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THE REGULATING INFLUENCE OF TRANSPIRATION AND SUCTION TENSION ON THE WATER AND SALT UPTAKE BY THE ROOTS OF INTACT VICIA FABA PLANTS

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CONTENTS

CHAPTER I

GENERAL INTRODUCTION

Though the processes of water uptake by the root and water transport through the plant are well known in general lines, little attention is paid to an important factor in these processes, viz. the resistance of the root tissue to the water current. Renner (1929) showed clearly that this resistance is considerable. Koehnlein (1930), Kramer (1933) and Brewig (1937) corroborated his observations. The most striking fact is, that this resistance is not a constant one but depends on various circumstances. Jost (1916) was the first physiologist to find that an increase in water uptake was not proportional to the increase in suction tension in the xylem vessels. He observed that at increasing suction tensions the water uptake per atmosphere pressure difference, increased, This can be explained in two different ways.

Firstly, it is possible that with a greater water deficit in the plant a considerable quantity of water is taken up by an active water pumping mechanism. The other possibility may be that in this case the resistance of the root tissue against the water current diminishes.

KOEHNLEIN (1930) observed that at stronger suction the suction tension in the vessels was more effective but that it was not possible to get the same efficiency when the outer solution was at 0° C. He suggested, therefore, that the first possibility mentioned by JOST was the right one. An active water uptake mechanism strengthens the effect of the mechanical suction. At 0° C this mechanism comes to a standstill and the root system behaves like a pure physical system.

BREWIG (1937) who elaborated a method of precisely determining the resistance of the various root zones, showed that the latter interpretation of JOST was the more likely one. It appeared that at low suction tension in the xylem vessels the resistance to the water current was greatest in the basal region and smaller in the zones near the tip. On enhancing the transpiration the resistance of the apical zones remains unchanged but the resistance of the basal root parts decreases and becomes as small as or smaller than the resistance in the apical zone.

Little attention was paid to these observations in the literature, probably because of the fact that no explanation of these changes in resistance could be given. The author was able to corroborate the results of Brewig's experiments completely. Using an improved method more regular results were obtained.

Moreover, it appeared possible to get some insight into the causal relations of the changes in resistance. In view of the importance of these changes in resistance to the water current for transpiration and salt uptake, a summary of the results of the preceding experiments is given in Chapter III.

The results of SIERP and BREWIG (1935), BREWIG (1936 cf) and BROUWER (1953*a*) confirmed the fact that the root of *Vicia faba* was permeable to water over a great length, at any rate up to 18 cm from the root tip. This seems contrary to the conceptions of SCOTT and PRIESTLEY (1928) and SCOTT (1928) who on account of anatomical data confined the water uptake to the apical 5 cm region, but it agrees with the experiments of POPESCU (1926), HÖHN (1934) and others who showed that the roots of various plants were able to absorb water over their whole length.

Though the methods used had some drawbacks (in fact all parts of the root, with exception of the tested one, have been prevented from the water uptake) it was clear that the capacity to absorb water was not limited to the apical region.

It is a surprising fact that, up to now, it is unknown which zone of the root of an intact plant shows the maximum salt uptake. Most experiments on this problem have been performed with excised roots or root systems. The experiments of PREVOT and STEWARD (1936) show nothing but the binding in the root tissue. It is not surprising that this binding capacity is most important in the root zone which shows the most rapid growth. The experiments of LUNDEGÅRDH (1945) deal with the same process and also the transfer to the vessels is determined. It is, however, a special uptake, viz. the uptake of an excised root (bleeding). KRAMER and WIEBE (1952) used intact plants but determined only that portion of the total uptake that has been withheld by the root tissue and this is nothing but the accumulation of ions as shown in the experiments of PREVOT and STEWARD.

The experiments described below show the uptake of the different zones of the root of an intact plant. The method used makes it possible to vary the water uptake of the tested root and in this way the problem of the influence of the water uptake on the salt uptake is drawn into the research. The anion uptake of the different zones can also be traced at different rates of water uptake.

There are few other problems in plant physiology that have attracted as much attention as this during the last 50 years. In spite of this no agreement has been reached as yet.

At first plant physiologists assumed that salts were taken up and transported passively by the transpiration stream. This concept could only be maintained if the amount of ions taken up was directly proportional to the amount of water absorbed. The experiments of HASSELBRING (1914), KIESSELBACH (1916), MENDIOLA (1922), MUENSCHER (1922), and GRACANIN (1932) threw doubt upon the validity of this concept, since no correlation could be demonstrated between water uptake and salt uptake.

On the contrary the experiments of HAAS and REED (1927), SCHMIDT (1936), FREELAND (1936, 1937), JAHNEL (1938), BÖTTICHER and BEHLING (1939), WRIGHT (1939) and PHILLIS and MASON (1940) showed clearly the existence of such a correlation.

During the same period the investigations of STEWARD and coworkers (1936), HOAGLAND and BROYER (1936), LUNDEGÅRDH and BURSTRÖM (1933), VAN DEN HONERT (1933, 1936), LUNDEGÅRDH (1940) and BROYER and HOAGLAND (1943) showed that, in contrast with the water absorption, the salt uptake was a fully active process dependent on the respiration and according to LUNDEGÅRDH more particularly dependent on the anion respiration. A passive uptake of ions in the transpiration stream is not consistent with this view. The consequence was that an influence of the water absorption on the salt uptake was thought to be of minor importance if any at all and in the newer textbooks on plant physiology this influence is barely discussed.

HUBER (1953) in a recent review supports an intermediate point of view. He assumes that there is an active salt uptake, on which at strong transpiration a passive carrying along in the transpiration stream is superposed. This idea is especially prompted by the results of his pupils SCHMIDT, JAHNEL and more recently by PETRITSCHECK (1953). A definite correlation between water uptake and salt uptake could also be shown by MICHAEL and WILBERG (1951), whereas HYLMÖ (1953) on account of his experiments with *Pisum sativum* reverted to the conception that the uptake of ions occurs completely passively with the water.

BUTLER (1953) does not object to this point of view, but shows with his material, *Triticum vulgare*, that the ion transport to the shoot can be inhibited by 2,4-dinitrophenol without any reduction of the water uptake.

In this connection it is worth noting that LONG (1943) could reduce the water uptake of tomato plants to about 20 % of the original value without inhibiting the nitrate uptake. The reduction of the water uptake was obtained by the application of an osmotic counter suction in the outer solution.

These methods, inhibition of the salt uptake without reduction of the water absorption and reduction of the water uptake without influencing the salt uptake will give a better insight into the mutual relation between water absorption and salt uptake.

From the facts, mentioned above, it is clear that the different points of view can only be reconciled with each other, by a theory which takes all these experiments into account. It is unlikely that the differing results must be attributed to methodical errors, since both types of results have been corroborated repeatedly. There may be, therefore, an influence of the transpiration on the salt uptake, but on the other hand there need not necessarily be such an influence. HOAGLAND and BROYER (1936) pointed to the importance of the salt status of the plant in this problem. In this investigation the attention will be drawn to a number of other factors which can be important.

At any rate, it has become apparent that a part of the total salt uptake is active, viz. it takes place at the expense of respiratory energy. This is, why some experiments are performed in order to determine the respiration intensity of the different root zones.

Finally it is of great importance to compare the physiological results with the anatomical structure. We stated above that an interpretation merely on the basis of the anatomical data, may lead us to misinterpretations.

CHAPTER II

MATERIAL AND GENERAL METHODS

§ 1. MATERIAL

In all the experiments described below, 5 to 7 weeks old *Vicia faba* plants were used. After germinating the seeds in wet river sand the seedlings were grown in jars containing tap water. After the removal of the tip of the main root long unbranched secondary roots develop. The length of these secondary roots amounted to 10–22 cm, the diameter was about 1 mm and was nearly constant over the entire length of the root.

§ 2. DETERMINATION OF THE WATER UPTAKE

The aim of a former investigation was the determination of the water uptake of the different root zones. Besides, it was necessary to measure the suction tension in the xylem vessels of the root according to a method developed by BREWIG (1937). For that purpose a rapid change of the outer solution of each zone was also necessary. Both conditions are fulfilled by an apparatus as described in detail in a former paper (BROUWER, 1953*a*). For the sake of brevity we may refer to that paper.

§ 3. DETERMINATION OF THE SALT UPTAKE

When determining the water uptake it was possible to change the outer solution after short periods since the experimental periods lasted only 20 or 30 minutes. For the salt uptake such short periods cannot be used, because the uptake of a 3 cm zone of the root is very small in proportion to the sensitivity of the determination methods.

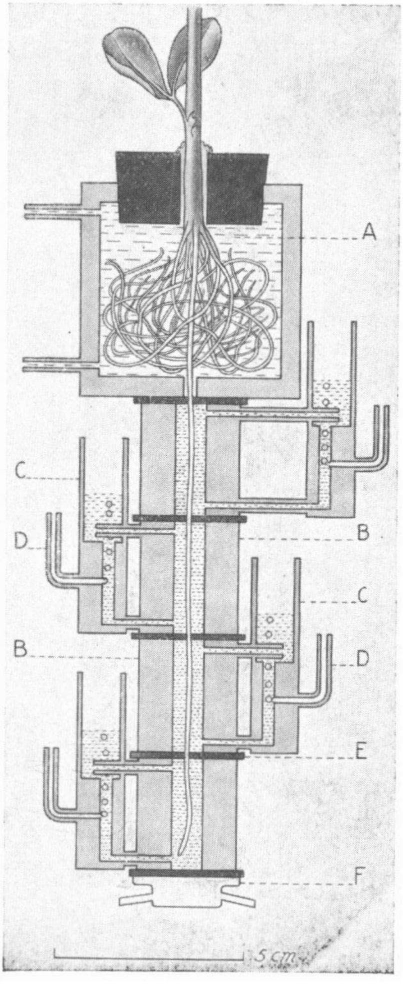

Fig. 1. Schematic longitudinal section of the apparatus used for experiments upon the salt uptake of the different root zones.

A = main vessel, containing the bulk of the roots;
B = zone vessel, containing a single zone of the tested root;
C = aeration vessel connected with B by two transverse tubes;
D = side tube connected with the air container, admitting a constant stream of air bubbles;
E = air- and water-tight rubber disk, fitting around the root, separating two adjacent zone vessels;
F = metal disk; strong springs attached to this disk press the series of zone vessels against the bottom of the main vessel A and hold the apparatus firmly together.

It is necessary, therefore, that the contents of the small vessels be aerated and stirred continually. To satisfy these conditions small vessels were designed which are drawn in section in figure 1. As with the apparatus formerly described for the water uptake, the ground plan is a metal standard on which the main vessel is mounted. Through a hole in the bottom of the main vessel a single root is hanging down. This root is enclosed in a series of small vessels, the zone-vessels.

These zone-vessels are separated from each other by rubber disks with a hole in the centre. Each vessel consists of a central cylindrical block(B) with a cylindrical boring of 8 mm diameter, through which the root hangs. With transverse connections at the top and at the bottom of the vessel it is connected with a second smaller vessel(C) which renders possible a stirring of the contents of vessel B. Via the side tube D a constant air stream enters each vessel C. In this way the solution of the two vessels can be circulated by means of air bubbles, and is aerated and stirred at the same time. Finally there is made a side tube at the bottom of B (not drawn on the figure) by which it is possible to drain off the contents of the vessels quantitatively. For this purpose the side tube is supplied with a rubber hose with a clamp.

The method used is as follows. With a 3 cc pipette a nutrient solution is brought into the vessels. The whole of vessel B and vessel C up to the level drawn on the figure is then filled. After an uptake period the remaining nutrient solution is drained and caught. Thereafter the vessel is rinsed twice with 5 cc distilled water. This is also caught.

To get information with regard to losses during the manipulations and the experiment we have always used one vessel at the bottom of the apparatus, in which no root segment was enclosed. It appeared that in this vessel the original amount of ions could always be recovered completely. With the set up described here the root remained in good condition for a long time.

We have investigated the nitrate, phosphate and chloride uptakes of the various root zones. This took place in separate experiments since the total amount of nutrient solution was always used up by a single determination. The experimental periods lasted from 8 to 24 hours, dependent on the ion investigated. The whole apparatus, with the exception of the metal standard, was made from perspex.

The nitrate analysis took place according to the colorimetric method with phenoldisulphonic acid. The precision amounted to about 3 γ.

The phosphate uptake was determined from solutions with labelled phosphate (P*). The results obtained with the counting method were checked by the colorimetric analysis using the molybdenumblue method. The use of P* made it possible to determine the uptake and the binding in the tissue at the same time.

The chloride analysis was performed by means of the electrometric titration with a 10 mM silver nitrate solution. With some practise the accuracy of this method is about 1 γ Cl. The added silver nitrate was determined gravimetrically.

The uptake periods lasted 24 hours for nitrate, 16 or 24 hours for phosphate and for chloride periods of 8 hours were sufficient.

§ 4. Determination of the oxygen uptake

For the determination of oxygen uptake from the medium the apparatus described for water uptake measurement was used. With a syringe pipette, as described by Van Dam (1935), a sample was taken from each of the potometers separately, sucking off the sample via the lowest side tube (Brouwer, 1953*a*, fig. 1D). The oxygen content of the samples was determined by Van Dam's adaptation of the micro-Winkler method. By determining this oxygen content before and after an experimental period of 45 or 60 minutes the amount of oxygen withdrawn from the medium could be calculated. The oxygen uptake of all the zones was determined separately at low and high water uptake, both with and without ions being present in the medium.

Chapter III

CHANGES IN WATER CONDUCTIVITY OF THE ROOT TISSUE

§ 1. Introduction

In the literature many experiments are described in which the water uptake by the different zones of the root was determined. At first they were performed by greasing with a fatty substance the whole root with exception of the tested zone, so that the water uptake in the greased zones was impossible. A second method frequently applied was the use of indicators. The roots were allowed to absorb water from a dye solution and after the experiment microscopic tracings showed what parts of the root held the highest concentrations of the dye.

The only thing that was proved by these experiments was the fact that the root was able to absorb water over its whole length. To get more exact data it was necessary to use micropotometers enclosing the different zones of the root.

In 1935 Sierp and Brewig succeeded in enclosing a single root of *Vicia faba* in a number of potometers, making it possible to determine separately the water uptake of each 2.5 cm zone. Rosene (1937) described a method for determining the water uptake of very small root segments at different distances from the apex. Moreover, Gregory and Woodford (1939) and Hayward, Blair and Skaling (1942) described potometer methods. Only the method of Sierp and Brewig (executed by Brewig) and the technique of Rosene appeared useful and following the first descriptions Brewig and Rosene published a series of papers, each of them acting with a distinct aspect of the water uptake.

The method used by the author is an improvement of the method of Gregory and Woodford.

As to the results of former potometer investigations only the results of Sierp and Brewig, Brewig, and Rosene need further discussion because the other investigations are more preliminary.

Sierp and Brewig showed that the water uptake of the different zones of the root strongly varied according to changes in transpiration of the shoot. At low transpiration the maximum water uptake took place near the tip. The basal zones showed a very small water uptake under these circumstances. At high transpiration the water uptake of all zones increased. The water uptake of the basal zones was then higher than the uptake of the apical zones. It is known that according to the formula of BRIEGER (1928) water uptake is determined by the suction tension in the xylem vessels and the water conductivity of the root tissue between medium and vessels: Uptake = c × S.

BREWIG (1937) showed that the suction tension in the xylem vessels in the apical parts is as high as in the basal zones. The conductivity for water, therefore, is highest in that region, where the water uptake is highest. Hence it is clear that at low transpiration the conductivity is highest in the apical zone and small in the basal ones. At high transpiration the conductivity of the apical zone remains the same as before but the conductivity of the basal zones shows an important increase. The sole assumption that we have to make is that the pathway of the water is the same at low and high transpiration. It might be conceivable that at high suction channels open up which are not available at low transpiration, for instance a lengthwise transport through the cortex. With regard to such a transport two possibilities can be considered. Firstly a transport through the walls is possible. In view of the narrow connecting canals (intermicellar spaces) and the length of the pathway such a transport would constitute an almost unfeasible task for the plant. As to an osmotic transport from cell to cell the same objection holds. Therefore, BREWIG is justified in stating that the resistance to water transport is localised in the tissue between medium and vessels.

The phenomenon that is at issue is the change in resistance against the water current, particularly in the basal zones. The changes in water conductivity are correlated with increases in suction tension. BREWIG (1939) even succeeded in getting the same changes in conductivity with excised roots by applying an osmotic suction (strong sucrose solution) in the medium, but only if at the same time, the contents of the xylem vessels were continually sucked away. By applying this sugar solution in the medium, water was withdrawn from the root segment as long as a water current was maintained lengthwise through the xylem vessels. As soon as this water stream stopped, the withdrawal by the sugar solution decreased rapidly. WIERSUM (1944) who showed with the same material, that sugars could diffuse rather easily through the tissue from medium to xylem vessels and vice versa, concluded from BREWIG's result that with a stagnating water stream in the xylem vessels sugar diffusion from the medium to those vessels would be responsible for a decreasing difference between the osmotic value of the contents of the vessels and the medium. This would result in the decreasing withdrawal of water by the sugar solution.

ROSENE (1941) working with both excised and intact onion roots, found that the water uptake was considerably increased following a treatment with sucrose in the medium. It seems that the treatment with sugar solution makes the tissue more permeable to water. Although this interpretation is not considered by the authors mentioned, it seems wise to bear in mind this possibility, in view of the experiments described below. The problem that is at issue, therefore is in the first place the causation of the changes in water conductivity.

Besides, it seems important to get some information regarding the area where these changes in conductivity take place; in the protoplasm, in the wall or in both. In the beginning BREWIG assumed the protoplasm to play an important role in these changes and he gives calculations from which it appears that the permeability of the protoplasm of the root of *Vicia faba* was of the same order as the permeability calculated by HUBER and HÖFLER (1930) for Salvinia and other objects. A transport through the protoplasm was rejected by him, however, after noting the results of STRUGGER (1938). STRUGGER showed by means of fluorescing dyes as indicators a wall transport of water in parenchymatous tissues. It should be remembered that the endodermis will raise a barrier for a transport through the walls.

§ 2. THE CAUSAL RELATIONS OF THE CHANGES IN WATER CONDUCTIVITY

If we look upon the distribution of water uptake over the different zones of the root, it appears that at low transpiration the maximum amount of water is taken up by either the apical zone or the zone adjacent to it. If the external circumstances are not altered a steady state is obtained which shows only slight fluctuations.

Enhancing the transpiration produces an immediate increase in uptake in such a way that the ratio between the rate of absorption, before and after enhancement, is the same for all zones. This must be due to the increased suction tension in the xylem vessels. For, according to the formula of BRIEGER (Uptake = Conductivity × Suction tension) the water uptake is determined by the suction tension in the xylem vessels and the water conductivity in the root tissue. This applies to the root system as a whole as well as to each zone separately. If the suction tension increases to twice its original value, the water uptake ought to increase to twice its original value, assuming the conductivity to be constant. After some time, however, we see that the water uptake of the more basal zones becomes greater and greater. After about three hours a new steady state has been established and a new constant distribution of the water absorption rates between the different zones has been reached. The difference with the former distribution (at low transpiration) is that the maximum uptake is shifted to the basal zones. As compared with the situation at low transpiration there is a difference in two respects. Firstly the suction tension is increased but this increase is the same for all zones. As a consequence of this fact therefore, the distribution of the uptake over the root axis would not change. On the other hand the conductivity is changed and these changes in conductivity caused the shifting of the uptake maximum.

It appears that the conductivity of the apical zones has not changed at all, whereas the conductivity of the basal zones is strongly increased. In this case, therefore, the changes in conductivity were produced by an increase of the suction tension in the xylem vessels as a consequence of an increased transpiration. However, an increased suction tension in the vessels can be produced in another way.

With the aid of the set up described it is easy to enhance the water absorption of the tested root by applying an osmotically active solution to the bulk of the roots in the main vessel. The water uptake of the plant as a whole is more or less hampered by this manipulation. In this way it is possible to get every desired suction tension in the xylem vessels and to investigate the correlation between suction tension and conductivity.

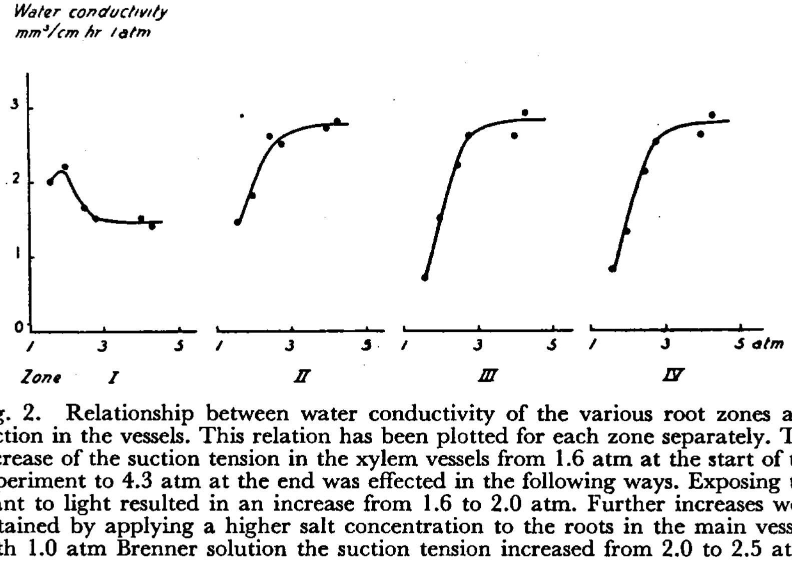

Fig. 2. Relationship between water conductivity of the various root zones and suction in the vessels. This relation has been plotted for each zone separately. The increase of the suction tension in the xylem vessels from 1.6 atm at the start of the experiment to 4.3 atm at the end was effected in the following ways. Exposing the plant to light resulted in an increase from 1.6 to 2.0 atm. Further increases were obtained by applying a higher salt concentration to the roots in the main vessel; with 1.0 atm Brenner solution the suction tension increased from 2.0 to 2.5 atm; with 1.5 atm Brenner solution from 2.5 to 2.8 atm; with 3.3 atm Brenner solution from 2.8 to 4.0 atm; and with a 3.9 atm Brenner solution from 4.0 to 4.3 atm. The conductivity values at a suction tension of 1.6 atm show that the normal distribution at low suction tension occurred at the start of the experiment. All the values plotted are determined at a steady state, the water uptake and the suction tension being constant for several hours.

The results of such an experiment are plotted in figure 2 (ample description of the experiment vide BROUWER 1953*a*). In this figure the conductivity of the different zones is plotted against values of the suction tension of 1.6; 2.0; 2.5; 2.8; 4.0 and 4.3 atmospheres. It is clear that the water conductivity of the basal zones increases from about 0.6 mm³/cm.hr. 1 atm at 1.6 atm to about 3 mm³/cm.hr. 1 atm at 4.0 atm. The conductivity of zone II is already comparatively great at low suction tension. In spite of this it further increases with increasing the suction tension. The conductivity of the tip zone (zone I) initially increases a little but thereafter decreases.

We have to consider two possibilities for this increase in water conductivity. It is possible either that the existing channels increase their capacity for water transport or that new channels, also running in radial direction, are added to the existing pathway. It is difficult to make a choice between these two possibilities on the basis of these experiments. It is clear, however, that there exists a strong correlation between the suction tension in the xylem vessels and the water conductivity of the root tissue.

On the basis of the result of ROSENE (1941) we may expect that the same correlation exists between suction tension in the outer solution and water conductivity of the root tissue. To check this assumption we carried out the following experiment; the results are plotted in figure 3.

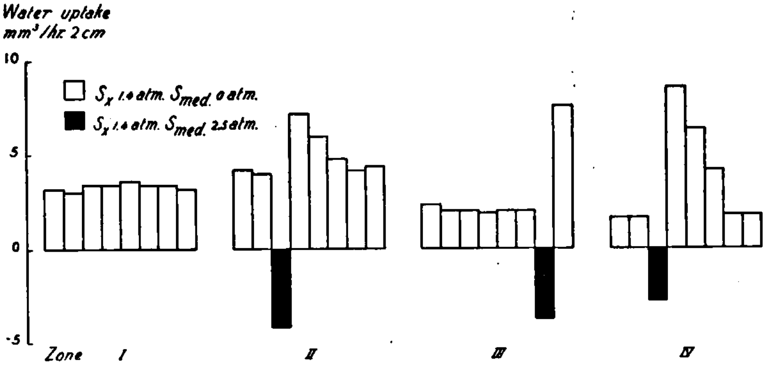

Fig. 3. Comparison of the water uptake of the different root zones, before, during and after application of a 2.5 atm sucrose solution. Each block represents the uptake value during one hour period in mm per hour per zone of 25 mm length. The suction tension in the xylem vessels amounted to 1.4 atm and remained constant throughout the experiment. Applying the 2.5 atm sucrose solution (black blocks) resulted in a withdrawal of water from the root. After this application the amount of water taken up was considerably greater than before. The ratio between the value before and after the application of sucrose is a measure of the changes in conductivity, the suction tension in the xylem vessels remaining the same in both cases.

The water uptake of three zones of the tested root of a plant with low transpiration and a low suction tension in the xylem vessels (1.4 atm) is measured before, during and after the application of a 2.5 atm sucrose solution in the medium. The tip zone is taken as control. The distribution of the water uptake over the different root zones at the start of the experiment was the normal one at low suction tension; the water uptake being highest in the apical region (zone I and II). During application of the sugar solution a withdrawal of water took place, the osmotic suction tension of the sugar solution being higher than the suction tension in the xylem vessels. The sugar solution is applied during the third one hour period to zones II and IV and during the seventh one hour period to zone III. During the sugar application the water moving power amounted to 2.5 — 1.4 =

1.1 atm. During the other periods this power amounted to 1.4 atm. For calculating the water conductivity during the successive periods we have to divide the water uptake by the effective suction tension. In table I the water conductivity values of the different root zones are given of the period directly before, during and directly after the sugar application. It appears from this table that the conductivity during a period of sugar application (one hour) is greater than in the preceding period. During the period directly after the sugar application the water conductivity is much higher than during the period before the treatment with sugar. It seems that because of the sugar application the conductivity increases and that this increase needs some time to get its highest value. The ratio between the conductivity values before and after the sugar treatment amounts to about 5.0 for zone IV, to about 4.0 for zone III and to about 1.6 for zone II. This means that the changes in water conductivity as caused by treatment with a sugar solution are most important in the basal zone. It is striking, therefore, that this behaviour resembles greatly the changes in conductivity obtained by an increase in suction tension in the xylem vessels.

TABLE I

The water conductivity of three zones of the tested root, directly before (*a*), during (*b*) and directly after (*c*) application of a 2.5 atm sucrose solution in the medium (vide fig. 3). The suction tension in the xylem vessels amounted to 1.4 atm.

Zone		Water transport mm³/cm. hr	Effective s. t. atm	Water conductivity mm³/cm. hr. 1 atm
IV	*a*	0.8	1.4	0.6
	b	1.4	1.1	1.3
	c	4.3	1.4	3.1
III	*a*	1.0	1.4	0.7
	b	1.9	1.1	1.7
	c	3.8	1.4	2.7
II	*a*	2.0	1.4	1.5
	b	2.1	1.1	1.9
	c	3.6	1.4	2.6

After some time the conductivity decreases to its original value. This is obvious from the steadily decreasing water uptake values after sugar application.

It is possible to get these changes in water conductivity at constant suction tension in the vessels in different ways. Instead of a sugar solution an osmotically active salt solution (Brenner solution) can be used. Moreover, it is possible to enhance the water conductivity of a root zone by means of a slight drying up of the tissue, e.g. by an air current through the potometer for 3 or 5 minutes.

All these methods have the same effect, namely, a water withdrawal from the tissue and a reduction of the turgescence. This holds for an increased suction tension in the xylem vessels caused by a loss of water during transpiration, as well as for an osmotic suction tension applied in the medium.

§ 3. LOCALISATION OF CHANGES IN WATER CONDUCTIVITY IN THE PROTOPLASM

To investigate the localisation of the resistance to the water current we have to look for the factors which influence the conductivity. RENNER (1929) showed already that the conductivity of a whole root system could be enhanced by killing the root tissue by means of high temperatures. From these results he concluded that the living root tissue and especially the living protoplasm, offered considerable resistance to the water current. KRAMER corroborated this observation and got the same result with ether narcosis. The cold-hardening experiments of LEVITT and SCARTH (1936) and the carbon-dioxide influence of CHANG and LOOMIS (1945) and of HOAGLAND and BROYER (1942) point in the same direction.

ROSENE (1944, 1947, 1950) described investigations into the influence of respiratory inhibitors and oxygen deficiency on the water uptake and exudation of excised onion roots. She found a strong inhibition at definite concentrations. It seems possible, however, that the salt secretion was inhibited in these experiments and that this inhibition caused a decreased water absorption. VAN ANDEL (1953) showed that even in a medium of distilled water a salt secretion into the xylem vessels is possible (tissue bleeding). BROUWER (1953*a*) found that the effects of a number of factors locally applied remained confined to the treated zones, so that without influencing the total water supply of the plant, the effects of several factors on the conductivity of the root tissue of the treated zone could be investigated.

By narcosis with chloroform the conductivity increases, confirming the ether experiment of KRAMER. Applying a carbon dioxide saturated solution reduced the conductivity (confirming the results of CHANG and LOOMIS and of HOAGLAND and BROYER), while, as KRAMER found, oxygen deficiency had little influence. Only the tip zone showed a gradual decrease in water uptake in the latter case.

All these experiments fail to detect an active water uptake during high transpiration. This also appeared from the influence of potassium cyanide on the water uptake. Concentrations of 10^{-4} and 10^{-5} molar, which inhibit respiration, do not influence the water uptake. Very high concentrations of 10^{-3} and $5.\ 10^{-3}$ molar have a distinct influence.

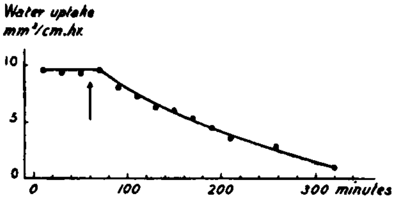

Fig. 4. Time course of the influence of potassium cyanide on the water uptake by one zone of a single root of an intact plant. The suction tension in the xylem vessels and the water uptake of the adjacent zones (non-treated) remained constant. More than 4 hours elapsed before the uptake had been diminished to about 10 % of its original value. The arrow indicates the beginning of the treatment.

Applying 5 × 10^{-3} M KCN stops the water uptake immediately. The time course of the influence of 10^{-3} M KCN on the water uptake is plotted in figure 4. The experimental plan was the following. At high water uptake, in the outer solution of a basal zone the tap water was replaced by a 10^{-3} M KCN solution. A gradual reduction of the water uptake was the result of this treatment. The water uptake of the adjacent non-treated zones remained unchanged, indicating that the suction tension in the xylem vessels did not alter. The water uptake of the treated zone was reduced gradually until after about 5 hours it completely stopped. Not until 36 hours after removing the potassium cyanide was this uptake normal again. It is unlikely that this gradual effect is attributable to an elimination of an active component of the water uptake. Rather we are dealing with a change in the structure of the protoplasm slowly setting in, causing a greater resistance to the water current. From these experiments we have to conclude that water conductivity is determined by the protoplasm to a high degree.

§ 4. Discussion

The experimental results show that by applying an osmotic suction tension in the medium the conductivity for water in the root tissue is increased. This increase is identical with that caused by normal changes of the suction tension in the xylem vessels. This demonstrates that the changes in conductivity are dependent on changes in suction tension.

Moreover, it is clear that this is not a polar process, since an enhancement of the suction tension in the xylem vessels has the same effect as an increase of the suction tension in the medium. In both cases the suction tension of the cells of the root tissue is influenced, the turgidity being reduced. It may be assumed, that this decrease in turgidity is responsible for the increase in conductivity.

Further, the experiments with inhibitors, oxygen deficiency and carbon dioxide treatment point to a plasmatic process, that changes the water conductivity of the tissue. Thus we may conclude that somewhere between the medium and the xylem vessels migration of water takes place through the cytoplasm. The exact area where this takes place is still unknown. Increasing the suction tension of the cell reduces the turgor pressure acting upon the protoplasm between vacuole and cell wall. It is supposed that this decrease in pressure upon the protoplasm causes a greater water conductivity of the protoplasm. The inhibitors etc. are assumed to be active via the structure of the protoplasm.

It is not necessary to suppose that there is any active water uptake.

It may be assumed that the same influence of an osmotic suction tension in the medium on the water uptake after treatment was found earlier but was not interpreted in this way.

CHAPTER IV

THE ANION UPTAKE OF THE DIFFERENT ROOT ZONES UNDER VARIOUS CIRCUMSTANCES

§ 1. INTRODUCTION

As stated in chapter I, up to now, little has been known about the salt uptake of the different zones of the root. The experiments of GREGORY and WOODFORD are preliminary and general conclusions are not yet possible. After developing a method for the determination of water uptake by the different zones, BREWIG discussed the desirability of a determination of the salt uptake. He was not successful in realizing his idea. It is clear that many physiologists before and after BREWIG have seen the desirability of such experiments. It is probable that technical difficulties have been responsible for the lack of such like experiments. The fact that the secondary roots of *Vicia faba* are strong enough and reach a length of about 20 cm before branching takes place, makes this material suitable for potometer experiments. Further, the possibilities offered by modern substances like perspex have contributed greatly to the mastery of the constructive difficulties.

As stated in chapter II a root of an intact *Vicia faba* plant is enclosed in a series of small vessels, each enclosing 3 cm of the root, and each of which can be easily drained. Joining an aeration mechanism to these vessels makes it possible to perform rather prolonged experiments. The plant was illuminated by a 300 Watt lamp at a distance of 40 cm and placed in a chamber of constant temperature, 24° C ± 0.5° with a relative humidity of 40–45 %.

In spite of these constant circumstances, it was possible to vary the water uptake of the root in the zone-vessels. This could be achieved by changing the osmotic suction tension of the solution in the main vessel in which the bulk of the root system was placed. As stated above this renders it possible to get each value of the suction tension in the xylem vessels and corresponding values for water uptake by the tested root. A salt solution according to BRENNER (1920) of different concentrations was used as an osmotically active solution. Because this solution contains much chloride a sucrose solution was used during experiments in which the chloride uptake was determined. A mannitol solution which has often been applied for this purpose, was not suitable in this case for it proved to reduce the water uptake very little.

The experiments were performed as follows. After enclosing the root in the zone vessels the main vessel was filled with tap water and the zone vessels were filled with 3 cc of the nutrient solution. After about 4 hours the contents of the zone vessels were drained and the vessels rinsed twice with 5 cc distilled water. Thereafter each zone vessel was refilled with 3 cc of the nutrient solution and the experiment was started. The preceding period of 4 hours was taken to allow an exchange equilibrium between the root and the nutrient solution to be established. After an uptake period of 24 hours (in a few cases

8, 12 or 16 hours) the nutrient solution was tapped off from the zone vessels and the vessels were rinsed twice with distilled water. This rinsing water was caught and mixed with the former sample. Apart from the number of vessels enclosing the root, there was always one vessel that served as a control.

The quantity of ions investigated was determined in:

a. 3 cc of the original nutrient solution;
b. the control after 24 hours aeration, rinsing etc.;
c. each of the zone-vessels after a 24 hour uptake period.

It appeared that the amounts found in *a* and *b* were the same within the limits of analytical errors.

This means that there are no losses as a consequence of the various manipulations. The difference between *b* and each of the zone vessels was taken as the uptake of the root zone concerned. All values were calculated in γ per 24 hours per 3 cm root length.

In previous experiments the constancy of the uptake under constant circumstances in successive periods of 24 hours had been investigated. It appeared that the uptake of the different zones was constant at least during three successive periods of 24 hours. This was the case with low water uptake as well as with high water uptake. This means that it is possible to investigate the influence of various factors on the ion uptake within a time period of 72 hours. The experiments described below, therefore, lasted 72 hours at the most.

§ 2. INFLUENCE OF WATER UPTAKE ON ANION UPTAKE BY THE DIFFERENT ZONES OF THE ROOT

The experimental planning of all these experiments was the following. The uptake of the nitrate, chloride or phosphate ions was determined during two successive periods of the same duration. During one of these periods there was tap water in the main vessel, during the other a Brenner salt solution or a sucrose solution of about 2.5 atm. This means, therefore, that on the ground of preceding experimental results the water uptake by the tested root can be assumed to be low with a maximum near the tip when tap water is in the main vessel and to be high with a maximum in the basal zones when an osmotically active solution is in the main vessel. The shoot remained under constant conditions during both periods. The anion uptake of the two periods was compared.

a. Chloride uptake

In connection with the fact that the analysis of chloride by means of the electrometric titration with silver nitrate makes possible very accurate determinations most of the experiments were performed with chloride. The chloride uptake from a 5 mM calcium chloride solution was determined. The chloride uptake of the different root zones at low and high water uptake is recorded in table II. From these data we see that the chloride uptake at high water uptake is considerably greater than at low water uptake.

TABLE II

Chloride uptake of the different root zones at low and high water uptake in γ Cl per 24 hours per 3 cm root length. Average values for 5 experiments

Zone	Water uptake by the tested root		Ratio
	low	high	
IV	12 γ Cl	42 γ Cl	3.5
III	19 „	52 „	2.7
II	26 „	43 „	1.7
I	26 „	33 „	1.4

At low water absorption the chloride uptake is highest in the apical zones and diminishes towards the base. At the transition from low water uptake to high water uptake the chloride uptake of the apical zones increases least. The greater the distance from the apex the greater the percentage increase in uptake. This agrees with the reaction which we found in the experiments on water uptake. There is, however, a quantitative difference. The maximum water uptake at a high suction tension in the xylem vessels was always found in the basal zones. This is not the case here. The relative increase in water uptake was greater than the increase in chloride uptake found here.

b. Nitrate uptake

In this case the difficulty arose that the nitrate uptake of the basal zones hardly exceeded the errors of the analysis when the water uptake was low. When water absorption was high, however, a significant nitrate uptake was obtained. To get a better view of the quantitative relations of this uptake at low and high water uptake the average values of this uptake in 8 experiments were determined. In table III all results are given and in a separate column the average values at low and high water uptake are shown. The corresponding experiments are indicated by experimental numbers one to eight. The ratio is calculated from the average values.

TABLE III

Nitrate uptake of the different root zones at low and high water uptake in γ N per 24 hours per 3 cm root length

Zone	Water uptake by the tested root																		Ratio
	low									high									
	Exp. nr.								Av. γN	Exp. nr.								Av. γN	
	1	2	3	4	5	6	7	8		1	2	3	4	5	6	7	8		
IV	8	4	2	9	3	5	–3	8	4.5	26	23	8	19	16	17	13	27	18.6	4.1
III	12	6	4	16	4	7	–2	10	7.1	30	26	8	20	15	19	12	25	18.4	2.6
II	11	8	6	19	9	8	4	10	9.4	28	24	16	23	18	23	10	29	21.4	2.3
I	13	12	6	18	12	10	7	12	11.6	19	17	10	24	17	16	14	18	16.9	1.4

The nutrient solution used was a 10 mM potassium nitrate solution. From the data of table III it appears that for nitrate the same holds good as for chloride, i.e. with an increased water uptake an increase of the nitrate uptake takes place simultaneously. This increase is also highest in the basal root parts.

c. Phosphate uptake

The phosphate uptake is determined with the aid of radio-active phosphorus. A full strength Hoagland solution containing labelled phosphate (activity about $15\mu^{\text{Curie}}/_{\text{L}}$) was used in the zone vessels. The activity before and after an uptake period was determined. The possibility exists that there has occurred some exchange of radio active phosphate against phosphate already present in the root tissue. This exchange, however, is probably negligible, for radio-active phosphate taken up by the root did not show any exchange with unlabelled phosphate in the medium. As is shown in table IV the phosphate uptake gives essentally the same picture as the chloride and nitrate uptake. The data reported refer to a single experiment. The root used for this experiment was 15 cm long. Zone I showed a rapid growth. The uptake of this zone (per 3 cm root length) was about the same under the various circumstances. The mature zones III and IV demonstrated the greatest influence of the water uptake on the phosphate uptake. The root segment V displayed an incipient side root formation during the experiment. We see that the phosphate uptake of this zone at low water uptake was higher than the uptake of the adjacent zone IV. On the other hand the influence of the water uptake was smaller in zone V. Thus the nature of the uptake resembles that of the apical zones. As will be described in one of the following chapters this is due to a relatively greater binding of phosphorus in the root tissue itself.

TABLE IV

Phosphate uptake of the different root zones at low and high water uptake in γ P per 24 hours per 3 cm root length

Zone	Water uptake by the tested root		Ratio
	low	high	
V	4.1 γ P	10.8 γ P	2.6
IV	1.5 „	9.9 „	6.6
III	1.6 „	10.4 „	6.5
II	5.2 „	9.3 „	1.8
I	10.1 „	11.3 „	1.1

It may be concluded from these experiments that the increase in water uptake is accompanied by an increased anion uptake. These results agree with experiments in which the uptake by the whole root system was determined at low and high transpiration (BROUWER, 1953*b*).

It is clearly demonstrated that this increase in anion uptake is in the main the consequence of an increase in uptake by the basal zones. The question arises how far this increased uptake at high water uptake is an active or a passive process.

§ 3. HAMPERING THE WATER UPTAKE DOES NOT INFLUENCE THE INCREASED ANION UPTAKE

These experiments were started in the same way as those of the preceding paragraph, viz. the first period with tap water in the main vessel, the second period with a 2.5 atm sucrose solution. In this series the chloride uptake from a 5 mM calcium chloride solution was determined for both periods. During the third period conditions in the main vessel remained as in the second period. The different root zones could then absorb from a 5 mM calcium chloride solution to which sucrose was added until an osmotic value of 2.5 atm was reached. The water uptake of the enclosed root zones may be assumed to be reduced compared with that uptake during the second period. No reduction of the chloride uptake can be detected, however. All the experiments performed in this way with sucrose as an osmotic substance showed this result.

The results of a single experiment are plotted in figure 5. From this figure we see that increasing the water uptake of the tested root (transition from period 1 to 2) is followed by an increase in chloride uptake. Reduction of this water uptake by a counter suction in the medium (period 3) does not reduce the increased chloride uptake, on the contrary this uptake continues to take place at the preceding high level.

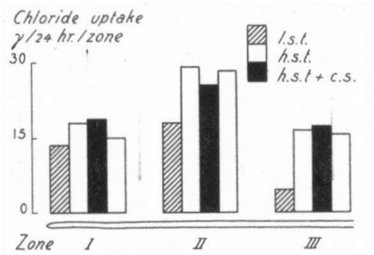

Fig. 5. The chloride uptake of the various root zones; shaded blocks, low suction tension in the vessels; white blocks, high suction tension in the vessels; black blocks, high suction tension in the vessels with an osmotic counter suction in the medium. The outer solution was 5 mM $CaCl_2$ (period 1,2 and 4) and 5 mM $CaCl_2$ + 100mM sucrose (period 3). Each period lasted 24 hours. The shoot was constantly illuminated. The suction tension in the vessels of the tested root was increased by enhancing the osmotic value of the solution in the main vessel.

We suppose, therefore, that the anions are not carried along passively with the increased transpiration stream during period 2, for in that case we would expect that the anion uptake, as a result of the reduction of the water uptake by the sugar solution in the zone vessels, would also decrease.

These experiments were repeated with mannitol as an osmotic agent. The results obtained were more variable. From parallel experiments upon the influence of mannitol on water uptake it appeared that this substance either inhibited water uptake to a variable extent or failed to do so. In some of the experiments with mannitol on the chloride uptake a leakage of chloride ions could be demonstrated. This leakage sometimes continued during a following period after the removal of the mannitol. An explanation of this phenomenon can not be given as yet.

§ 4. INHIBITION OF ANION UPTAKE AT LOW AND HIGH WATER UPTAKE

From the preceding experiments we may conclude that the anion uptake increases simultaneously with water absorption, but that the anion uptake is not directly dependent on water uptake since it is possible to reduce the latter without influencing the anion uptake.

It was expected that by means of inhibitors the reverse could be reached. The experiments were performed in the same way as described above. The inhibitors used were applied in concentrations which did not influence the water uptake according to former experiments.

The experiments were carried out in order to be able to decide whether the increased anion uptake at high water uptake was an active or a passive process. In the former case it must be possible to inhibit a greater portion of the total anion uptake than the amount of ions already taken up at low water uptake. From table V it is clear that the inhibition (difference between columns 2 and 3), recorded there, is slightly higher than the uptake values at low water uptake. Thus, although these data do not prove definitely that the inhibition is greater than the uptake at low suction tension, this seems very likely.

TABLE V

Chloride uptake of the different root zones in γ Cl per 24 hours per 3 cm root length at low and at high water uptake. Column 3 shows Cl uptake at high water uptake and with 10^{-5} M DNP added to the nutrient solution

Zone	Water uptake by the tested root		
	low	high	high + 10^{-5}M DNP
IV	7 γ Cl	22 γ Cl	12 γ Cl
III	11 „	27 „	14 „
II	13 „	24 „	9 „
I	12 „	19 „	6 „

When higher concentrations of the inhibitor are used it is possible to completely inhibit the chloride uptake. The drawback of such experiments is, however, that sometimes a leakage from the root can be measured which renders the interpretation more suspicious.

Investigating the inhibition by definite concentrations of the inhibitors at low and high water uptake it was apparent that different plants showed a great difference in sensitivity for inhibitors. Moreover, in no case did this inhibition appear to be fully reversible, i.e. the anion uptake before and after application of the inhibitor did not reach the same level. This rendered it impossible to investigate the inhibition at low and high water uptake with the same root. The consequence is that we are obliged to derive our conclusions from a comparison of different experiments, accepting the variability of the sensivity of the individual plants. We traced the effect of potassium cyanide 5 × 10^{-6}, 2–4-dinitrophenol 10^{-5}, 0_2-deficiency by nitrogen bubbling through the solution, 0_2-deficiency by stopping the aeration, and CO_2 bubbling through the solution.

The results of inhibition of the chloride uptake by 10^{-5} M 2,4-dinitrophenol (DNP), 5 10^{-6}M potassium cyanide and nitrogen are given in table VI, viz. at low water uptake in VI*a* and at high water

TABLE VI

Inhibition of the chloride uptake by various inhibitors at low and at high water uptake of the tested root

A. Chloride uptake of the different root zones at low water uptake with and without inhibitor

Zone	DNP 10^{-5} M			KCN 5.10^{-6} M			N_2		
	—	+	% inh.	—	+	% inh.	—	+	% inh.
IV	7	4	43				10	6	40
III	16	4	75	5	2	60	11	7	36
II	15	0	100	29	11	63	13	6	52
I	27	6	78	27	6	78	20	7	66

B. Chloride uptake of the different root zones at high water uptake with and without inhibitor

Zone	DNP 10^{-5} M			KCN 5.10^{-6} M			N_2		
	—	+	% inh.	—	+	% inh.	—	+	% inh.
IV	41	25	40	49	30	40			
III	39	26	35	53	28	47	57	23	40
II	49	32	37	50	26	48	40	18	55
I	42	19	55	36	11	70	31	12	61

uptake in VI*b*. From these data it can be concluded that the percentages of inhibiton differ somewhat. The inhibition is always strongest in the apical zone. We can state that we cannot observe any essential difference between the experiments at low water uptake and those at high water uptake. It is a pity that the best method, the direct comparison of the inhibition at low and high water uptake with the same root, cannot be used. This is possible, however, with the inhibition of the chloride uptake obtained by stopping the aeration, such inhibition being fully reversible.

The data of table VII may illustrate this fact. Comparing the values of columns 1 and 3, on the one hand, and columns 5 and 7 on the other, we see that the uptake after a period without aeration reaches about the same level as that during the period preceding the treatment. Moreover, it appears that the inhibition obtained by stopping the aeration is equally strong both at low and high water uptake.

This strongly supports the fact that the chloride uptake at high water uptake is as active as the uptake at low water uptake.

TABLE VII

Chloride uptake of the different root zones in γ Cl per 24 hours per 3 cm root length with and without aeration at low and high water uptake

Water uptake	low				high			
Aeration	on	off	on	% inh.	on	off	on	% inh.
Zone								
III.	23	19	21	14	58	33	64	46
II.	33	16	35	53	40	18	38	50
I.	31	13	29	57	32	12	26	59

§ 5. Discussion

The experiments on the determination of the anion uptake by the different root zones, described in this chapter, are new with the exception of a few preliminary communications. It is clear that at low water uptake, the anion uptake is strongest within a distance of 5 cm from the tip. On the other hand it is clear that under these circumstances the more basal zones, up to at least 15 cm from the apex are able to absorb anions. This is not surprising since we know that water can be absorbed over the whole root length.

Brouwer (1953*b*) demonstrated that the material used, showed an increased anion uptake after increasing the water uptake. It could be expected, therefore, that one single root would give the same phenomenon as the whole root system. It is interesting that especially the basal zones strongly increase their anion uptake just as they did for water. The anion uptake of the tip zone showed hardly any change. This fact is particularly interesting for we know that the water uptake of the tip zone increased almost in proportion with the increase in suction tension in the xylem vessels, according to the fact that the water conductivity of the tip zone remains about the same. This suggests a probable mechanism as to the influence of the transpiration on the anion uptake.

If the extra amount of ions taken up is assumed to be transported passively by the water stream the tip zone would show an increased anion uptake simultaneously with an increased water uptake. A passive transport in the transpiration stream is the more improbable because we are able to hamper the water uptake by applying an osmotic counter suction in the outer solution without any reduction in anion uptake.

The experiments with inhibitors support this assumption, although the results were variable.

One drawback of the experimental method described in this chapter is the fact that it was impossible to compare directly the water uptake with the anion uptake.

CHAPTER V

DIRECT COMPARISON OF WATER UPTAKE AND ANION UPTAKE IN THE SAME ROOT

§ 1. INTRODUCTION

The drawback of the experiments described in the preceding chapter, viz. the impossibility of getting direct information on the water uptake, made a direct comparison of both water and salt uptake values desirable. The experiments described below were performed in such a way, that the water uptake of two zones of the tested root was determined, simultaneously with a determination of the anion uptake of the other zones. The water uptake was determined during two periods of one hour, one at the start and one at the end of each experimental period. Between these periods a continuous flow of aerated nutrient solution was circulated through the potometers used for measuring the water uptake. In this way conditions in the water uptake vessels and the salt uptake vessels were maintained as equal as possible.

§ 2. SIMULTANEOUS DETERMINATION OF THE INCREASE IN CHLORIDE UPTAKE AND THE INCREASE IN WATER UPTAKE ON ENHANCING THE SUCTION TENSION IN THE XYLEM VESSELS

The suction tension in the xylem vessels was varied in the usual way, viz. by changing the osmotic value of the solution in the main vessel. Tapwater and a 2.5 atm. sucrose solution were used alternately. The uptake values during a period with low suction tension (l.s.t.) and a period with high suction tension (h.s.t.) in the xylem vessels were compared. The results of two experiments are given in table VIII. Table VIIIA gives the data of an experiment in which the chloride uptake of zones I and III and the water uptake of zones II and IV were determined. On the contrary, table VIIIB indicates the water uptake of zones I, III and V, and the chloride uptake of zones II and IV. Though not completely comparable (the experiments

TABLE VIII

Increase in chloride and water uptake of the various root zones as caused by an increased suction tension in the vessels. Chloride uptake in γ Cl, water uptake in mm^3 per 24 hours per zone

Experiment A			Experiment B		
Zone	L. s. t.	H. s. t.	Zone	L. s. t.	H. s. t.
			V$_{\text{water}}$	64	704
IV$_{\text{water}}$	56	352	IV$_{\text{chloride}}$	18	37
III$_{\text{chloride}}$	7	25	III$_{\text{water}}$	84	672
II$_{\text{water}}$	112	320	II$_{\text{chloride}}$	25	35
I$_{\text{chloride}}$	32	34	I$_{\text{water}}$	152	278

were performed with different roots) the data give a good picture of the influence of an increased suction tension in the xylem vessels on the water and anion uptake of the different root zones. It is, in a sense, a combination of an experiment on the influence of an increased suction tension on the water uptake and an experiment on such an influence on the anion uptake. It is not surprising that the results reported in table VIII confirm the results described above. It is possible to derive from this table a number of important data.

At first it appears that the increase in water uptake is greater than the increase in chloride uptake. This holds good for both experiments.

The chloride uptake of the tip zone does not increase at all although the water uptake does. This is reasonable since the suction tension is enhanced. The increase in water uptake of the tip zone is more or less a measure of the increase in suction tension in the xylem vessels.

The more basal zones show a greater increase in water uptake partly caused by an enhancement of the suction tension and partly by an increase in water conductivity.

The increase in chloride uptake of the basal zones is lesser than the increase in water uptake of these zones.

§ 3. THE INFLUENCE OF AN OSMOTIC COUNTER SUCTION ON WATER UPTAKE AND CHLORIDE UPTAKE

These experiments were performed in the same manner as those described in § 3 of the preceding chapter. During the first period the water and chloride uptake from a 5 mM calcium chloride solution was determined at low suction tension in the xylem vessels. In the second period the only difference with regards to period 1 was the enhanced suction tension in the xylem vessels. During the third period the water and chloride uptake from a 5 mM calcium chloride solution

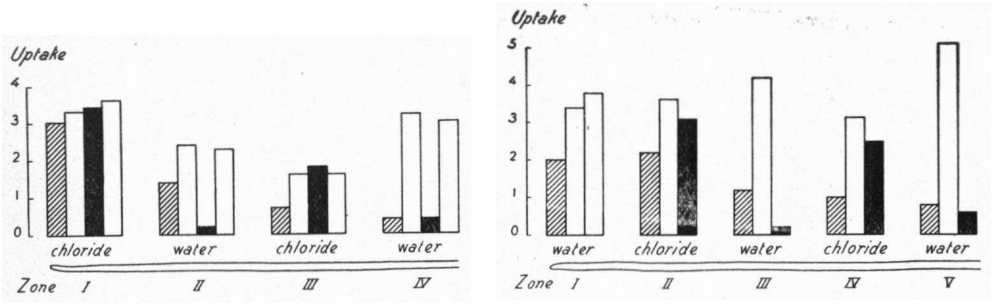

Fig. 6*a*. and *b*. Comparison of chloride uptake and water uptake of the various root zones, at low suction tension (shaded blocks), at high suction tension without a counter suction in the medium (white blocks) and at high suction tension with an osmotic counter suction in the medium (black blocks). Solutions used as indicated in fig. 5. *a* and *b* represent different experiments; *a* with the tip zone enclosed in a salt uptake vessel, *b* with the tip zone in a potometer. With a counter suction in the medium the water uptake strongly decreases without a reduction in chloride uptake. Scale unity = 14 γ Cl or 200 mm^3 water per zone per 24 hr. Experimental periods 16 hours.

with added sucrose (2.5 atm) was determined at a high suction tension in the xylem vessels.

We see from figures 6*a* and 6*b*, showing respectively chloride uptake of the tip zone and water uptake of the tip zone, that on increasing the suction tension in the xylem vessels the normal enhancement of the water and chloride uptake takes place and that during the third period, when an osmotic suction tension in the medium was applied, the water uptake is strongly reduced, without any decrease of the chloride uptake. We have, therefore, a distinct affirmation of the supposition stated in the preceding chapter.

In figure 7 the results are plotted of an experiment in which the counter suction in the medium was applied at low suction tension in the xylem vessels. The osmotic value of these solution was 2.5 atm, as in the former experiments. After applying this solution we see an immediate water withdrawal from the root. This withdrawal is diminishing gradually and reverted into a water uptake at the end of the experimental period. As a result there was only a slight total withdrawal of water from zone II and even a slight total water uptake by zone IV. The total amount of water uptake of the whole root will have been about zero. The chloride uptake of zone I is not influenced by the treatment but in zone III we see an increased chloride uptake. Repetition of this experiment gave the same results. For the interpretation we may refer to the increased salt uptake following an increase in the suction tension of the outer solution (Brouwer, 1953*b*, fig. 9) and the increase in water conductivity (Chapter III, fig. 3) by the application of a sugar solution in the medium.

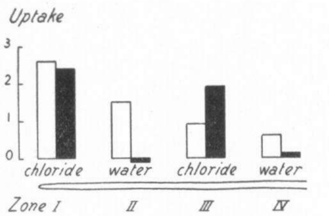

Fig. 7. Comparison of chloride uptake and water uptake of the various root zones at low suction tension in the xylem vessels without an osmotic counter suction in the medium (white blocks) and with such a counter suction (black blocks). The water uptake during application of the sugar solution is strongly reduced whereas the chloride uptake of zone I did not change and that of zone III increased. Values vide fig. 6.

§ 4. Influence of inhibitors on water uptake and anion uptake

These experiments were performed at high suction tension in the xylem vessels. The uptake values from a nutrient solution with and without inhibitors were compared. We have investigated the effect of 2,4-dinitrophenol and oxygen deficiency on the chloride and phosphate uptake. For the chloride uptake a 5 mM calcium chloride solution was used; for the phosphate uptake a Hoagland solution with labelled phosphate.

From all the experiments it appeared that the anion uptake was much more sensitive to a treatment with inhibitors than the water uptake. The results of two experiments with 2,4-dinitrophenol (10^{-5}M) are given in table IX (IX *A* chloride; IX *B* phosphate). In the latter case the inhibitor is only added to the medium of zones II and III.

TABLE IX

Influence of 2.4-dinitrophenol, 10^{-5} M, on the water and anion uptake at high suction tension in the vessels. Water uptake in mm^3, chloride uptake in γ Cl per 24 hours per 3 cm root length. Application of inhibitor indicated by italics.

A (chloride) B (phosphate)

Zone			Zone		
IV_{water}	730	*746*	$IV_{phosphate}$ . . .	6	5
$III_{chloride}$	40	*18*	III_{water}	640	*655*
II_{water}	560	*540*	$II_{phosphate}$ . . .	11	*6*
$I_{chloride}$	31	*12*	I_{water}	470	455

TABLE X

The influence of oxygen deficiency on the water and chloride uptake of the different root zones at high suction tension in the vessels. Uptake in mm^3 water and γ Cl per 24 hr per 3 cm root length

Zone	Aeration		
	on	off	on
IV_{Cl}	37	23	35
III_{water}	580	600	570
II_{Cl}	39	18	38
I_{Cl}	32	11	24

In both cases the water uptake was not inhibited, but the anion uptake was strongly inhibited. These data strongly confirm the results of the preceding chapter.

The reversible inhibition caused by stopping the aeration of the zone vessels is shown in table X. It appears that the anion uptake is extremely sensitive to this oxygen deficiency while the water uptake, as expected on the ground of many preceding experiments, does not decrease. The inhibition of the anion uptake by a period with oxygen deficiency is probably not fully reversible for the tip zone.

§ 5. DISCUSSION

The results of the experiments of this chapter confirm the conclusions which were drawn from the experiments of the preceding chapter. The most striking result is that on the one hand there was a distinct correlation between water and anion uptake, on the other hand it was possible to separate both processes. By means of inhibitors it is possible to reduce the anion uptake without influencing the water uptake. Moreover, an osmotic counter suction in the medium diminishes the water uptake without reducing the anion uptake. It seems, therefore, that the total anion uptake, including the increased anion uptake at an enhanced suction tension in the xylem vessels, is a fully active process. These experiments do not indicate that there is any passive transport of ions from the medium to the xylem vessels in the transpiration stream.

It seems appropriate to record briefly the results obtained so far.

a. by increasing the suction tension in the xylem vessels an enhanced water uptake and an enhanced anion uptake take place;
b. the water uptake increases in all zones, most strongly, however, in the basal zones;
c. the anion uptake of the root tip does not increase, that of the other zones does;
d. the increase in anion uptake is proportionally strongest in the basal parts;
e. the anion uptake increases to a lesser degree than the water uptake;
f. the increased anion uptake did not prove to be caused by a passive transport in the transpiration stream.

Each of these facts is important and will be discussed more extensively in the general discussion. In particular, it is interesting to know the nature of the relation between water uptake and anion uptake. To get an answer to this question we have to consider the nature of the salt uptake mechanism. The results of the experiments of HOAGLAND, STEWARD, LUNDEGÅRDH, ROBERTSON and their co-workers, suggest the desirability of studying respiration in connection with the observed phenomena.

CHAPTER VI

THE RESPIRATION OF THE DIFFERENT ROOT ZONES

§ 1. INTRODUCTION

In the literature several experiments are described concerning the respiration of the different root zones. The methods used differ strongly, viz. from a reduction of methylene blue absorbed (LUND and KENYON 1927) to a determination of the oxygen consumption in Warburg vessels (MACHLIS, 1944*b*). The material used also differred. But all authors agree in stating that the oxygen consumption of the tip is much greater than the oxygen consumption of the more basal parts.

Only a few experiments deal with the difference in oxygen consumption in distilled water and in a salt solution. LUNDEGÅRDH (1940) has shown that respiration in a salt solution is stronger than in distilled water. Using excised roots he found the strongest respiration occurred in the tip segments (0–3 cm from the tip). The respiration of the following zones, 3–6 and 6–9 cm from the tip, amounted to about one third of this value. With intact plants GREGORY and WOODFORD (1939), using the main root of *Vicia faba*, found a similar gradation from tip to base. The experiments described below show the oxygen absorption of the different root zones from the outer solution, both with high and low water uptake, in distilled water and in a salt solution. It should be considered that only the oxygen uptake from the medium is determined. There might be an oxygen supply from the shoot via the intercellular spaces in the cortex. If so, it is not an important one, for it appeared that stopping the aeration of the outer solution caused a strong reduction of the anion uptake. VLAMIS and DAVIS (1944) showed that the salt uptake of roots which are provided with oxygen from the shoot is independent of aeration of the medium. After cutting off the shoot the salt uptake was dependent on aeration. It is likely, therefore, that in the apparatus used the roots of *Vicia faba* do not obtain oxygen from the shoot.

The method followed has been described in chapter II.

§ 2. RESPIRATION OF THE VARIOUS ROOT ZONES IN DISTILLED WATER AT LOW AND HIGH WATER UPTAKE

In these experiments the respiration of the different zones at low and high values of water uptake are compared under conditions of constant light and temperature. As communicated elsewhere (BROUWER, 1953*a*) no difference in respiration intensity was found. This appears from the results which are plotted in figure 8. This

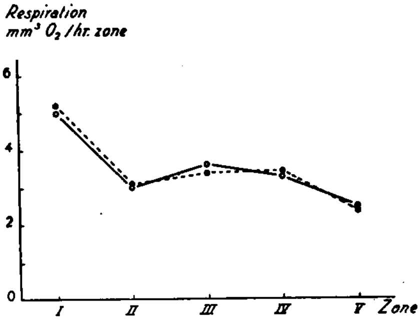

Fig. 8. The oxygen uptake of the different zones from the medium at low transpiration (solid line) and at high transpiration (dotted line).

observation agreed with the results of KRAMER and WILSON (1949), LOWENECK (1930) and others. One can conclude from these experiments that there is no correlation between water uptake and oxygen consumption. The oxygen consumption of the tip zone is always stronger than the oxygen consumption of the more basal root parts.

§ 3. RESPIRATION IN A SALT SOLUTION AS COMPARED WITH RESPIRATION IN DISTILLED WATER

A number of investigators have shown that the presence of ions in the outer solution has an influence on the level of respiration. Working with wheat roots Lundegårdh and Burström (1933) were the first to mention this phenomenon. Thereafter, various investigators (Van Eijk, 1939; Steward and Preston, 1941; Robertson and co-workers, 1941 cf.) observed it with other material. The difference between respiration in a salt solution and respiration in distilled water was called salt respiration or anion respiration.

It appeared that the different zones of the root of *Vicia faba* also showed such a salt respiration. At first the respiration of the different root zones was measured after a long period (16 hours) in distilled water. Immediately after this determination the distilled water was replaced by a 10 mM potassium nitrate solution and the respiration was measured again. In all cases the level of respiration was then higher. An example of such an experiment is plotted in figure 9. Not only is the level of respiration in distilled water highest in the apical region, but also the difference between respiration in a salt solution and respiration in distilled water is highest in this region. The difference between these two levels is plotted in fig. 9 as salt respiration. Expressed as percentages of the respiration in distilled water the salt respiration in *Vicia faba* roots is much smaller than that found by Lundegårdh for wheat roots.

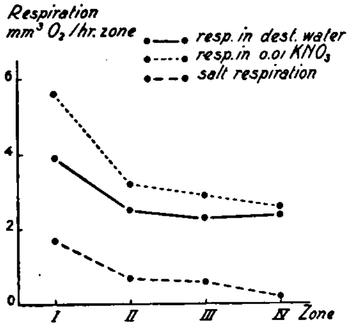

Fig. 9. The oxygen uptake from the outer solution of the different root zones in distilled water and in a 10 mM KCl solution. The difference between these two values is plotted as salt respiration.

§ 4. SALT RESPIRATION AT LOW AND INCREASED SALT UPTAKE

Milthorpe and Robertson (1948) showed that for barley roots a long period elapsed before the salt respiration ceased after transferring the roots from a salt solution to distilled water. Also with my material there was an after effect which lasted for some hours. To compare the salt respiration at a low suction tension in the vessels with that at a high suction tension in the xylem vessels the experiments were performed in the following way.

After a pretreatment in distilled water (16 hours) the salt respiration at low suction tension in the xylem vessels (tap water in the main vessel) was determined as indicated in § 3. Thereafter the salt solution was replaced by distilled water. At the same time the tap-water in the main vessel was replaced by a 2.5 atm Brenner solution and after 6 to 8 hours the respiration in distilled water was again determined. The level of this respiration appeared to be nearly as high as with the former determination in distilled water. During the next period the respiration in a 10 mM potassium nitrate solution was measured. In spite of repeated efforts no difference between the salt respiration at low and at high suction tension in the xylem vessels could be demonstrated. The values for salt respiration of the different zones are plotted in figure 10. The black dots represent the salt respiration at low suction tension, the open dots the salt respiration at high suction tension. We have shown in the preceding chapters that the salt uptake was considerably higher in the latter case. This is a remarkable fact, for we know that it is likely that the anion uptake, even with increased water absorption, is an energy consuming process.

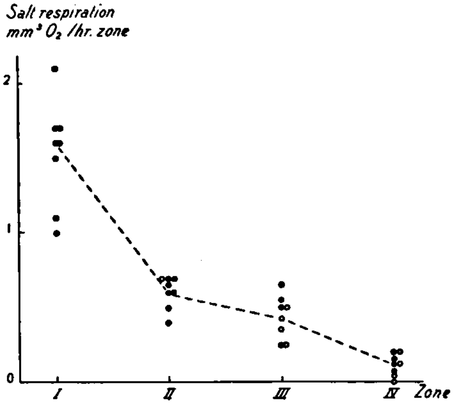

Fig. 10. The salt respiration (difference between respiration in 10 mM KNO_3 and in distilled water) of the various root zones at low and high water uptake. The values found in 4 experiments have been plotted. Black dots represent the salt respiration at low water uptake, open dots the salt respiration at high water uptake.

§ 5. DISCUSSION

From the results described above, it is clear that respiration is highest in the apical region. The values found are expressed per unity of root length, for the roots used have a uniform diameter over the whole length. The fresh weights per centimeter of root length, therefore, differ only slightly.

As compared with other investigations the tip zone in these experiments is very long, viz. 25 to 30 mm, so that it seems likely that it can not be considered uniform tissue with regard to respiration activity. On the contrary, some difference can be expected between the extreme tip and the rest of the tip zone. At distances greater than 30 mm from the tip respiration is more or less uniformly distributed and diminishes only at about 12 to 15 cm from the tip.

The higher respiration of the tip zone must be ascribed to the presence of a differentiating tissue and, therefore, consists partly of growth respiration.

Further, it is possible to show a salt respiration with *Vicia faba*. It is not surprising that the salt respiration found here is relatively smaller than that of wheat roots, because the former are much thicker and show a smaller salt uptake than grasses.

As stated in the introduction it has not been proved that salt respiration provides the energy for salt uptake. According to the strong correlation between salt respiration and salt uptake LUNDEGÅRDH assumes that this correlation is causal. Such an interpretation is accepted by most investigators.

If we follow this view, it can be concluded from the results given, that the ratio between the number of ions taken up and the oxygen consumed is not a constant one. The amounts of ions taken up at low suction tension and at high suction tension differ distinctly, the oxygen consumption being the same in both cases. In table XI a comparison of the ratios between anion uptake and anion respiration at low and at high suction tension is given. The values recorded in this table are average figures for 8 experiments, both for salt respiration and salt uptake. Salt respiration and salt uptake were determined with different plants. The salt solution used was in both cases a 10 mM potassium nitrate solution. Given the uniformity of the material used we can expect a reasonable picture from these comparative experiments.

TABLE XI

Comparison of the ratio $\frac{\text{milliaeq. } NO_3 \text{ absorbed}}{\text{milliaeq. } O_2 \text{ respired}}$ at low and high water uptake for the different root zones

Zone	low water uptake			high water uptake		
	m. aeq. O_2	m.aeq. NO_3	Ratio	m. aeq. O_2	m.aeq. NO_3	Ratio
IV . . .	0.32	0.23	0.7	0.31	0.74	2.4
III . . .	0.36	0.33	0.9	0.36	0.90	2.5
II . . .	0.45	0.37	0.8	0.46	1.05	2.3
I . . .	0.80	0.56	0.7	0.78	0.62	0.8

In the first place it is striking that the efficiency of the salt respiration of the different zones, which does not essentially differ at low suction (column 3), does differ at high suction tension. In the latter case (column 6) the efficiency of the apical zone has not changed, while the efficiency of the basal zones is strongly increased. Thus, an increased suction tension in the vessels renders the anion uptake easier which means that with the same expenditure of energy more ions are taken up.

Though not comparable with this phenomenon ROBERTSON found that the efficiency of salt respiration increased with rising concentration of the nutrient solution untill a maximum was reached. This followed from the fact that the relation between salt uptake and salt concentration differed from the relation between salt respiration and salt concentration.

The above reasoning stands or falls on the question of whether or not the salt respiration causes the salt uptake. The experiments of investigators who are engaged in this problem, moreover, have a bearing on the accumulation in the tissue itself, rather than on the total uptake, that is the sum of the amount withheld in the root tissue and the amount transferred to the shoot. It is of great importance, therefore, to trace to what extent these two processes occur.

CHAPTER VII

UPTAKE AND BINDING IN THE TISSUE

§ 1. INTRODUCTION

Little difference is made in the literature between accumulation and total absorption, though we may assume that there is a great difference between these two processes, especially with regard to the roots. Conclusions as to the absorption capacity are often based on experiments on the accumulation in the tissue. I am not aware of any attempt to compare the relation between these two processes, accumulation and uptake, with regard to the distribution along the root axis.

To make such a comparison it would be advantageous to use ions, which are not present in the root at the start of the experiments. Most investigators have used ions like bromide or radio-active isotopes.

Since it was necessary to determine an amount of plant material as small as 1 cm of a single root, labelled phosphate (P*) was chosen for the experiments described below. It can be estimated in small amounts. A drawback is that in addition to an active binding a more or less passive exchange against phosphate already present in the tissue, may probably occur. In the experiments described below the total amount of P* present in the tissue was determined and no correction for such an exchange was made. Hence the actual amounts, actively taken up by the root tissue may be somewhat lower than the amounts found in the experiments.

§ 2. METHOD

The plants were mounted on the apparatus in the usual way. The uptake periods lasted 24 hours. During these periods a full strength Hoagland solution with P* served as a nutrient solution in the zone vessels. The total uptake by the different zones was determined as described above. After the uptake period the root zones were rinsed with distilled water to remove loosely adsorbed phosphate. Thereafter, the zone vessels were removed and the root was cut into 1 cm segments. These segments were placed on small planchets and allowed to dry. After drying the activity of each was determined and compared with the activity of a small drop of the nutrient solution of known weight. The latter was also placed on the planchet and evaporated to dryness. The experiments were performed partly at low and partly at high suction tension in the xylem vessels.

Further, a number of experiments were performed in which labelled phosphate solution was applied to a single zone and a Hoagland solution without P^{32} to the other zones. The activity of all segments was determined in order to get an impression of the transport in the root tissue itself.

§ 3. THE RATIO BETWEEN TOTAL UPTAKE AND THE AMOUNT WITHHELD IN THE TISSUE

From the experiments it appeared that there was a great difference between the growing tip and the rest of the root. The occurrence of growth seemed to be particularly important. For this reason the growth was measured in each experiment in addition to the uptake and the binding in the root tissue. This can be easily performed with the apparatus used. An example of the uptake and the binding by the root is given in table XII.

TABLE XII

The ratio between phosphate uptake and phosphate binding of the different zones of the root at low water uptake. During the experiment the length of the tip zone increased from 25 to 40 mm

Zone	Uptake	Binding	% transfer
III	6.1 γ P	0.89 γ P	86
II	8.2 „	0.99 „	88
I	12.3 „	5.9 „	52

At the start of the experiment the length of the tip zone amounted to 25 mm, at the end, i.e. after 24 hours, it amounted to 40 mm. The mean length during the uptake period was $(25+40)/(2) = 33$ mm. The total uptake amounted to 12.3 γ P, or calculated per 3 cm root length, 11.2 γ P per 24 hours. The activity determinations showed that in the tip itself 5.9 γ P was present (per 3 cm root length 4.4 γ P). Thus, of the total uptake 48 % $((5.9 \times 100)/(12.3))$ was bound in the root tissue and 52 % was transferred to the shoot or the other parts of the root system. For zone II we found a binding percentage of 12 %, whereas 88 % was transferred. Zone III showed 14 % bound and 86 % transferred.

This experiment was performed at low suction tension in the xylem vessels. The ratio between binding and transfer as found here for the tip was about the average value for the experiments performed. In a single case we found that the amount withheld by the tissue of the root tip was about 83 % of the total uptake. On the other hand a non-growing tip showed about the same ratio as the rest of the root. It can be concluded that the ratio between binding and uptake of the apical zone is strongly dependent on its original length and growth.

At a higher water uptake the ratios between uptake and binding

TABLE XIII

The ratio between phosphate uptake of the different root zones and phosphate binding in the tissue at high water uptake. Tip zone showed no growth

Zone	Uptake	Binding	% transfer
IV	10.4 γ P	0.6 γ P	94
III	water uptake determ.	0.01 „	—
II	8.8 „	1.1 „	88
I	7.0 „	1.05 „	85

are somewhat different depending on the amounts of ions taken up. An experiment in which the uptake was rather low is given in table XIII. The root tip showed no growth in this case. The phosphate uptake of zones I, II and IV was determined from a Hoagland solution with P*. Zone III (5 cm long) was used for determining the water uptake from a Hoagland solution without P*. We see that the phosphate uptake of zone IV is highest, the binding in the root tissue being lowest at the same time. The binding in zone II does not differ essentially from that of zone II of table XII. It is a striking fact of all these experiments that the amount of ions withheld in the tissue is rather independent of the rate of water uptake. At high suction tension a higher percentage of the total uptake is involved in transfer to the shoot.

Moreover, there appears to be only a small amount of P* withheld in zone III, which was not in direct contact with the labelled phosphate solution, though about 13 γ (difference between uptake and binding of zones I and II) must have passed through the vessels of this zone. This phenomenon will be further analysed in the next section.

§ 4. LONGITUDINAL TRANSPORT IN THE ROOT

In the preceding section the binding by the tissue of each zone was given. Seeing that the length of the tip zone varied from 25 to 40 mm the distribution must be further analysed. By making the partition as small as possible a difference between the various parts of the apical zone can be observed. In figure 11 *A* the distribution of the amount of phosphate withheld per cm root length is given. The series of numbers right and left show the results of one experiment each. The apical 10 cm (shaded) of these roots were able to absorb phosphate from a Hoagland solution with P* during 24 hours. The more basal parts, 11 to 16 cm inclusive and 11 to 13 cm inclusive from the apex, were in the main vessel in tap water. The numbers beside the root in fig. 11 *A* refer to the counting-activity (1000 countings corresponds to 1.07 γ P). It appears from these data that most phosphate is withheld by the apical 1 cm, and from there to the base a gradually decreasing amount of phosphate is withheld. Those parts of the root which were not in contact with the P^+ solution held only very small fractions of these amounts, though the whole amount that is taken up by the lower parts and that is not withheld there, must have passed through the vessels of these parts of the roots. So it seems that there is only a small exchange between phosphate present in the vessels and that in the tissues.

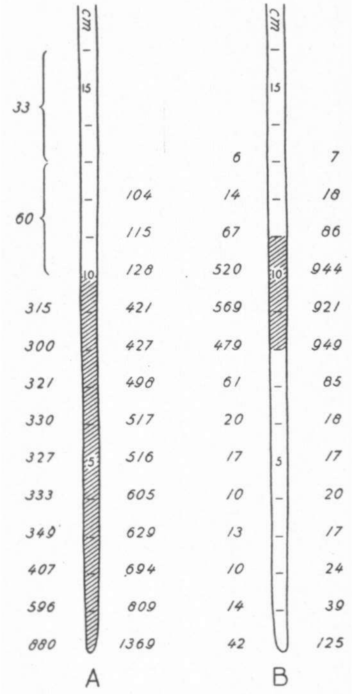

Fig. 11*A*. The distribution of the activity (counts per minute) of dried, 1 cm long segments, along the root axis after an uptake period of 24 hours. During this period a Hoagland solution with labelled phosphate served as nutrient solution for the apical 10 cm of the root (shaded). The more basal parts were in tap water. The countings left and right represent the results of different roots. (1000 counts = 1.07 γP)

Fig. 11*B*. The distribution of the activity (counts per minute) of dried, 1 cm long root segments along the root axis, after an uptake period of 24 hours during which zone IV (shaded) was able to absorb phosphate from a Hoagland solution with labelled phosphate. The other root parts were in a Hoagland solution without P^{32}. The countings left and right represent the results of different roots. (1000 counts = 0.6 γ P)

Figure 11*B* shows the distribution of phosphorus (two experiments also) absorbed by zone IV (9–11 cm inclusive from the apex) which was the only zone that was allowed to absorb the labelled phosphate. Zones I, II, III and V were placed in a Hoagland solution without P*. At the end of the experiment the whole root was divided into 1 cm segments and, after drying, the activity was determined. It appears from the data that the treated zone shows the normal picture, or a piling-up of about 0.7 to 1.5 γ P (1000 counts/min = 0.6 γ P). The first cm bordering on this zone towards the root apex as well as towards the base, shows an activity of about $^1/_{12}$ of the treated zone, whereas the zones further removed have an activity of about $^1/_{50}$ of this zone. The utmost tip makes an exception, showing a distinctly greater activity than the rest of the root not in contact with the P^+ solution.

How can these results be explained?

It seems to be most obvious to assume that the high activity of the root parts bordering the treated zones are due to a diffusion in the cortex. The large influx of phosphate ions, however, will enter the central cylinder where an upward transport in the xylem vessels and a downward transport to the tip will take place. Nothwithstanding the fact that the apical zone is able to absorb phosphate from the medium, it shows a distinct piling-up of phosphate absorbed in the more basal region.

In connection with the anatomical proportions of the root, impermeability of the endodermis etc., it is noteworthy that the ions taken up by a distinct zone penetrate the endodermis. LUNDEGÅRDH and also KRAMER assumed that a longitudinal transport through the cortex in the direction of the tip takes place after which a transfer to the stele occurs in the apical region. This concept is not supported by these experiments. Given a somewhat important transport through the cortex in a longitudinal direction we should expect that the activity of the root segments would gradually decrease from the place of application of the active nutrient solution along the root in both directions.

The data of figure 11*B* are obtained by cutting the root in 1 cm sections. If we take shorter segments it appears that the radiation is limited to the first 3 mm on both sides of the treated zone. This renders a longitudinal transport in the cortex unlikely.

§ 5. RELEASE AND EXCHANGE OF PHOSPHATE

If we determine in an experiment as described above the activity of the outer solution of the zones which have not been in direct contact with the P* solution, it appears that no P* is released by those parts of the root. The ions present in the stele show no exchange with the phosphate present in the medium. The phosphate transported to the apex of the root, to the base of the root or to the shoot is protected against leakage and exchange.

§ 6. DISCUSSION

The experiments described in this chapter are interesting in various respects. Firstly, it is clear that the ions absorbed are transported for the greater part to the shoot. On the other hand it appears that the distribution of the binding in the tissue can not be used for conclusions on the absorption capacity. Uptake by and consequent binding in the tissue are two different processes.

Further, it seems to be obvious that the ions absorbed by a distinct root zone are mainly transported to the stele in radial direction. KRAMER and WIEBE, who investigated the accumulation of P in barley roots, found that this accumulation was highest in the root hair zone and that it decreased in apical as well as in basal directions. As in the experiments described here, they showed that with an uptake limited to the growing tip, no transport to more basal parts occurred. The total uptake was withheld in the tissue of the growing region. An uptake by the root hair zone showed a transport both in the direction of the root tip and in the direction of the base. No exact data are given, however, as to this subject.

From the fact that in our experiments such a rapid fall of the P* content of the root tissue occurs upwards and downwards from the zone of application, it can be assumed that longitudinal transport in the cortex meets with considerable resistance. It is likely that this holds good both for salt transport and for water transport. For that reason an upward current of ions in a longitudinal direction through the cortex, as suggested by HYLMÖ, seems to be unlikely.

Further attention must be drawn to the relation of the distribution of binding in the tissue and salt respiration along the root axis. Thus, it appears that salt respiration is running parallel to both uptake at low suction tension and binding in the tissue. We have to be careful, therefore, with our conclusions on the connection between salt uptake and salt respiration. It is not inconceivable that salt respiration is only responsible for the piling-up in the tissue, as occurs in storage tissues, and that on the other hand the transport of ions from medium to vessels is based on another mechanism.

The results of these experiments on phosphate binding in the tissue agree fully with the bromide accumulation described by PREVOT and STEWARD for the same material. It seems justified, therefore, to attach some general meaning to these phosphate experiments.

CHAPTER VIII

THE ANATOMY OF THE ROOTS

§ 1. INTRODUCTION

A survey of the anatomy of roots in connection with the problem of uptake from the surroundings can be found in a paper of WIERSUM (1947). On the basis of the previous literature he divides the root from tip to base, in three zones, viz. the undifferentiated tip zone, the more or less differentiated zone with root hairs and with an endodermis in its primary or secondary stage, and the basal root zone which is covered by an isolating suberized layer.

It is reasonable to suggest that there is no uptake of water or salts in the latter zone because of the presence of this tightly fitting layer of suberized cells. As soon as lenticells are present, it is possible for water and solutes to enter the root. ADDOMS (1946) and also KRAMER (1946) showed that this really happens. For the normal water and salt uptake this zone will be of little importance.

If we consider the undifferentiated tip zone, no anatomical features are apparent which can be considered to prevent water and ions from entering this part of the root. The growing cells present here can easily provide themselves with salts and water. The uptake will be limited, however, by the direct requirements of the cells.

Between the two zones mentioned lies the zone with fully differentiated tissues and without a suberized surface layer. This is the zone where root hairs normally occur. An uptake of water and salts from the medium is possible. The possibility of a transport to the stele is dependent on the developmental stage of the endodermis. A radial transport through the walls is unlikely because of the presence of

Casparian strips in the radial walls of the endodermal cells. Transport through the protoplasm is the only possible alternative.

Finally, the endodermis in the secondary stage is fully suberized, with the exception of the passage cells which remain in the primary stage.

Of course, the zones mentioned above are not clearly separated. There exist conversions of which, in particular, that between the undifferentiated tip and the differentiated parts is of special interest. This region constitutes that part of the root in which a transport to the xylem vessels begins.

§ 2. ANATOMY OF THE ROOTS USED FOR THE EXPERIMENTS

The anatomy was investigated on long, unbranched, secondary roots which were used for the experiments. Transverse, tangential and longitudinal free-hand sections were used, representing segments at different distances from the root tip. The roots investigated were grown in tap water. The root tip is protected by a root cap. The tip itself, 1 to 3 mm, contains small isodiametrical, parenchymatous cells and no tissue differentiation can be observed.

The greater the distance from the apex, the greater the diameter of the cortex cells, whereas the cells of the central cylinder show only a small enlargement in diameter but an important increase in length. All these features refer to the first few mm of the root apex. At a distance of about 4–5 mm from the tip the first xylem elements develop and a distinct difference between cortex and stele becomes visible. The extension of the cells and a further differentiation takes place between 4 and 7 mm from the tip. The transition to a more or less complete differentiation occurs in the region between 7 and 10 to 15 mm from the tip. A distinct wood reaction can be demonstrated there.

At a distance of 10 to 15 mm from the tip the endodermis can be noticed, but a suberisation of the radial walls does not yet exist. Casparian strips can not be found. In the cortex the first intercellular spaces originate. The epidermis is colourless.

At a distance of about 20 mm from the tip the tissues are fully differentiated. The largest cells of the cortex are situated in the outer layers, more inwardly the cells become smaller. The endodermis can clearly be identified by the suberisation of the radial walls. In the central cylinder the vascular bundles are fully developed. The rest of the tissue of the stele consists of long stretched cells with a small diameter.

This state exists over the whole root length up to the insertion on the main root. The epidermis becomes yellowish. The endodermis remains in its primary stage. At greater distancess from the apex, in the longitudinal walls of the endodermis cells thickenings arise which are somewhat suberized, but a complete suberisation of these walls does not occur.

Root hairs exist only at those parts of the root which arise above the liquid.

§ 3. Discussion

The anatomical data described above are in full agreement with the results of physiological investigations. Because of the absence of vascular bundles in the apical 1 cm of the root the uptake of water there is virtually impossible. Salts can be absorbed but only as far as they can be used for growth.

The adjacent differentiating zone, 1 to 2 cm from the apex, will also consume an important part of the ions taken up for its own growth. The experiments described in chapter VII can be easily explained when these physiological factors are considered.

That the endodermis over the whole root length is in its primary stage was not to be expected. This fact, however, makes it clear that a transport in radial direction can take place over the whole root. On the other hand, on account of the anatomical features, we should expect that the uptake of water and salts would occur equally easily over the whole length. The reverse is true, however. Therefore, the existing differences must be ascribed to physiological differences, possibly connected with differences in age.

Chapter ix

GENERAL DISCUSSION

In order to obtain a survey of the experiments and theoretical aspects of this study reference will also be made to the preceding papers of the author. It is a well known fact that the water uptake is determined by the suction tension in the xylem vessels and the water conductivity of the root tissue between the medium and these vessels, as is briefly indicated by the formula of Brieger: Uptake = conductivity × suction tension. If a suction tension exists in the medium, the effective water moving power amounts to: $S_{xylem} - S_{medium}$.

On enhancing the transpiration the suction tension in the xylem vessels increases. If no changes in water conductivity occurred, the uptake should increase in proportion to the increase in suction tension, resulting either from the enhanced transpiration or from a hampered water absorption.

In fact the water uptake increased to a higher degree than the suction tension did. This must have been due to an increase in water conductivity. Brewig showed for the root of *Vicia faba* that the increase in water conductivity was dependent on the region of the root involved. He found that the changes in conductivity occurred especially in the mature root parts and he assumed they were caused by the enhanced suction tension. This is made more probable by the results obtained from the experiments of this investigation (chapter III). Moreover, not only did an enhanced suction tension in the xylem vessels appear to increase the water conductivity but an enhanced osmotic suction tension in the medium had the same effect. This is in accordance with similar findings of Rosene.

From the fact that both an increase in suction tension in the xylem vessels and an identical increase in osmotic suction tension in the medium cause the same changes in water conductivity of the tissue it can be concluded that the point of attack does not matter. Both suction tensions will cause an increase in suction tension of the cells of the root tissue, these cells being in equilibrium with the surroundings. An increased suction tension in the cells means a decreased turgor pressure. It is supposed that this decrease in turgescence is the direct cause of an increased water conductivity. The latter is not only effective for a water current from medium to xylem vessels but also for a current from the xylem vessels to the medium, if the suction tension of the medium exceeds the suction tension in the xylem vessels.

The water transport itself is a fully passive process. This appears, among other things, from the fact that it is independent of the oxygen supply. Further, inhibitors in concentrations that inhibit the respiration, have no influence on the water uptake. The fact that potassium cyanide in higher concentrations (5.10^{-3} and 10^{-3}) decreases the water uptake (at the concentration 5.10^{-3} directly and completely) does not prove that an active portion of the water uptake process is eliminated. We have to assume that as a result of these high concentrations the conductivity of the tissue between medium and xylem vessels decreases and after some time becomes infinitely small, so that no water uptake at all can take place. Presumably this will be due to changes in the structure of the protoplasm.

With chloroform we get the reverse of this process, viz. a temporary increase in conductivity. (Brouwer, 1953).

An excess of carbon dioxide in the medium causes an initial decrease followed by an increase in conductivity.

These facts, supported by many investigations of other physiologists (vide Kramer, 1932 cf.), argue in favour of a complete regulation of the conductivity by the protoplasm.

It must be left an open question whether this regulation of the conductivity takes place in the endodermis or over the whole pathway between medium and xylem vessels.

From the above it is clear that the changes in water conductivity must take place in the protoplasm. From the correlation between the turgescence of the tissue and water conductivity it may tentatively be assumed that the pressure upon the protoplasm between vacuole and cell wall is an important factor. A decrease of this pressure makes possible a certain expansion of the protoplasm that is perhaps coupled with a change in hydration, thus causing an increased water conductivity.

Another problem closely related to this question is that of different reactions to an increased suction tension in the various root zones. We have seen above that the water conductivity of the apical zone does not increase in this case whereas that of the more basal zones does. It appears that the changes in water conductivity are inversely proportional to the turgor stretching of the zones. This means that the tip which does not show any changes in water conductivity possesses the greatest turgor stretching. On the contrary, the basal parts have only a little elastic cell wall and in these very zones the changes in water conductivity are greatest. In these older zones a water withdrawal from the vacuole will not be followed by a decrase in volume of the cell to such an extent as will be the case in the apical region. The consequence of this fact is that a reduction of the pressure on the protoplasm occurs primarily in the mature root parts.

Much attention has been called to the changes in water conductivity, and not without good reason, for the effect of these changes for both water and salts is very similar.

The experiments on salt uptake were started with the intention of gaining an insight into the distribution of the uptake along the root axis. In this respect most of the experiments described in the literature give only an impression of the accumulation in the root tissue. Though many conclusions as to the uptake by the root are based on this accumulation they do not seem to be justified (ARISZ, 1945). It appears that the anion uptake at low water uptake is greatest in the apical region, and rather small in the basal zones. The binding in the tissue, though on a much lower level, shows the same gradient from tip to base. At high water uptake, however, we get the same binding gradient but the total uptake has another distribution along the root axis. The amount of anions taken up by the tip does not change but that in the more basal zones increases in an inverse proportion to their distance from the tip. The maximum anion uptake at high water uptake lies in zone II or III. More basal zones show the relatively strongest increase but the total level does not reach that of zone II or III, because of the low ion uptake at low water uptake. Thus, the distribution of the anion uptake at high water uptake deviates from the distribution of the binding capacity.

With phosphate we have examined what portion of the total uptake was withheld by the root tissue and what part was transferred to the shoot. It appeared that the growing root tip fixed a rather great part of the total phosphate absorption varying from 30 to 80 %, whereas this percentage was much lower in the other zones. As to the distribution along the root axis the binding of phosphate as determined in these experiments, and which is assumed to take place mainly in the protoplasm, strongly resembles the bromide accumulation found by PREVOT and STEWARD with the same material.

If we first consider the anion uptake at low water uptake the question can be asked why the uptake of the apical zones is so much greater than the uptake of the basal zones. Anatomically only the uttermost root tip is different from the rest of the root. It seems likely that the differences between the zones are physiological in nature. If we consider the respiration of the different zones it appears that the same gradient is found. The same holds good for salt respiration. A disparity of age may be responsible for the differences.

After increasing the water uptake (the anion uptake also increases) the distribution of the anion uptake more or less resembles that expected on the basis of the anatomical structure. The differences between the various zones almost disappear. This is in contrast with what is found with the water uptake where at high suction tension a steep gradient of the water absorption value is found from base to tip.

The influence of the suction tension on the water uptake by the different zones on the one hand and on the nitrate, chloride and phosphate uptake on the other hand can be compared by means of figure 12. It appears that the water uptake increases more than the anion uptake. This is due to an increase in the water uptake owing to an increased suction tension and an enhanced conductivity. If, however, a correction is made for the difference in the driving force, i.e. if the water conductivity values are compared instead of the water absorption values, a totally different picture is obtained. In figure 12 that part of the increased water uptake that is due to increased suction tension in the xylem vessels is recorded above the dotted line. It appears that the ratio $\frac{\text{white}}{\text{black}}$ for anions and the ratio

$$\frac{\text{white up to the dotted line}}{\text{black}}$$

for water for the different zones is about the same. This means that on increasing the suction tension in the xylem vessels the anion uptake increases to the same extent as the water conductivity.

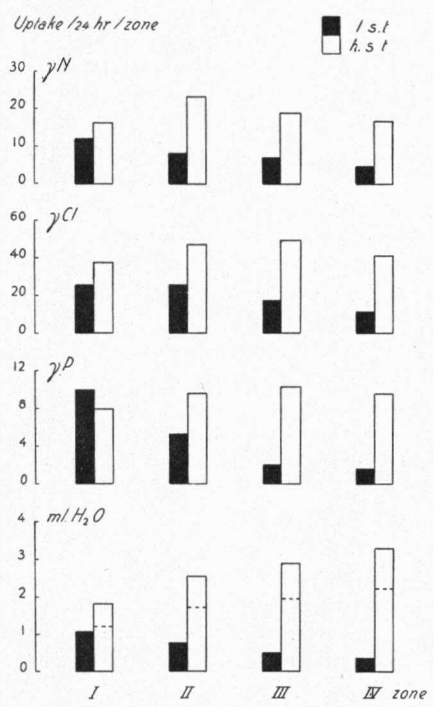

Fig. 12. The nitrate, chloride, phosphate, and water uptakes of the different root zones at low (l. s. t.) and high suction tension (h. s. t.) in the vessels. In the columns representing water uptake at high suction tension (white blocks) the amount above the dotted line is due to the increase in suction tension, the difference between black and white blocks up to the dotted line being due to an increase in conductivity.

In spite of extensive investigations on the mechanism of the salt uptake, for which we may refer to the recent reviews of ARISZ (1945), HOAGLAND (1948), BROYER (1951), ROBERTSON (1951), BURSTRÖM (1951), OVERSTREET and JACOBSON (1952) and SUTCLIFF (1953) it is not yet sure how the energy transfer from respiration to ion uptake is brought about. It is certain, however, that this energy is supplied by respiration, possibly only by the salt respiration. Both total respiration and salt respiration proved to be equal at low and high suction tensions in the xylem vessels, and there seems to be no reason, therefore, to assume that the energy available for salt uptake is greater at high suction tension than at low suction tension. If this is true, this process of uptake is more effective at high suction tension than at low suction tension, or in other words the resistance against salt transport is lower. It seems, therefore, that enhancing the suction tension in the xylem vessels causes a similar increase in conductivity both for water and anions, as a result of a decreased turgescence of the tissue. It is not surprising, therefore, that increasing the suction tension in the medium at constant low suction tension in the xylem vessels, does increase the anion uptake (figure 7).

One assumption for this view is that the salt uptake at high as well as at low suction tension is a fully active process. This seems probable from the experiments on the influence of inhibitors and countersuction. Both methods have also been applied by other investigators. In particular inhibitors are often applied to get a better view of the mechanism of uptake processes (ARISZ, 1953; LUNDEGARDH; ROBERTSON). High concentrations, however, are always injurious, but even the very low concentrations used here, caused an inhibition that was not completely reversible, probably because of the long periods of application. Withholding oxygen from the medium showed the same inhibition, however, and appeared to be reversible.

Any after-effect of the application of the sucrose solution which was used for counter suction was, in contrast to a mannitol solution, not noticeable.

These experiments present a new point of view on the influence of transpiration on the salt uptake. To check the general validity of this view, the experiments were repeated with a number of different plants. It appeared that with increased transpiration, in general, the water uptake increased more than the anion uptake. This phenomenon was also found by PETRITSCHEK (1953) with *Clematis*. Using inhibitors and counter suction it was possible to separate water uptake and salt uptake from one another. It appeared that there was no reason to suppose a passive transport of ions in the transpiration stream for the materials used. That is why the interpretation given above is more satisfying. It remains possible, however, that there is a passive transport as HYLMÖ suggested. Unpublished experiments of the author with HYLMÖ's material, *Pisum sativum*, showed a rectilinear connection between water uptake and anion uptake. This confirmed the results found by HYLMÖ. Using 2,4-dinitrophenol, however, a definite inhibition of chloride uptake could be found without any reduction in water uptake. A counter suction with sucrose did not give the results expected. In one experiment the water uptake was not influenced at all, while in another the water uptake was irreversibly reduced, accompanied by a reduction of the chloride uptake.

Investigating the influence of water uptake on anion uptake with rye plants, which were pretreated in a different way (BROUWER, 1953*b*), it appeared that the salt content, and in particular, the content of the ion investigated, determines the uptake of this ion to some degree. HELDER (1952) showed that in some cases utilisation assigns a limit to the uptake. If this limit is already reached at low transpiration we may expect that an increase in transpiration does not further promote the uptake. It is likely that this is the case if the ion content of the plant is high and the utilisation is limited. Hence a greater uptake by enhancing the transpiration can not be realised.

The resultant uptake from the medium is the difference between the entrance of ions into the root and leakage into the medium. Now the question arises whether the unchanged uptake is the result of a greater entrance into the root, an equally greater efflux from the shoot and subsequent leakage into the medium, or of an unchanged entrance of ions. The efflux from the shoot is discussed by ALBERDA (1948) and others and was demonstrated by PHILLIS and MASON and by MOORE (1945).

As indicated by the experiments of HOAGLAND and BROYER the relative importance of the accumulation in the root system is dependent on the salt status. The latter will also influence the reaction of the salt uptake upon an increased water uptake.

Finally it may be stated, that the phenomenon described in this paper can only be expected if the transport of ions in the root tissue is the limiting factor in the uptake process.

A well-rounded picture of all these processes will only be possible if we are able to determine uptake, accumulation, transfer to the shoot and eventually efflux from the shoot at the same time.

SUMMARY

In the above a survey is given of the experiments on the water uptake described in preceding papers. It appeared that the water uptake is dependent on the suction tension in the xylem vessels and the water conductivity of the root tissue between medium and vessels.

Important stress is laid upon the water conductivity of the tissue, this conductivity being not the same under various circumstances. It is shown that the water conductivity increases on enhancing the suction tension in the xylem vessels. As to the different root zones it appeared that in this case the conductivity of the tip zone did not alter, whereas the conductivity of the basal zones strongly increased.

Moreover, such an increase in conductivity could be caused by enhancing the osmotic suction of the outer solution.

Both the increased suction tension in the xylem vessels and the

osmotic suction in the outer solution, cause a decreased turgescence of the root tissue. It is assumed that this decrease in turgescence is the direct cause of the enhanced water conductivity.

Applying various inhibitors in different concentrations it could be stated that water uptake and water transport are completely passive processes. In spite of this the water transport is controlled by the living protoplasm, because of the fact that inhibitors in high concentrations reduced the water uptake. It is assumed that this is caused by changes in the structure of the protoplasm.

Besides, the anion uptake of the various root zones is investigated. An apparatus to make possible these measurements is described.

At low water uptake the maximum anion uptake is found in the apical root zones, the basal zones showing a rather small anion uptake in this case. Increasing the water uptake the anion uptake increases, this increase being most important in the basal zones.

Moreover, on using respiration inhibitors in various concentrations it appeared to be possible to inhibit the anion uptake without reducing the water uptake. On the other hand an osmotic counter suction in the outer solution reduces water uptake without diminishing the anion uptake. Both processes water uptake and anion uptake, therefore, are related to one another, but this is not a direct relation. Comparing the water uptake and the anion uptake of the different root zones at low and high suction tension in the xylem vessels, it appears that the water uptake increases more than the anion uptake. The water conductivity of the different zones, however, increases to the same ratio as the anion uptake.

An explanation of this phenomenon can be obtained via the respiration. The respiration of the different root zones and also the salt respiration is the same at low and high water uptake. Accepting the hypothesis that the respiration delivers the energy for the anion uptake there is reason to assume that the driving force of the salt uptake is the same at low and high water uptake values, therefore, also at low and high anion uptake values. As a consequence of this fact the anion uptake at high suction tension in the xylem vessels is as much facilitated as the water uptake, or in other words the conductivity for water and anions is increased to the same degree. It is assumed that this is a reasonable explanation as to the influence of the water uptake on the salt uptake. Moreover, such an influence can only be expected if the resistance to the salt transport is the limiting factor in the salt uptake process.

A number of experiments are described dealing with the ratio between the total uptake and the amounts bound in the root tissue. The latter is more or less independent of the water uptake.

Anatomical studies show that the whole root up to at least 20 cm from the root tip, has a non-suberized epidermis and an endodermis in its primary stage. The difference between the various root zones, therefore, must be ascribed to physiological features such as a difference in age.

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REFERENCES

ADDOMS, R. M. 1946. Entrance of water into suberized roots of trees. Pl. Phys. 21: 109–111.

ALBERDA, TH. 1948. The influence of some external factors on growth and phosphate uptake of maize plants of different salt conditions. Extr. Rec. d. Trav. Bot. Neerl. 41: 541–601.

ANDEL, O. M. VAN. 1953. The influence of salts on the exudation of tomato plants. Acta Bot. Neerl. 2: 445–521.

ARISZ, W. H. 1945. Contribution to a theory on the absorption of salts by the plant and their transport in parenchymatous tissue. Proc. Kon. Ak. Wet. 48: 420–446.

ARISZ, W. H. 1953. Active uptake, vacuole-secretion and plasmatic transport of chloride ions in leaves of Vallisneria spiralis. Acta Bot. Neerl. 1: 506–515.

BERRY, L. JOE and M. J. BROCK. 1946. Polar distribution of respiration rate in the onion root tip. Pl. Phys. 21: 542–549.

BIDDULPH, O. 1951. The translocation of minerals in plants. Mineral Nutrition of Plants. Univ. Wisc. Press 261–275.

BÖTTICHER, R. and L. BEHLING. 1939. Licht, Transpiration, Salzaufnahme und Blattstruktur. Flora (N.T.) 34: 1–44.

BRENNER, W. 1920. Über die Wirkung von Neutralsalzen auf die Säureresistenz, Permeabilität und Lebensdauer der Protoplasten. Ber. D. Bot. Ges. 38: 277–298.

BREWIG, A. 1936*a*. Beobachtungen über den Einflusz der Sproszsaugung auf die Stoffdurchlässigkeit der Wurzel. Ber. D. Bot. Ges. 54: 80–85.

BREWIG, A. 1936*b*. Die Regulationserscheinungen bei der Wasseraufnahme und die Wasserleitgeschwindigkeit in Vicia Faba Wurzeln. Jahrb. Wiss. Bot. 82: 803–828.

BREWIG, A. 1937. Permeabilitätsänderungen der Wurzelgewebe die vom Sprosz beeinfluszt werden. Zeitschr. f. Bot. 31: 481–540.

BREWIG, A. 1939. Auslösung leichter Wasserdurchlässigkeit an Wurzeln von Vicia Faba. Planta 29: 341–360.

BRIEGER, F. 1928. Untersuchungen über die Wasseraufnahme ganzer Pflanzen. Jahrb. Wiss. Bot. 69: 295–330.

BROUWER, R. 1953*a*. Waterabsorption by the roots of Vicia Faba plants at various transpiration strengths
 I. Analysis of the uptake and the factors determining it. Proc. Kon. Ak. Wet. C 56: 105–115.
 II. Causal relations between suction tension, resistance and uptake. Proc. Kon. Ak. Wet. C 56: 129–136.
 III. Changes in water conductivity artificially obtained. Proc. Kon. Ak. Wet. C 57: 68–80.

BROUWER, R. 1953*b*. Transpiration and anion uptake. Proc. Kon. Ak. Wet. C 56: 639–649.

BROYER, T. C. 1950. Further observations on the absorption and translocation of inorganic solutes using radioactive isotopes with plants. Pl. Phys. 25: 367–377.

BROYER, T. C. 1951. The nature of the process of inorganic solute accumulation in the roots. Mineral Nutrition of Plants. Univ. Wisc. Press. 187–249.

BROYER, T. C. and D. R. HOAGLAND. 1943. Metabolic activities of roots and their bearing on the relation of upward movement of salts and water in plants. Am. J. Bot. 30: 261–273.

BURSTRÖM, H. 1951. The mechanism of ion absorption. Mineral Nutrition of Plants. Univ. Wisc. Press 251–260.

BUTLER, G. W. 1953. Ion uptake by young wheat plants. II. The "apparent free space" of wheat roots. Phys. Plant. 6: 617–635.

CHANG, H. T. and W. E. LOOMIS, 1945. Effect of carbon dioxide on absorption of water and nutrients by roots. Pl. Phys. 20: 221–232.

CRAFTS, A. S. and T. C. BROYER. 1938. Migration of salts and water into xylem of the roots of higher plants. Am. J. Bot. 25: 529–535.

DAM, A. VAN. 1935. A method for determining the amount of oxygen dissolved in 1 cc of water. J. Exp. Biol. 12: 80–85.

EIJK, M. VAN. 1939. Analyse der Wirkung des NaCl auf die Entwicklung, Sukkulenz und Transpiration bei Salicornia Herbacea, sowie Untersuchungen über den Einflusz der Salzaufnahme auf die Wurzelatmung bei Aster Tripolium. Rec. Trav. Bot. Neerl. 36: 561–657.

FREELAND, R. O. 1936. Effect of transpiration upon the absorption and distribution of mineral salts in plants. Am. J. Bot. 23: 355–362.

FREELAND, R. O. 1937. Effect of transpiration upon the absorption of mineral salts. Am. J. Bot. 24: 373–374.

GRACANIN, M. 1932. Contribution a l'étude de la relation entre la transpiration et la résorption des ions. Compt. Rend. Ac. Sc. Paris 195: 899–935.

GREGORY, F. G. and H. K. WOODFORD. 1939. An apparatus for the study of the oxygen, salt, and water uptake of various zones of the root, with some preliminary results with Vicia Faba. Ann. Bot. 3: 147–154.

HAAS, A. R. C. and H. S. REED. 1927. Relation of desiccating winds to fluctuation in ash content of citrus leaves and phenomenon of mottle-leaf. Bot. Gaz. 83: 161–172.

HASSELBRING, H. 1914. The relation between the transpiration stream and the absorption of salts. Bot. Gaz. 57: 72–73.

HAYWARD, H. E., W. M. BLAIR and P. E. SKALING. 1942. Device of measuring entry of water into roots. Bot. Gaz. 104: 152–160.

HELDER, R. J. 1952. Analysis of the process of anion uptake of intact maize plants. Acta Bot. Neerl. 1: 361–434.

HUBER, B. 1953. Vergleichende Betrachtung der pflanzlichen Saftströme. Die Naturwissensch. 40: 180–185.

HUBER, B. and K. HÖFLER. 1930. Die Wasserpermeabilität des Protoplasmas Jahrb. wiss. Bot. 73: 351–511.

HOAGLAND, D. R. 1948. Lectures on the inorganic nutrition of plants. Chron. Bot. Comp. Waltham, Mas. (Sec. print).

HOAGLAND, D. R. and T. C. BROYER. 1936. General nature of the process of salt accumulation by roots with description of experimental methods. Pl. Phys. 11: 471–507.

HOAGLAND, D. R. and T. C. BROYER. 1942. Accumulation of salts and permeability in plant cells. J. Gen. Phys. 25: 865–880.

HÖHN, K. 1934. Die Bedeutung der Wurzelhaare für die Wasseraufnahme der Pflanzen. Z. f. Bot. 27: 529–564.

HONERT, T. H. VAN DEN. 1933. The phosphate absorption by sugar cane. Leiden.

HONERT, T. H. VAN DEN. 1936. Beperkende factoren bij de phosphaatopname. (with English translation) Leiden.

HYLMÖ, B. 1953. Transpiration and ion absorption. Phys. Plant. 6: 333–405.

JAHNEL, B. 1938. Untersuchungen über die Elektrolytkonzentration des Transpirationsströmes. Jahrb. wiss. Bot. 86: 527–555.

JOST, L. 1916. Versuche über die Wasserleitung in der Pflanze. Zeitschr. f. Bot. 8: 1–55.

KIESSELBACH, T. A. 1916. Transpiration as a factor in crop production. Un. Nebr. Agr. Exp. Stat. Res. Bull. 6: 170–184.

KOEHNLEIN, E. 1930. Untersuchungen über die Höhe des Wurzelwiderstandes und die Bedeutung aktiver Wurzeltätigkeit fur die Wasserversorgung der Pflanzen. Planta 10: 381–423.

KRAMER, P. J. 1932. The absorption of water by root systems of plants. Am. J. Bot. 19: 148–164.

KRAMER, P. J. 1933. The intake of water through dead root systems and its relation to the problem of absorption by transpiring plants. Am. J. Bot. 20: 481–492.

KRAMER, P. J. 1938. Root resistance as a cause of the absorption lag. Am. J. Bot. 25: 110–113.

KRAMER, P. J. 1940*a*. Root resistance as a cause of decreased water absorption by plants at low temperature. Pl. Phys. 15: 63–79.

KRAMER, P. J. 1940*b*. Causes of decreased absorption of water by plants in poorly aerated media. Am. J. Bot. 27: 216–220.

KRAMER, P. J. 1946. Absorption of water through suberized roots of trees. Pl. Phys. 21: 37–41.

KRAMER, P. J. 1949. Plant and soil water relationships. Mc. Graw Hill Book Comp. Inc. New York 1949.

KRAMER, P. J. 1951. Effects of respiration inhibitors on accumulation of radioactive phosphorus by roots of loblolly pine. Pl. Phys. 26: 30–36.

KRAMER, P. J. and H. H. WIEBE. 1952. Longitudinal gradients of P^{32}-absorption in roots. Pl. Phys. 27: 661–674.

KRAMER, P. J. and K. M. WILBUR. 1949. Absorption of radioactive phosphorus by mycorrhizal roots of pine. Science 110: 8–9.

LEVITT, J. and G. W. SCARTH. 1936. Frost-hardening studies with living cells. II. Permeability in relation to frost resistence and the seasonal cycle. Can. J. Res. C 14: 285–305.

LONG, E. M. 1943. The effect of salt additions to the substrate on uptake of water and nutrients by roots of approachgrafted tomato plants. Am. J. Bot. 30: 594–601.

LOWENECK, M. 1930. Untersuchungen über die Wurzelatmung. Planta 10: 185–228.

LUND, E. J. and W. A. KENYON. 1927. Relation between continuous bioelectric currents and cell respiration. Electric correlation potentials in growing root tips. J. Exp. Zool. 48: 333–357.

LUNDEGÅRDH, H. 1940. Investigations as to the absorption and accumulation of inorganic ions. Ann. Agric. Coll. Sweden 8: 234–404.

LUNDEGÅRDH, H. 1945. Absorption, transport and exudation of inorganic ions by the roots. Ark. f. Bot. 32*A*: 1–139.

LUNDEGÅRDH, H. 1950. The translocation of salts and water through wheat roots. Phys. Plant. 3: 103–151.

LUNDEGÅRDH, H. and H. BURSTRÖM. 1933. Untersuchungen über die Salzaufnahme der Pflanzen. III. Quantitative Beziehungen zwischen Atmung und Anionenaufnahme. Bioch. Zeit. 261: 235–251.

MACHLIS, L. 1944*a*. The influence of some respiratory inhibitors and intermediates on respiration and salt accumulation by excised barley roots. Am. J. Bot. 31: 183–192.

MACHLIS, L. 1944*b*. The respiratory gradient in barley roots. Am. J. Bot. 31: 281–282.

MENDIOLA, N. B. 1932. Effect of different rates of transpiration on the dry weight and ash content of the tobacco plant. Phill. J. Sc. 20: 639–655.

MICHAEL, G. and E. WILBERG. 1951. Untersuchungen über die Stoffaufnahme der höheren Pflanzen. Zeitschr. Pflanzenernahrung. 52: 242–268.

MIKA, J. 1939. Die exakten Methoden der Mikromaszanalyse. Band 42 "Die chemische Analyse" v. Wilhelm Böttger. Stuttgart. 1939.

MILTHORPE, J. and H. N. ROBERTSON. 1948. Studies in the metabolism of plant cells. 6. Salt respiration and accumulation in barley roots. Austr. J. Exp. Biol. and Med. Sc. 26: 189–197.

MOORE, R. F. 1949. Downward translocation of phosphorus in separated maize roots. Am. J. Bot. 36: 166–169.

MUENSCHER, W. C. 1922. The effect of transpiration on the absorption by plants. Am. J. Bot. 9: 311–330.

OVERSTREET, R. and L. JACOBSON. 1952. Mechanism of ion absorption by roots. Ann. Rev. Pl. Phys. 3: 189–206.

PETRITSCHEK, K. 1953. Über die Beziehungen zwischen Geschwindigkeit und Elektrolytgehalt des aufsteigenden Saftströmes. Flora 140: 345–385.

Phillis, E. and T. G. Mason. 1940. The effect of ringing and transpiration on mineral uptake. Ann. Bot. N. S. 4: 645–650.

Popescu, L. 1926. Recherches sur la region absorbante de la racine. Bull. agric. 4: 7–18.

Prevot, P. and F. C. Steward. 1936. Salient features of the root system relative to the problem of salt absorption. Pl. Phys. 11: 509–534.

Renner, O. 1929. Versuche zur Bestimmung des Filtrationswiderstandes der Wurzeln. Jahrb. wiss. Bot. 70: 805–838.

Robertson, R. N. 1940. Studies on the metabolism of plant cells. I. Accumulation of chlorides by plant cells and its relation to respiration. Austr. J. Exp. Biol. Med. Sc. 19: 264–278.

Robertson, R. N. 1951. Mechanism of absorption and transport of inorganic nutrients. Ann. Rev. Pl. Phys. 2: 1–24.

Robertson, R. N., J. S. Turner and M. J. Wilkins. 1947. Studies in the metabolism of plant cells. V. Salt respiration and accumulation in red beet tissue. Ibid. 25: 1–8.

Robertson, R. N. and M. J. Wilkins. 1948. Studies in the metabolism of plant cells. VII. The quantitative relation between salt accumulation and salt respiration. Austr. J. Sc. Res. 1: 17–37.

Rosene, H. F. 1937. Distribution of the velocities of absorption of water in the onion root. Pl. Phys. 12: 1–19.

Rosene, H. F. 1941. Control of water transport in local root regions of attached and isolated roots by means of the osmotic pressure of the external solution. Am. J. Bot. 28: 402–410.

Rosene, H. F. 1944. Effect of cyanide on rate of exudation in excised onion roots. Am. J. Bot. 31: 172–174.

Rosene, H. F. 1947. Reversible azide inhibition of oxygen consumption and water transfer in root tissue. J. Cell. Comp. Phys. 35: 179–193.

Rosene, H. F. 1950. The effect of anoxia on water exchange and oxygen consumption of onion root tissues. J. Cell. Comp. Phys. 35: 179–193.

Schmidt, O. 1936. Die Mineralstoffaufnahme der höheren Pflanze als Funktion einer Wechselbeziehung zwischen inneren und äusseren Faktoren. Zeitschr. f. Bot. 30: 289–334.

Scott. L. I. 1928. The root as an absorbing organ. II. The delimitation of the absorbing zone. New. Phyt. 27: 141–174.

Scott, L. I. and J. H. Priestly. 1928. The root as an absorbing organ. I. A reconsideration of the entry of water and salt in the absorbing region. New Phyt. 27: 125–140.

Sierp, H. and A. Brewig. 1935. Quantitative Untersuchungen über die Wasserabsorptionszone der Wurzeln. Jahrb. wiss. Bot. 82: 99–122.

Steward, F. C., W. E. Berry and T. C. Broyer. 1936. The absorption and accumulation of solutes by living plant cells. VIII. The effect of oxygen upon respiration and salt accumulation. Am. J. Bot. 50:

Steward, F. C. and Preston. 1940. Metabolic processes of potato discs under conditions conducive to salt accumulation. Pl. Phys. 15: 23–61.

Steward, F. C., P. Prevot and J. A. Harrison. 1942. Absorption and accumulation of Rubidiumbromide by barley plants. Localisation in the root of cation accumulation and of transfer to the shoot. Pl. Phys. 17: 411–421.

Strugger, S. 1938. Die lumineszenzmikroskopische Analyse des Transpirationsströmes in Parenchymen. Flora 33: 56–68.

Sutcliffe, J. F. 1953. Ion secretion in plants. Int. Rev. Cyt. 2: 179–200.

Vlamis, J. and A. R. Davis. 1944. Effects of oxygen tension on certain physiological responses of rice, barley and tomato. Pl. Phys. 19: 33–51.

Wiersum, L. K. 1944. Een methode van doorstroming der houtvaten in de wortel in verband met het stoftransport in radiale richting. Versl. Ned. Ac. Wet. C 53: 38–47.

Wiersum, L. K. 1947. Transfer of solutes across the young roots. Rec. Trav. Bot. Neerl. 41: 1–74.

Wilson, C. C. and P. J. Kramer. 1949. Relation between root respiration and absorption. Pl. Phys. 24: 55–59.

Wright, K. E. 1939. Transpiration and the absorption of mineral salts. Pl. Phys. 14: 171–174.