will be given in the further discussion. One point however should be stressed here, since it has been the immediate motive for the experiments to be described. As far as we know, there has not yet been published any extensive study concerning active wall growth during water absorption in potatoes, yet one might readily believe that real cell enlargement is a possible factor in this process, although many workers in this field do not seem to expect anything like this.

---

[1] University Lovanium, P.O.B. 2158, Leopoldville, Belgian Congo.

In 1951 some preliminary experiments, which were carried out in our laboratory at Louvain by P. VANDERVEKEN, showed very convincingly that during a ten day water intake the cellulose content of potato slices was considerably increased (see VANDERVEKEN, 1955). Afterwards a much more detailed research has been undertaken in the same laboratory, in which we studied not only cellulose formation, but also the building up of furfurogenic substances in the potato cell walls, as well as the influence of growth substance on these synthesis processes. As some facts which were found recently might throw a new light on the *immediate* factors of auxin action upon elongation, we think it will be interesting to communicate the more important of them.

The question of the primary action mechanism of auxins will be left out of consideration here. We only refer to the interesting suggestion of ANKER (1953), assuming the action of auxins on lipoid phase bound-aries around enzymatic centres within the protoplasm (e.g. chondrio-somes, see also KETELLAPPER, 1953).

Some parallel results on cell wall extensibility will be described along with the findings of biochemical nature.

The influence of mannitol on water absorption and polysaccharide metabolism has also been studied. ·

MATERIAL AND EXPERIMENTAL PROCEDURE

The experiments were carried out with the central reserve paren-chyma of tubers from the potato variety 'Bintje'. Special attention was paid to aseptic conditions during water intake. Starting from the finding of HACKETT (1952) that considerable water absorption may occur when the tissue is kept at the water surface, no aerating devices then being necessary, we put our potato blocks in large culture tubes upon a 1 % agar gel. The blocks, about 2″ long, ½″ large and ¼″ thick, were cut out aseptically by means of a special apparatus made from monel alloy. All manipulations at the beginning of the experiment were done in orange light. Afterwards the blocks were left in the dark in a thermostatic room at 20° C.

Every experimental series comprised from 12 to 15 blocks. After water intake, which lasted for several days, the rare infected tubes could be easily recognized. Since the blocks were kept individually per culture tube, they could be eliminated without troubling the experimental series as a whole.

At the end of the experiment water absorption was measured from the increase in fresh weight of the blocks. Immediately afterwards they were dried by lyophilization. [1] Following this treatment the tissue is easily ground to a fine uniform powder, which may be kept apart for chemical analysis.

Extensibility measurements were effectuated on blocks of the same

initial dimensions as indicated above, which were plasmolysed in 20 % mannitol solution. The further mentioned figures (Table IV) give the % elongation with a total load of 52.2 gram, the two black ink marks on the plasmolysed tissue being at a distance of 40 mm.

In order to get some insight into the question, whether cell wall formation is a cause or a consequence of the surface increase of the wall, mannitol has been added in some experiments to the agar in the culture tubes. By the osmotic pressure which it brings about, this substance hinders water intake to a considerable extent. As may be seen from Table I a 4 % concentration reduced the fresh weight increase for about 60 %. Mannitol has much been used as an osmotic growth retarder, because it permeates very slowly and because in general it is looked upon as a metabolically inert material, which cannot be used as a nutrient factor by the cells. Some of the present results suggest however, that this substance is not quite so harmless, as it might seem.

TABLE I

Auxins and water absorption

Date	W	IAA	$\dfrac{IAA}{W}$	NAA	$\dfrac{NAA}{W}$	WM	$\dfrac{WM}{W}$	NAAM	$\dfrac{NAAM}{W}$
IX 52	29.4 ± 0.7	35.5 ± 1.7	1.21						
X 52	23.0 ± 1.3	32.0 ± 1.5	1.39						
IV 53	36.0 ± 1.6	51.4 ± 1.6	1.43	65.5 ± 3.1	1.82				
V 53	38.0 ± 2.2	45.4 ± 3.3	1.19	54.0 ± 2.0	1.42				
X 53	21.0 ± 0.7			37.0 ± 1.0	1.76	10.0 ± 0.6	0.48	15.0 ± 0.5	0.71
X 53	22.0 ± 1.4			35.0 ± 1.6	1.59	7.0 ± 0.5	0.32	11.0 ± 1.2	0.50
II 54	34.0 ± 1.0			55.3 ± 2.0	1.63	16.6 ± 0.6	0.49	25.0 ± 1.0	0.74
II 54	32.0 ± 3.1			51.0 ± 2.9	1.60	13.0 ± 0.7	0.41	22.0 ± 1.1	0.69
II 54	32.0 ± 2.1			59.0 ± 2.1	1.84	15.0 ± 1.0	0.47	29.0 ± 2.4	0.91
Mean	29.7 ± 2.1	41.1	1.30 ±0.06	51.0	1.67 ± 0.055	12.3	0.43 ±0.03	20.4	0.71 ± 0.065
% Increase over W			+ 30		+ 67		— 57		— 29

Most of our experiments comprised 5 series of blocks:

(1) the fresh reserve parenchyma (F), which was lyophilized immediately after cutting out from the tuber;
(2) tissue which was kept upon pure agar for ten days (W);
(3) the same as (2) but with a naphthyl acetic acid concentration of 10 mg per litre (NAA);
(4) tissue kept on pure agar with 4 % mannitol (WM);

(5) the same as (4) but with an identic concentration of naphthyl acetic acid as (3) (NAAM).

In our first experiments indole-3-acetic acid, (10 mg per litre) was used, but afterwards this growth substance was left out, when it was found that NAA is a much better activator of water absorption (see Table I).

ANALYTICAL DATA

Since in freshly cut reserve tissue about 70 to 80 % of the dry weight is made up from starch, it is not so easy to quantitatively determine the cell wall substances. Indeed, as for the components studied here, they form together only about 5 % of the dry weight.

After thorough extraction of the soluble sugars, starch in the powder was determined by enzymatic hydrolysis to glucose, by means of the Mylase P preparation from the Wallerstein Laboratories (New York), followed by titration of the sugar according to a method of LAMPITT, FULLER, GOLDENBERG and GREEN (1947).

As could be expected lignin was not detectable by the phloroglucin hydrochloric acid test, although it gives a strong reaction in the xylem zone lying at the periphery of the tuber.

Cellulose was determined according to the usual procedure of crude fibre estimation, by successive boiling during half an hour in diluted sulfuric acid and sodium hydroxide.

The determination of the softer cell wall substances, pectins and pentosans, was based on the production of furfurol and carbon dioxide after four hours distillation with 12 % hydrochloric acid. Before then the powder was thoroughly extracted with water at 50° C, in order to eliminate all soluble furfurogenic and uronic materials. According to STEWARD c.s. (1940) a considerable quantity of soluble pectins is formed during aerobic water intake by potato discs. We have not taken into account such soluble pectins as they probably have no importance for cell wall texture itself.

It was found that under the same distillation conditions CO_2 is also formed from the starch present, at a rate of 5.6 $\pm$ 0.1 mg per gram of starch. As the starch content for every sample was known, CO_2 produced from it could be calculated and subtracted from the total caught up during distillation. Pectic acid was calculated from the difference, through multiplication by a factor 4, which is the proportion of the molecular weights of one uronic unit in the pectin chain and of the carbon dioxide molecule (176/44). Uronic acids in fact give a nearly 100 % production of CO_2 per carboxylgroup, pointing to a complete decarboxylation (KLEIN, 1932).

The furfurol distilled over in 12 % HCl was determined according to the barbituric acid method of UNGER and JAEGER (1903), the furfurol being precipitated, dried and weighed as a yellow complex with this acid. This procedure is more satisfactory than that ·of TOLLENS and LEFÈVRE with phloroglucin, because the hydroxymethyl-furfurol produced from hexoses and hexosans is not precipitated with barbituric acid. However, as we realized subsequently, the conversion of

the furfurol figures into absolute values for pectic substances and pentosans separately is very complicated. In fact, in the presence of starch the weight of the barbituric acid complex obtained from furfurogens may be raised considerably, as was shown by UNGER and JAEGER. When starch was present in a twentyfold excess over xylose for example, they observed that the precipitate was no longer of a pure yellow colour and that its weight exceeded by about 23 % that obtained from xylose alone.

Because of the very large excess of starch in our samples we may estimate that the real furfurol figures representing the pectic substances and pentosans, are about 20–25 % less than those actually determined. It should be remarked, however, that our conclusions are based not on the absolute but on the relative furfurol values which were found. Because starch and furfurogens vary inversely during water absorption in our samples, the real changes in furfurogen content are even more significant than is evident from our tables.

Galactan, which also may be a constituent of the wall, has not been included in the analysis.

The figures for cellulose, furfurol, pectic substances and starch have all been recalculated in mgs per gram dry weight of the fresh tissue (F). Every figure is the mean of at least two determinations, on a powder sample obtained from 12–15 blocks. The significance of differences has been calculated according to MATHER (1949).

RESULTS

1. Auxin, water absorption and cell wall growth

It will be seen from Table II that water absorbing potato tissue has a quite remarkable capacity of building up cell wall substances.

Upon pure agar the mean water absorption of nine experimental

TABLE II

Auxin and the Synthesis of Cellulose and Furfurogens
($^0/_{00}$ Content, on dry weight basis of F)

Date	F		W		NAA	
	Cellul.	Furfur.	Cellul.	Furfur.	Cellul.	Furfur.
IX 52	30.6	6.03	44.4	7.36		
X 52	32.6	5.55	40.6	7.76		
IV 53	25.1	6.78	38.8	8.81	39.4	9.91
X 53	27.6	6.60	44.6	8.30	40.3	8.89
X 53	26.7	6.84	43.6	8.13	40.2	8.80
II 54	33.1	6.45	47.6	8.45	46.0	9.73
Mean	29.3 ± 1.4	6.38 ± 0.20	43.3 ± 1.3	8.14 ± 0.21	41.5 ± 1.3	9.33 ± 0.28
Water absorption (see table I)			29.7		51.0	
Pectic substances (from CO_2-production)	16.4 ± 0.5 (5)		21.1 ± 0.7 (4)		23.0 ± 0.8 (3)	

series was 29.7 ± 2.1 % of the fresh weight (see also Table I). During this process cellulose increased from 29.3 ± 1.4 to 43.3 ± 1.3 % of the dry weight of F. Whereas NAA raised the water absorption very considerably, it will be seen however that the cellulose content of the NAA treated blocks is not larger than of those on pure agar.

At the same time there are no less important changes in the furfurogenic substances. Upon pure agar the furfurogenic capacity rises from 6.38 ± 0.20 to 8.14 ± 0.21. Moreover, in striking contrast to cellulose, furfurogens are further considerably increased by the NAA treatment. By a parallel study of carbon dioxide production it was found that uronic acid chains and furfurogens in general rise in an almost direct proportion.

The activating influence of the growth substance on the synthesis of furfurogens in contrast to cellulose formation is a very striking fact. It may be further emphasized by comparing the quotients of furfurol and cellulose increase in presence and absence of the NAA. Table III shows these values calculated from the four complete experiments of Table II. The proportions show very convincingly that the synthesis of the furfurogens is favoured more than that of cellulose by application of the auxin.

TABLE III

The influence of NAA on the furfurogen/cellulose balance during wall growth

Date	W			NAA			% Increase of Furf./Cell.
	$\triangle$ Furf.	$\triangle$ Cell.	Ratio	$\triangle$ Furf.	$\triangle$ Cell.	Ratio	
IV 53	2.03	13.7	0.148	3.13	14.3	0.219	+ 48
X 53	1.70	17.0	0.100	2.29	12.7	0.180	+ 80
X 53	1.29	16.9	0.076	1.96	13.5	0.145	+ 91
II 54	2.00	14.5	0.138	3.28	12.9	0.254	+ 84
Mean		0.113 ± 0.017			0.199 ± 0.024		+ 76 ± 10 P < 0.001

It may be worth while to mention here another observation as to the change of cell wall composition. In the fresh tissue the furfurol/cellulose proportion was equal to 0.218 ± 0.016. Since this figure gives the mean proportion of furfurol and cellulose increase during the whole development of the tubers, it is clear that during water intake of the potato pieces upon pure agar there is a relatively greater cellulose formation, the corresponding proportion of furfurol increase to cellulose increase being about 50 % lower (0.113 ± 0.017). On agar with auxin the proportion is but little if any less than in fresh tissue (0.199 ± 0.024). Consequently it can be said that the NAA treatment has prevented the furfurol/cellulose proportion from falling down. This observation will be discussed further on in relation to the changes of cell wall extensibility during aging, described in the next section.

From the results just mentioned it can hardly be doubted that the so-called 'water absorption' of the potato tissue is a real growth

phenomenon, accompanied by an active building up of at least two important cell wall materials. We think therefore that there is no longer any reason of suspecting that the mechanism of auxin action in this water absorption might be fully independent of what is going on in young growing tissues, as was suggested for instance by AUDUS some years ago (1949) in his interesting review on the mechanism of auxin action. It may be worth while to recall the recent work of Plaisted (see LOOMIS, 1953, p. 4), from which it appears rather clearly, that potato tubers should not be taken for 'adult' organs. Plaisted finds a continuous growth with slow cell multiplication in tubers up to weights of 200 grams.

It must be mentioned moreover that STEWARD, STOUT and PRESTON (1940, p. 421) found a considerable increase of proteins (about 37 %) in water absorbing potato discs. This increase may stand comparison with that in elongating corn coleoptiles and Oenothera hypanthia found by BLANK and FREY-WYSSLING (1941, 1944), the water intake being much larger in these latter cases.
Furthermore the furfurogens and especially polyuronides found by us, together with the surface growth of the cells, point to the primary character of the cell walls in potato tuber parenchyma.

2. Auxin, cell wall extensibility and aging of the potato tissue

Although our method of studying the mechanical cell wall properties was seriously hampered by a considerable variability of the wall extensibility, some changes during the water intake were so large, that their statistical significance is well established. In Table IV we give the results of the two experiments which have been performed.

TABLE IV

The influence of N A A on the loss of wall extensibility
($^0/_{00}$ elongation of plasmolysed tissue blocks)
Means for 8–14 blocks. Load: 52.2 grams

Date	November 1953	February 1954
F	70.0 ± 2.3	71.8 ± 8.0
W	29.5 ± 2.0	29.6 ± 4.2
NAA	33.3 ± 1.5	49.8 ± 3.9
Probability of accidental difference	F-W : $P < 0.001$ F-NAA : $P < 0.001$ W-NAA: $P \approx 0.15$	F-W : $P < 0.001$ F-NAA : $P \approx 0.02$ W-NAA: $P \approx 0.002$

The first fact which should be stressed is the very distinct fall of the wall extensibility in tissue which has been on pure agar for ten days. Furthermore, in both cases the extensibility of the NAA treated blocks is intermediary between that of fresh tissue and of tissue on pure agar. In the November experiment the W-NAA difference is unsatisfactory, but in that of February 1954 it is very safe, the probability of accidental difference being of the order of $1\,^0/_{00}$.
It may thus be said that the auxin brings about a relative rise in

wall extensibility, comparable to that generally found in young growing tissues.

The stiffening of the cell walls found after water intake has much in common with the well known aging phenomenon, which has been studied in the oat coleoptile and other growing tissues, after exhaustion of the auxin reserve. Several workers e.g. HEYN, 1931, WENT and THIMANN, 1939, who studied the extensibility loss during aging, ascribed it upon indirect grounds to cell wall thickening. As in the present case the synthesis of wall substances had been followed in a quantitative way, the wall thickening, or at least the increase of wall material per unit surface area, could be calculated. Let us compare for instance the auxin treated tissue with the freshly cut material. It should be remembered here, that the proportions of pectic material to cellulose are very nearly the same in both cases. In the experiment of February 1954 water absorption was 51 %. From this figure a surface increase of the walls of about 31 % may be deduced. At the same time the increase of pectic substances and cellulose was 45 %. From these values it may be calculated, that there was an increase of wall material per unit surface of about 11 % only (145/131 = 111). As the wall extensibility decreased however for 31 %, it does not seem possible to ascribe it to wall thickening alone. Although, unfortunately, exact figures are lacking for water intake and cell wall formation in the November experiment, the disproportion of wall thickening and extensibility loss was still larger there.

Consequently, there are very probably other factors which intervene in wall aging. We may think, for instance, of an increase in cellulose crystallization, or perhaps of a formation of cation bridges with Ca^{++} and Mg^{++}-ions between the carboxyls of the protopectin chains. In the growing primary wall we may look for a positive function of methylation in preventing the formation of such bridges. Such a methylation could also explain the mobilizing or sparing action on calcium, which has been found by STRUCKMEYER (1951).

On the other hand it may be accepted that the lower extensibility of the W-blocks, as compared with the auxin treated ones, should be explained partly by the higher cellulose content of the walls in this case, partly also by the fact that surface growth is much less here, for a nearly identical synthesis of wall substances (Table II: sum of pectic substances and cellulose).

3.   *The influence of mannitol on water absorption and cell wall growth*

In three experiments mannitol was added to the agar bottoms, in order to study the possible dependence of wall substance formation from extension. It has been suggested by SACHS (see HEYN, 1931) that wall extension could be an immediate requisite for the building up of wall substances, by the formation of free submicroscopic spaces within the wall texture.

Table V shows the changes of water absorption, cellulose formation and synthesis of furfurogenic substances, when mannitol is present in the agar.

TABLE V

The influence of mannitol on water absorption and polysaccharide metabolism of potato blocks

Treatment	F	W	NAA	WM	NAAM
Water intake (Table I)		100	167 ± 5.5	43 ± 3	71 ± 6.5
Cellulose	29.3 ± 1.4	43.3 ± 1.3	41.5 ± 1.3	41.8 ± 1.2	37.4 ± 1.0
Furfurogens (furfurol)	6.38 ± 0.20	8.14 ± 0.20	9.33 ± 0.28	7.52 ± 0.16	8.16 ± 0.05
Dry weight % (Mean of 3)	14.41 ± 0.18	12.49 ± 0.20	11.70 ± 0.30	13.01 ± 0.29	12.42 ± 0.11
Starch	100	75.1 ± 0.8	64.1 ± 2.1	79.6 ± 2.3	68.5 ± 1.6

WM and NAAM values are means of 3 experiments.

When no auxin is added, the mannitol brings about a doubtful and certainly slight decrease of cellulose synthesis. The decrease of the furfurogens is more important and statistically significant ($P < 0.01$). It may be concluded from these facts, that the furfurogen/cellulose balance is shifted still further to the cellulose side by the mannitol treatment. In any case the decrease of cell wall synthesis is slight and not proportional to the considerable decrease of 57 % in water absorption itself. It would not seem probable then, that wall formation depends directly on the free spaces available in the wall. It is a rather independent process, which is regulated by the general metabolism of the cells.

When NAA is present, the decrease of wall synthesis by the action of mannitol is much more considerable, although once again it is not proportional to the reduction of water intake.

These facts suggest that the retarding influence of mannitol on wall synthesis is not brought about by its osmotic effect on growth, but through some other mechanism. This is indicated still more clearly when making a comparative study of the loss of dry weight and of starch during the water intake, as shown by Table V. As already claimed by other workers on potato parenchyma (REINDERS, 1942; LEVITT, 1948), auxin treatment causes a somewhat greater loss of both dry weight and starch content. This loss is counteracted by the mannitol. As shown by REINDERS and by STEWARD et al. (1940), the dry weight loss should be ascribed, at least partly, to respiration of the starch reserves of the tissue. Consequently the sparing action of mannitol on dry weight and starch would suggest that this substance does to some extent reduce the respiration of the cells. It would be worth while to confirm this by direct respiration measurements. We could suggest that mannitol through its structural resemblance to glucose may supplant this sugar and may thus inhibit its metabolic working up, retarding by this way the loss of starch and the formation of new wall materials.

As to the results of the WM and NAAM treatments shown in Table

V, attention must once more be called to the opposite effect of NAA on cellulose and furfurogens. In the presence of mannitol NAA probably diminishes the cellulose content (P ≈ 0.05), whereas the furfurogens are increased (P ≈ 0.02).

The importance of the furfurogens in cell enlargement may be shown in a very suggestive way, by comparing water intake and increase of cellulose and furfurogens (see diagram). We find a striking parallelism of water absorption with the latter, but not with the former.

RELATIVE VALUES FOR W = 100

W   NAA   WM   NAAM
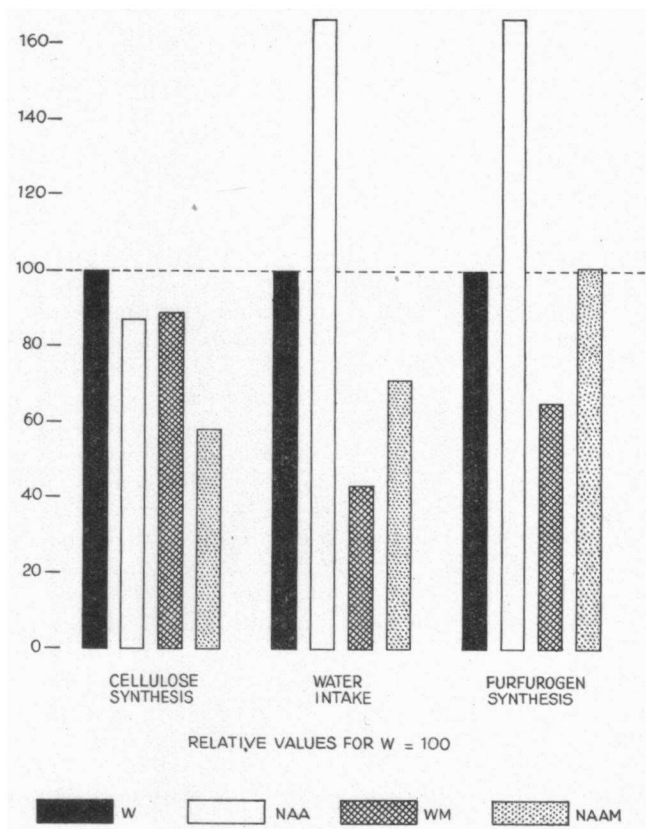
DISCUSSION IN RELATION TO OTHER RESEARCHES ON WATER ABSORPTION BY RESERVE TISSUES

In the course of the present work we became acquainted with the study of BRAUNER and HASMAN (1952), in which they examined the osmotic properties and mechanical cell wall characters of potato parenchyma during water intake. These authors ascertained a slight increase of wall extensibility in the first day of the absorption process. After 48 hours they already observed a small decrease of the extensibility, which was however not yet less than in fresh tissue. Our observations are

only apparently in contradiction with theirs, since the already mentioned decrease far below that of fresh tissue is probably a continuation of the beginning decrease which Brauner and Hasman have found.

The relative increase of the extensibility by auxin observed in both researches may at least partly be ascribed to the fact, that softer wall substances are synthesized during this treatment. Since, on the other hand, during the first day the extensibility is raised above that of the fresh tissue, this might mean that in the very first period of water absorption there is a still greater formation of furfurogens, which gradually shifts to a relatively more important cellulose synthesis during the aging of the tissue.

We may come now to a short discussion of the mechanism of water absorption itself.

In the early study of REINDERS (1938–1942) this author suggested that water absorption was brought about by starch hydrolysis, the sugars which are thus formed increasing the suction force of the cells through raising of the inner osmotic pressure. Later on it was suggested by COMMONER et al. (1942, 1943, 1944) that auxins may raise the osmotic pressure of the cells by accelerating the active uptake of salts. It should be remarked immediately, however, that water uptake may proceed just as well in distilled water. Consequently it looks rather improbable that salt uptake may be the fundamental factor of water absorption. Furthermore it seems to be well established now by the studies of VAN OVERBEEK (1944), LEVITT (1948), HACKETT (1952) and BRAUNER and HASMAN (1952) that during water absorption the osmotic pressure does actually decrease instead of increasing. It would at first seem, therefore, that the explication· of water absorption should be sought either in changes within the cell walls themselves, or possibly in some so-called 'meta-osmotic' water absorption, as suggested by BONNER and BANDURSKI (1952).

LEVITT (1953) is a very decided opponent of the view of a meta-osmotic water intake. In our opinion there is one of his experiments which so far seems especially significant, viz. the temperature experiments mentioned in his study of 1948. Potato tissue was brought to a temperature of 2° C and did not loose the water which had been absorbed before. If an energy supply is really indispensable to hold the water within the cells, as is implicated in the process of meta-osmotic absorption, one would expect that water would be lost again at such a low temperature.

From the known facts in the literature and the present results we do not see any necessity of supposing the intervention of a non-osmotic water intake in potato parenchyma. It has already been emphasized above that the so-called water absorption does not differ from a real growth of the cell enlargement type. The situation here may once more be compared with that in young elongating tissues, for instance coleoptiles, where extensibility of the walls is raised in a similar way (HEYN, 1931), whereas the osmotic value is decreased (KETELLAPPER, 1953), by the action of auxins. The importance of active growth in area of coleoptile cell walls was adequately stressed by Ketellapper in the latter publi-

cation. No doubt the water intake process, in potato parenchyma too, can be explained sufficiently by the lowering of wall pressure, partly by the increase of wall extensibility, partly and perhaps chiefly by the continuous building up of soft wall constituents.

As to the relation of water absorption with oxidative metabolism, this would seem to have a twofold aspect. On the one hand it was found by BRAUNER and HASMAN (1952) that wall extensibility does increase only in aerobic conditions. Oxygen would thus at first be necessary for the loosening of the wall structure. On the other hand there is good evidence that oxygen will be indispensable too for the synthesis of wall constituents, especially pectic substances, which was found in the present work. It may even be suggested that this building up of pectic materials and the increase of wall extensibility are one and the same process, as pectins are 'weaker' substances than cellulose, since they seem to crystallize very difficultly and are more strongly hydrated.

It may be readily accepted that the galacturonic acid units of the pectins are derived directly or indirectly from the glucose or starch reserve of the cells, as was shown by STEWARD et al. (1940) for the soluble potato pectins, in the carbon balance made up by them. Since the formation of a carboxylgroup on the 6th carbon atom is an oxidative process, this may be one important link of cell enlargement to the respiration of the cells.

Although rather few experimental facts can be found in the botanical literature concerning the role of pectic substances in growth, suggestions as to their possible importance have been made several times recently. KERR (1951) for instance suggests that the properties of the primary cell wall might be explained more easily by assuming that the continuous phase is formed by protopectin and not by cellulose, as is supposed by FREY-WYSSLING (1950).

VAN OVERBEEK too (1952) presents the view that there is a close relation between pectins and cell enlargement. He points to the work of NEELY et al. (1950) who found an activation of pectin-methylesterase in tissues treated with 2-4-D, and he suggests that demethylation might lead to the breakdown of protopectin, thus loosening the wall structure. It should be emphasized however that in the present research an obvious increase of protopectin substances in the cell walls was found in consequence of auxin treatment. We think therefore that in the present case the above mentioned hypothesis cannot hold true. The importance of methylesterase activation might as well be sought in the reverse direction, viz. in its preventing bridge formation by Ca and Mg ions by methylation of the pectic acid chains, methylgroups being supplied by the activated metabolism. A research on the influence of auxins upon methylation would probably give further valuable information on the role of pectic substances in growth. We have also made some calculations on the results of STUART (1938) on pieces of bean stalks treated with indole-acetic acid. From the few pectin determinations which this author made, it seems probable once more that the pectin content of the dry material increases as compared with the control plants. We would prefer to suggest, therefore, that auxins

stimulate pectin formation, by the activation of a respiratory system in the outer layers of the cytoplasm, which catalyses the oxydation of the 6th carbon atom of the substrate sugar, after conversion to galactose.

STEWARD *et al.* (1940, p. 434) also insist on the apparent relation of soluble pectin formation in potato discs to aerobic metabolism.

In relation to the latter suggestions, attention may be called upon the work of NEWCOMB (1951), who found a very striking activation of ascorbic acid oxidase in tobacco pith sections by indole-acetic acid. He believes the enzyme to be located near the boundary layers of the cell. Perhaps this or an allied respiratory system might be responsible for the transformation of galactose to galacturonic acid.

SUMMARY

We may summarize our communication in the next four points:

(1) The water absorption in potato reserve tissue was found to be a real growth phenomenon, which is accompanied by an active synthesis of cellulose and furfurogens. As a consequence, the activation of water intake by auxins may be regarded as a normal growth activation of the same type as found in the common growing zones of plants.

(2) Auxins cause a shift in cell wall composition, since they bring about a more rapid building up of pectic substances, in opposition to cellulose.

(3) We suggest that this shift is the immediate cause of the increased extensibility of the cell walls, the pectic substances being more hydrated and less crystallizable than cellulose.

(4) Hindrance of water intake by 4 % mannitol is accompanied by reduction of starch loss, of furfurogen and probably of cellulose synthesis. These reductions however are not proportional to the large decrease of water intake. It is suggested that mannitol is not only an osmotic growth retarder but also interferes directly with glucose metabolism.

REFERENCES

ANKER, L. 1953. Acta Bot. Neerl. 2:22.
AUDUS, L. J. 1949. Biol. Rev. 24, 51.
BLANK, F. and A. FREY-WYSSLING. 1941. Ber. Schweiz. bot. Ges. 51:116.
BLANK, F. and A. FREY-WYSSLING. 1944. Ann. Bot. Lond. 8:71.
BONNER, J. and R. S. BANDURSKI. 1952. Ann. Rev. Plant Physiol. 3:59.
BRAUNER, L. and M. HASMAN. 1952. Protoplasma 41:302.
CARLIER, A. 1954. Thesis, Leuven (in press).
COMMONER, B. C. and D. MAZIA. 1942. Plant Physiol. 17:682.
COMMONER, B. C., S. FOGEL and W. H. MULLER. 1943. Am. J. Bot. 30:23.
COMMONER, B. C. and D. MAZIA. 1944. Am. J. Bot. 31 suppl.:8.
FREY-WYSSLING, A. 1950. Ann. Rev. of Plant Physiol. 1:169.
HACKETT, D. P. 1952. Plant Physiol. 27, 279.
HEYN, A. N. J. 1931. Rec. Trav. Bot. Néerl. 28, 113.

KERR, TH. 1951. In: Skoog, Plant Growth Substances, Wisconsin, p. 37.
KETELLAPPER, H. J. 1953. Acta Bot. Neerl. 2, 387.
KLEIN. 1932. Handbuch der Pflanzenanalyse. Vol. III. Wien.
LAMPITT, L. H., C. H. FULLER, N. GOLDENBERG and G. H. GREEN. 1947. J. Soc. Chem. Industr. 66:68.
LEVITT, J. 1948. Plant Physiol. 23:505.
LEVITT, J. 1953. Physiologia Plantarum 6:240.
LOOMIS, W. E. 1953. Growth and Differentiation in Plants. The Iowa State Coll. Press, Iowa.
MATHER, K. 1949. Statistical Analysis in Biology, London.
NEELEY, W. B., C. D. BALL, C. L. HAMMER and H. M. SELL. 1950. Plant Physiol. 25:525.
NEWCOMB, E. H. 1951. Proc. Soc. Exptl. Biol. Med. 76:504.
OVERBEEK, J. VAN. 1944. Am. J. Bot. 31:265.
OVERBEEK, J. VAN. 1952. Ann. Rev. Plant Physiol. 3:87.
REINDERS, D. E. 1938. Proc. Kon. Ned. Akad. Wet. Amsterdam 41:520.
REINDERS, D. E. 1942. Rec. Trav. Bot. Néerl. 39:1.
STEWARD, F. C., P. R. STOUT and C. PRESTON. 1940. Plant Physiol. 15:409.
STILES, W. and I. JÖRGENSEN. 1917. Ann. Bot. 31:415.
STRUCKMEYER, B. E. 1951. In: Skoog: Plant Growth Substances. Univ. Wisconsin Press.
STUART, N. W. 1938. Bot. Gaz. 100:298.
UNGER, E. and R. JAEGER. 1903. Ber. D. Chem. Ges. Berlin 36:1222.
VANDERVEKEN, P. 1951. Thesis, Leuven (unpublished).
VANDERVEKEN, P. 1955. Med. Kon. Vlaamse Akad. Wet. etc. (Belgium) 17: nr. 2.
WENT, F. W. and K. V. THIMANN. 1937. Phytohormones. The MacMillan Co. New York.