TRANSFER OF SOLUTES ACROSS THE YOUNG ROOT

by

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CONTENTS

CHAPTER I

INTRODUCTION.

For the ordinary higher plant the roots are the part of the organism which is in contact with the normal source of water and most of the nutricious substances. Besides their function of fixing the plant in the soil it is chiefly their power of resorption that is important. In fact, the roots of by far the most plants form a specific apparatus for the absorption of water and nutrients. The latter — both mineral and organic — are nearly always available as solutions. Recent investigations, by JENNY et al. have, however, made it probable that this is not necessary for ions, and that direct absorption from a more fixed condition, by means of interchange, is also possible.

However specific the function of absorption may be, it has gradually become evident that an absolutely unilateral direction of the transfer of matter does not exist. It has long been known that water could again be given off by roots and the secretion of some products has also been observed.

The forces governing the transportation of water have for the greater part become comprehensible after numerous experiments.

It seems that the intake and ascent of water can be completely effected under the influence of known physical or physico-chemical forces. The latter however, are ultimately dependent upon the vital metabolism of the organism and here in most cases all deeper insight is lacking. In the intact, strongly transpiring plant, the prevailing under-pressure in the root cells, caused by the cohesive tension in the xylem, will effect a suction force which is capable of causing transportation. With much certainty we may regard osmotic phenomena as the driving force when guttation or bleeding occurs. The transmission of water in these processes is subject to known forces, and the process can be imitated in models. However we usually speak of „active", i.e. vital, processes, because the living organism must continuously be forming and maintaining the potentials and conditions requisite for the transfer of water. Also when water is given off by the roots the forces are essentially always fully known. The direction of water-transport in the root is in fact not subject to "polarity".

In the following speculations we shall restrict ourselves to the study of the behaviour of "dissolved" substances.

As a consequence of increasing knowledge in the course of years a gradual change of explanatory hypotheses took place. With the exception of the cases, where the explanation was sought in the "vital power" of the plant, there was a general tendency to try and find simple mechanical principles: the transmission of water was considered to be merely passive, a consequence of the given conditions. Therefore it was obvious that one should think of a carrying along of the dissolved substances by the transpiration current, or of penetration by simple diffusion. The discovery of higher concentrations in the plant than in the surroundings, necessitated other hypotheses: adsorption theories, Donnan equilibria and differences of solubility. Under the influence of the newer general views of the process of absorption of salts, one has come to acknowledge the active part of the metabolism of the plant in this matter. This is why, in the last few years, the accumulation and transportation problems and the dependence of both on the vital processes in the organism, have been emphasized. Several theories were broached, mostly for inorganic substances: among others by ARISZ, CRAFTS and BROYER, HOAGLAND, LUNDEGÅRDH and STEWARD. With regard to the present state of knowledge concerning the problem of uptake one question remains undecided. It still has to be demonstrated clearly if the "*active mechanisms*" are wholly predominant or if, besides these, passive processes can occur. In view of this, results obtained from experiments, regarding the influence of the transpiration current on the absorption of salt, demand attention. A carrying along of the ions would at once make comprehensible the great correlation between transpiration and Ca-absorption, found, among others, by BÖTTICHER and BEHLING.

Besides this, the problem of polarity has come to the fore during the last few years. The data about the transportation of some phytohormones especially have given rise to the question whether, here too, the normal direction of the transfer from the surroundings into the plant, really was the only possible one. Here it is of great importance that some recent data show that giving off of ions from the roots is also possible. The results of LUTTKUS and BÖTTICHER especially point to the fact that a considerable amount of K, which had first been absorbed by the plant, comes out again, via the root system, if the plant is subjected to altered conditions. Investigations about interchange of ions, too, lead us to the supposition of the possibility of simultaneous transportation in two directions.

It is not necessary for us to consider the process of accumulation of matter in the vacuole, for the apprehension of uptake, as we shall conceive it. In many cases vacuole-accumulation and intake and

transfer up to the xylem will indeed occur simultaneously, but it is desirable to keep the two processes separated, because in the two cases different membranes are passed. We shall restrict ourselves exclusively to the process of "absorption", so far as this takes substances into the xylem. It appears that we are justified in doing so from data given by HOAGLAND, BROYER and STOUT (1946), which demonstrate that the concentration of the xylem-exudate may very rapidly become increased, even before the root tissue has reached its top capacity for the accumulation of salts in the root as a whole.

The whole complex of processes that leads up to the uptake of matter can only be correctly judged on the base of a sound knowledge of the anatomical structure of the root. One must be able to test the conceived hypotheses in relation to the conditions existing in the plant. For example the outmost layer of root cells becoming corky, will prevent contact with the surroundings, and intake is only possible in a zone with permeable cellwalls. Further analysis must decide whether the zone of absorption actually coincides with the zone anatomically suited for this. Finally the ever present endodermis will force all transmission of matter to pass the living plasm here at least.

The aim of the following investigation is to get a deeper insight into those properties of the symplast which are important to this complex of problems. It is a matter of course that the choice has been limited to no more than a few problems. In addition to other results data were obtained about the extension of the zone of absorption. More attention, however, has been paid to the properties of permeability of the symplast as a whole. Some attention has also been paid to the problem of polarity. The possibility for these investigations was offered by working out a new method of letting liquid flow through the vessels of cut root cylinders (WIERSUM). This new technique will be called briefly "*flowing-method*" in this paper.

CHAPTER II

METHOD.

§ 1. *General Procedure.*

In order to subject a number of properties of the living root tissue to an exact analysis it is necessary that one should be able to regulate arbitrarily both the surroundings and the liquid in the xylem-channels. In order to have them available for investigation one

should also be able to remove the substances, disengaged into the vessels, quickly, independently of the normal mechanism of longitudinal transportation — bleeding or transpiration-suction.

When we consider the extent of the area capable of absorption of matter, it immediately appears that here attention is paid only to the first phase of the process. It was not examined whether lateral transportation of the absorbed substances to the xylem and freeing into the vessels actually occur in the whole zone that is assumed for these processes. Here it is supposed that — under normal conditions — the absorbed substances, especially the mineral ones, get to the shoot via the xylem. The possibility of longitudinal transportation to the shoot through the living cells, although existing, is considered to be of secondary importance. It has never been properly investigated in what area of the root salts are given off to the xylem.

Up till now, only a few publications have dealt with estimations as to what extent the symplasm is permeable for solutes. With reference to ARISZ' publication (1945) we shall henceforth mean by *plasm-permeability*: the capacity of the cells to let substances pass by the route plasmalemma — cytoplasm — plasmalemma. Tonoplast and vacuole will not be considered. The same restriction holds good for the use of the conception "*permeable*" for the complete root tissue. When speaking of *transmeation* we mean transport from surroundings to all the zones as far as the vacuole.

It was endeavoured to obtain most of the data concerning permeability from the analysis of the correlation between uptake of salts and the amount of water absorbed by transpiration. Apart from some older publications, among others by DE RUFZ DE LAVISON, who worked with dyes and microchemical reactions, we find only one investigation (PERIS) in recent publications, which was carried out for the solving of this problem. The method used here, however, is, on account of the indirect way of analysis, insufficient to lead to indisputable results. Again an experimental procedure is necessary in which a certain substance is put either in the exterior medium round the root, or in the interior solution in the vessels, after which the permeation of the substance through the tissue to the other surroundings is studied by chemical analysis. At the same time the question can be investigated if, for this passive process too, there is polarity. A "f l o w i n g-m e t h o d" for cut pieces of a root, in which an artificial flowing through with water of the vessels is brought about, thus quickly washing out the disengaged products and maintaining the concentration-gradient, or a method in which any liquid is introduced into the xylem, will suit the purpose.

Finally such a method also makes it possible for a differentiated O_2-supply of the cortex and central cylinder to be applied. Interior or exterior surroundings can be made rich or poor in O_2 and even the percentage of CO_2 can be varied arbitrarily. This may be of importance in connection with the study of transport-mechanism. In the theory of CRAFTS and BROYER (1938) it is thought that, among other reasons, on the ground of anatomical data, it can be assumed that the difference of O_2-potentials between cortex and central cylinder would cause the lateral transfer and the freeing of the minerals into the vessels. By fixing an arbitrary O_2-potential this theory might be tested experimentally.

By using a "flowing-method", in which rather long pieces of primary root, cut open at both ends must be used, only a very limited choice of experimental plants remained. Referring to the experiments by SIERP and BREWIG, and by BREWIG alone, concerning the uptake of water, and made with a special potetometer-construction, Vicia Faba was chosen. It is possible to cultivate a bundle of long single side-roots to this object, which are to all intents and purposes without any root hairs and almost completely in their primary stage of development. It had also been observed that they were capable of absorption of water from one end to the other. Potentially speaking, therefore, the possibility of uptake of solutes existed. BREWIG had also managed to cause movement of water in the xylem in an artificial way. In connection with the necessary activity of the tissue some data of GREGORY and WOODFORD, as well as of PREVOT and STEWARD were at our disposal. A root system, however suitable it may be, would be unsuited to our purpose if it should not have sufficient power to absorb substances. Besides, the side-roots had the advantage of not being too thin, and of having a very constant thickness for a long stretch.

Three sets of seed of the broad bean, Vicia Faba, were used for the experiments. The coarser varieties, with thick brown or grey beans of 1½ to 2½ cm in length, appeared to be most suitable. The finer horticultural varieties were much less satisfactory. One of the kinds much used was the "Lange Hangers" variety. The seed of one of the sets had been dusted with "Fusarion", which appeared to have no influence, however. As a rule the seed was used the first or second year after the harvest.

The seeds were germinated in large petri-dishes. For this purpose these dishes had some tap-water on the bottom mixed with saw-dust and covered with a slip of filter-paper. 5 beans were put in a dish of $\pm$ 13 cm diameter. The dishes were covered with their lids.

They were then placed in the dark at room temperature. After a week one could start taking the seeds that had satisfactory long roots — 6 cm or more — to their nursery-pots. Jam-pots of 450 cm^3 with pierced lids were used for the purpose. These were covered with a coat of black paint and on top of this one of white lacquer. First the extreme root end was cut away for 3 to 6 mm from the primary mainroot, next the seed with the root through a perforated cork-plate, was placed on the completely filled pot. The rootend was cut off in order to prevent the mainroot from growing further, so that the plant would be stimulated to a more regular development of the side-roots. The solution in the pots consisted of mere tapwater. (Fig. 1, pag. 7).

The further cultivation of the plants was done in daylight in a hothouse which was heated in winter and where there was a high degree of moisture. There were rather great fluctuations of temperature, the average being 20° C. The developing shoot, which was rather weak, was kept erect by some supporting rings. After a total lapse of about three weeks, the material was fit for experiments and the side-roots were over 10 cm long.

Every week 20 beans were laid out for germination and the material thus obtained was used the third or fourth week. In this manner a sufficient number of plants in the most suitable stage was always at our disposal. So all the time roots of the same age were used, in order to comply with the requirements of homogeneous material.

This way of cultivation leads to a peculiar condition of the material. The main cause is the medium, viz. tap-water. These rather natural surroundings are in this way a poor source of minerals for the plant, the water in the pots not being supplemented or renewed. Thus we obtain a root system poor in salts and with a strong power of accumulation. With this goes a

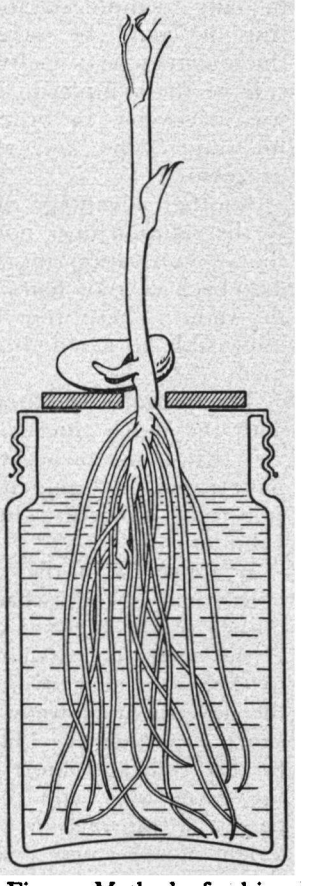

Fig. 1. Method of cultivating unbranched side-roots of Vicia Faba in jam-pots filled with tap-water.

high sugar-content, probably, for the greater part, originated from the seed-lobes. Thus the roots can essentially be compared with objects used by HOAGLAND and others for experiments on the uptake of salts. The only difference is that no aeration was applied here during the cultivation, this being considered superfluous as the solution was not shut off from the free air. The "poverty in salts" simplified the condition of our material in a way, and thus it could be assumed that disengaged ions originated from the quantity artificially made available in the solution at the other side of the symplasm. The high percentage of respiratory material was necessary in order to keep the isolated pieces of root alive for some time and also as a source of energy for absorption processes.

Another advantage of tap-water was that the "low salt-content" of the solution does not check the longitudinal growth of the side-roots. Cultivation in moist air was also possible, but it has the drawback of root hairs developing abundantly, which is prevented in water. These root hairs were undesirable, since it would be impossible to avoid their being damaged during the further treatment.

Theoretically speaking it would have been possible to experiment with the much thicker main-root. This has a drawback, however, viz. that a narrow cavity often develops in the centre. Then the penetration of liquid into this could not have been prevented. The side-roots, however, always had a complete compactly-built central cylinder.

It appeared in the course of the investigation that the chosen object had one drawback, as a consequence of which, some possibilities of the method could not be turned to account. The cause of this were the narrow wood-vessels, which offered high resistance to the water sucked or forced through. The consequence was that the movement of water caused in the xylem was not sufficient for short experiments. The possibility of obtaining a good O_2-supply for the central cylinder requires a supply greater than the amount the tissue uses, and this can only be attained when much water passes. A transmission of water of $^1/_2$ cm^3 in $\pm$ 20 hours through the xylem of one single piece of root was but rarely effected. To make the most of the technical possibilities of the designed apparatus, one should have a primary root with very wide wood-vessels. Since there are other demands on the object, however, we have not been able to make a better choice.

The purpose of the system was to obtain a young root of sufficient

length, cut open at both ends, which, as a consequence of the uninterrupted bundles of vessels lying in it, formed a kind of hollow tube surrounded by living tissue on all sides. If a suitable method for connecting both ends to a supply-tube and an outlet tube could be devised, it would be possible to let a liquid flow through artificially. In this way the symplast could be put into contact with arbitrary media at both sides, since the solution can be varied as well.

We must first and foremost consider what method of connection with rather tender pieces of root might be used. It would also be important to know what pressure-gradient would be necessary to effect a water-current in the narrow xylem-tracts of the roots, which are usually less than 1 mm thick.

In the first trial experiments the root cylinder was connected with a vessel containing $K_4Fe(CN)_6$ at one end only, the other end leading to the outer surroundings, which consisted of a dilute $FeCl_3$ solution. The connection with the supply-pipe was obtained by fitting the root in a little opening of a flat rubber stopper cut in two, which plugged the opening of a glass tube of $\pm 2^1/_2$ cm diameter (Fig. 2, pag. 9). In doing so, care had to be taken that the root was slightly squeezed in, without the wood-vessels being obstructed however, or the cortex tissue damaged. A pressure of 30 to 50 cm liquid appeared to be sufficient to make a little jet of the blue reaction product come from the free end of the piece of root. In addition to this it was studied how much had to be cut off from the tip of the root to remove the undifferentiated part of the xylem and to open the vessels sufficiently. It appeared that it was sufficient to cut away 1 cm of the tip of the root, this being confirmed by anatomical

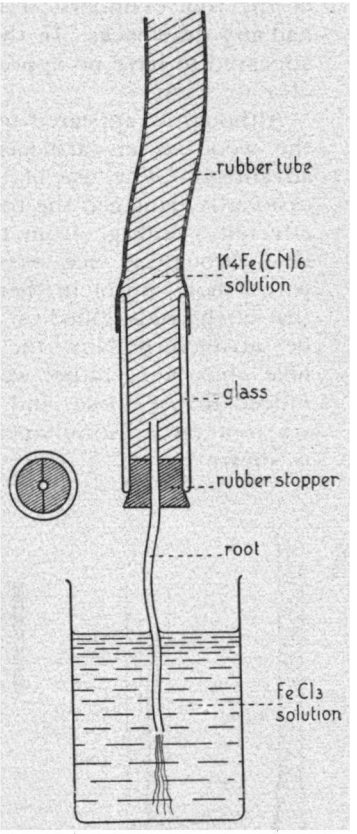

Fig. 2. Pressure method for bringing about a flow of solution through the xylem of excised pieces of root. The piece of root is fixed to the supply tube by means of a rubber stopper cut in two and containing a hole in the centre, just a little narrower than the average thickness of the roots.

investigation. Often, however, more was cut away. Since the resistance was bound to increase proportionate to the increase of length of the root cylinders, it had also to be investigated whether this had any drawbacks. In the set-up we used, the length of the pieces appeared to have no appreciable effect, not at least over the distance of 2 to 7 cm.

Although it appeared to be possible to let a liquid flow through the wood-vessels artificially, this simple method had some disadvantages. The use of the two halves of the rubber stopper occasionally damaged the root, or the plugging was insufficient, which effected a leakage from the surroundings to the liquid caused to flow through, or vice versa. Then it was tried whether rubber films with a hole, burnt in them, having a diameter a little smaller than that of the root, glued on to the supply-pipe and outlet-tube, would be satisfactory. Now the rootpieces could be pushed through the hole, thus being rather well fixed. It turned out to be quite possible indeed to use these, and an apparatus was made to experiment on six root pieces simultaneously. The construction of the apparatus is shown in fig. 3, pag. 1c. Six glass tubes with perforated films glued on to them were connected with a forked glass tube with a connected pipe for applying the pressure required. A glass tube, serving as surrounding-vessel, could be pushed over these and was joined to a piece of rubber tube at the lower end. Short celluloid tubes served as receiving vessels; these too had rubber films luted at the bottom. The whole apparatus was fixed to a rack with a number of clips.

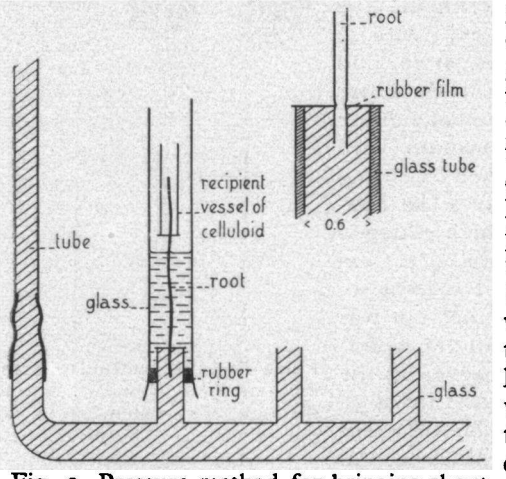

Fig. 3. Pressure method for bringing about a flow of solution through the wood-vessels of root pieces.

Though regular flowing through occurred in this apparatus and rather many experiments were made with it, a better method appeared desirable. An apparatus was designed in which the same root connection was used. Passage of water was brought about by underpressure, maintained by an air-pump. This was done in order to get a better

current. The rubber films, however did not stand the pressure, and leakage occurred frequently.

It was evident that the connection with supply and outlet should be brought about by elastic rubber tubes. The difficulty was that a tube of such a small inner diameter — less than 1 mm — could not easily be obtained. We first tried to get sufficient connection with the root, by taking a piece of a valve-tube and wrapping 2 or 3 short pieces of the same tubing round the end of it. Thus a pretty good joint was obtained which when used, however, had too many drawbacks to be satisfatory, though a number of experiments was made with it.

At last a solution was found by a method resembling the one used by WHITE in his experiments on the root pressure of tomato-roots grown in tissue culture. Thanks to the collaboration of the "RIJKS RUBBER BUREAU" (Government Rubber Institution) we could use rubber tubes of sufficiently small inner diameter — $\pm$ 0,7 mm. These were tubes with walls $\pm$ 1 mm thick, obtained by removal of the metal kernel from insulated electric wire. The little roots could be carefully pushed into these and with the help of a metal clip the tube was carefully squeezed a little, so that a leak-proof connection with the root was obtained. The bore of the clip was first carefully tried, and the only remaining difficulty was the selection, gained by experience, of the right root thickness. Too thin roots caused insufficient plugging, too thick pieces were damaged by the pressure of the clip. Since we were now able to work with a high vacuum even, we passed on to the construction of two apparatuses with which the experiments were chiefly made.

Most of the experiments in which the transmission of dissolved substances from the inside to the outside was studied, were made with the simple first apparatus — the small one (Fig. 4, pag 12).

This consisted of a solid brass basin, $16^1/_2 \times 11$ cm, divided into three parts by two partitions. The lower part A, $1^1/_2$ cm high, served as supply-chamber for the liquid caused to flow through. This was completely closed by a layer of rubber and a lid screwed on to it. In the lid a lengthening-piece had been inserted, with which a bent glass tube was connected (2). It was filled through this end, and on the glass tube the displacement of the water as a consequence of the flowing through, could be read. Six tapered glass tubes (3) leading to chamber B had been fixed in the partition in little metal flanges.

In the large central chamber B there were six root cylinders, each fixed separately in a glass tube. This separate fixing of each

root, with its own surroundings, was done in order to prevent the failure of the whole experiment in case of a leak somewhere in the system. The results thus obtained from each root formed separate observations. This precaution appeared to be by no means superfluous, since one or more outer media were regularly unfit for analysis, on account of insufficient plugging.

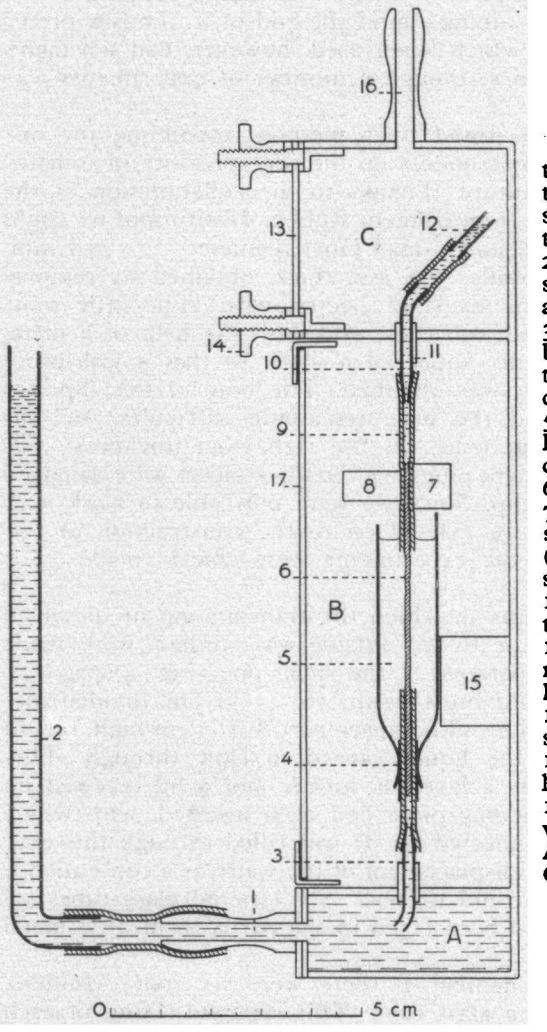

Fig. 4. Section through the small apparatus used in the "flowing-method". 1. small brass pipe for attaching rubber-tube.
2. glass tube with supply solution for maintaining an overpressure.
3, 10. glass tube. 4. rubber tube for connecting the root to the supply chamber.
5. glass vessel containing liquid, cubic capacity 3½ cm^3.
6. excised piece of root.
7, 8. bridge of clips for softly pressing rubber tube (9) against the root to secure a tight connexion.
11. metal tube for fixing the glass tube.
12. narrow glass tube for receiving the circulating liquid.
13. celluloid lid of the suction-chamber.
14. nut to screw on the lid tightly.
16. tube for attachment to vacuum pump.
A. supply-chamber.
C. suction-chamber.

The connection with the supply-chamber is made by the rubber tube (4) — wire-insulation around which a layer of rubber film had been glued in the middle — which debouches into the glass tube (5). The glass tubes are about $5^1/_2$ cm long and about 1,3 cm wide. At one end they have been drawn to narrow tubes in such a manner that the rubber tube is slightly squeezed but can yet be pushed up and down in it with a little trouble. The root of $\pm$ 0,8 mm (6) is in free contact with its medium for $\pm$ 4 cm, these surroundings being an aqueous solution in tube 5. Further down, the root continues in tube 4 till below the narrowing of the glass tube, where a closure is found by a slight narrowing. Upwards, the root is in tube 9 for $\pm$ 1 cm, the bridge of clips bringing about a good connection.

Finally, the six glass tubes are also supported by a wooden block with pegs in it (15). In order to prevent the drying up of the little free end of the root, chamber B can be closed by a lid (17), which is, for the greater part a glass plate.

The third chamber C of the apparatus is the suction chamber. Into this six other glass tubes (10) debouch, with which a piece of thin glass tube of about 6 cm (12) is connected. The liquid sucked through, can be observed inside these and thus the quantity of liquid flowing through each of the six roots, can be noted. Chamber C can be shut off airtight by a celluloid plate (13) which is screwed on to a rubber under-layer by a metal edge and six nuts (14). Finally a lengthening-piece (16) debouches into chamber C, which piece is connected with the vacuum-tube leading to the air-pump.

Afterwards a slightly more complicated apparatus (Fig. 5, pag. 14) — the large apparatus — was constructed, into which some improvements were introduced. It consisted again of a brass basin, $17^1/_2$ cm long and 19 cm wide, also divided into three chambers. Supply-chamber (A) is a closed space, into which two glass tubes (1) debouch at the top, with a tap in each. At the bottom there is a pierced celluloid tube (2), passing through the side wall and fixed to a glass lengthening-piece to connect it with a rubber tube. By means of this, the liquid in chamber A can be saturated by arbitrary gas mixtures before flowing through the root pieces, e.g. by leading N_2 through it, the inner solution can be made anaerobic. The twelve supply-tubes (4) inside, have been bent upwards in order to prevent gas-bubbles from being sucked into the root and plugging the xylem.

The large central chamber B has now been divided into two parts by a lengthwise partition, for 4 and 8 roots separately. Each part has two celluloid tubes (8) for its own aeration. This division into two chambers makes it possible for two different experiments to

be carried out simultaneously, or for a blank observation to be made in otherwise identical conditions. Part of the roots is again pushed into the rubber tubes (5) and fixed by a twofold clip-system (6 and 7), so that there is no leaking. The two chambers B are closed by a glass lid (9) with metal edges and can be filled with any arbitrary solution.

The quantity of liquid sucked through, passes, via the tubes bent

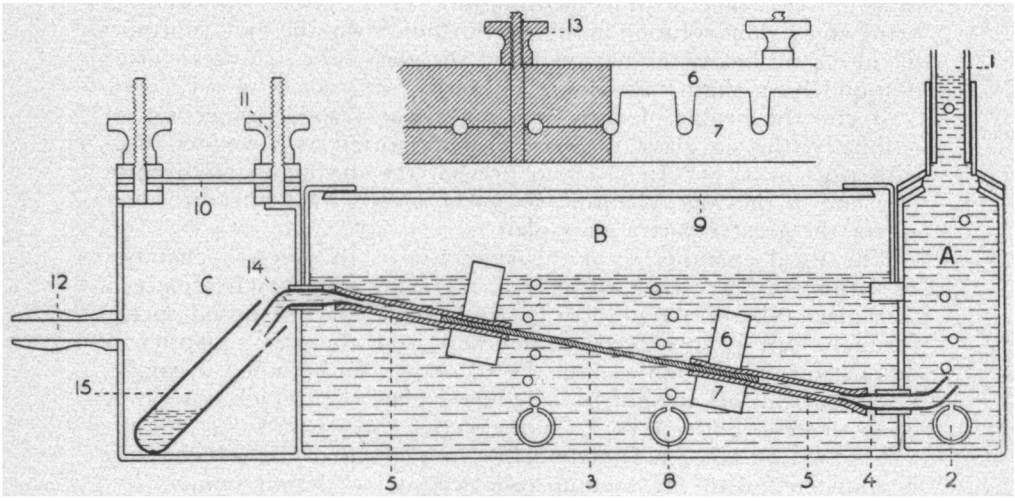

Fig. 5. The more elaborate "large apparatus" used in bringing about a flow of liquid through the xylem vessels. Section through the whole apparatus and side view and section through the clip system.
A. supply chamber. B. compartment containing liquid. C. suction chamber.
2, 8. celluloid aeration tubes.
3. piece of root.
15. receiving vessel containing the liquid that has passed through the xylem.

downward (14), into chamber C. The liquid that has passed the root can be collected in tube 15, and used for analysis. The liquid is received from each root separately so that in case of leakage it is only the root concerned that drops out. The suction-chamber can again be closed by a celluloid plate (10) that fits in with a rubber edge and must be fixed by a brass frame and 12 nuts (11).

This construction of the large apparatus makes it especially suitable for absorption experiments under different conditions. Both inner and outer surroundings can be completely regulated, also with regard to the O_2-supply of the tissues. The narrow xylem-tracts in the root however form a restriction in supplying the inner

root tissue with a desired amount of oxygen. The quantity of liquid sucked through in each root (3) can be read in the receiving-vessels (15).

The apparatus had originally been put in a room of constant temperature, but afterwards, the apparatus itself was put for economical reasons in a thermostat; the pump-installation standing outside. The temperature during the experiments was usually 20° C.

The motive power for the flow of liquid through the root was generally an underpressure of 55 to 65 cm Hg, which was obtained by means of a pump-installation connected to the suction-chamber. The suction-chamber was connected with an air-pump, a wash-bottle and a larger brass vacuum-vessel being inserted in between. The air-pump was a Hyvac vacuum pump, Cenco made. With the help of a mercury pressure gauge with points of contact melted into it, and of a relay connection (Fig. 6, pg. 15), the underpressure could automatically be kept within the limits of 55 to 65 cm Hg. If, through a leak, air should have been sucked in so quickly that the pump could not maintain this underpressure, the switch had been constructed in such a manner that, at an underpressure of ± 30 cm Hg, the $^1/_4$ h.p. engine, driving the pump stopped altogether.

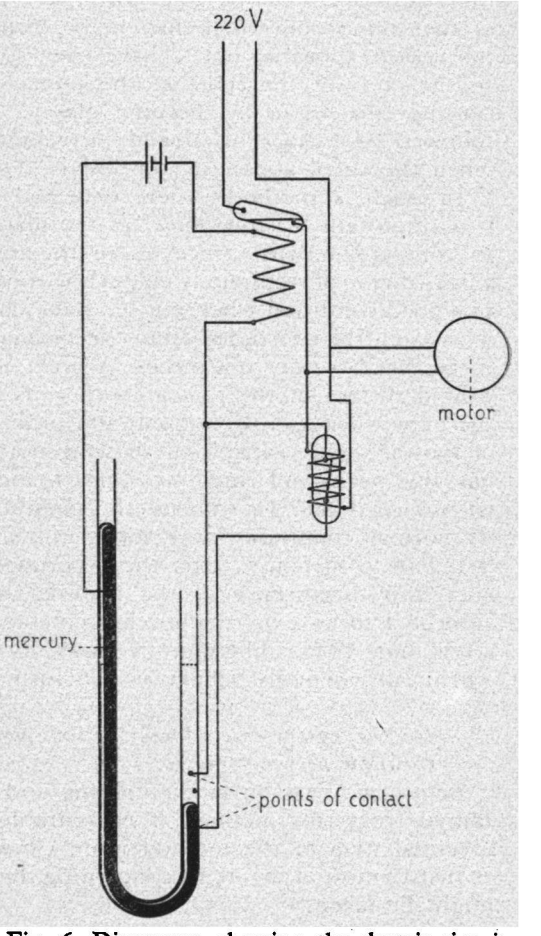

Fig. 6. Diagramm showing the electric circuit, relay and manometer for maintaining a constant vacuum of 55—65 cm Hg.

Surveying the method we are able to say that after some experience, it was possible to get a usually leak-proof connection between the root and rubber tube in spite of the great pressure-gradient at one of the joints. Indigocarmine, added to the surroundings, functioned as an indicator of possible leakage in absorption experiments. In experiments on the giving off of solutes, uranin or K-fluoresceinate was added to the solution passing inside, since these substances can be observed in very minute quantities. In spite of the connection which had to be formed under slight pressure, the vessels appeared not to have been squeezed. The damage done to the ends of the roots at the joints was generally slight, only causing the tissue to become glassy. Worse damage was often followed by leakage of air and shrivelling of the tissue, at any rate when the small apparatus was used.

In such a method, where detached pieces of tissue are used, it is important to study how far the material admits of conclusions in comparison with processes in the intact plant-root. Owing to a due reserve of respiratory matter the root pieces appeared to remain in a good condition when put for some days into a few cm^3 of water. This could be concluded from the normal appearance and from the turgescence of the root pieces. A great advantage is the very small wound-surface of the root at both ends. Besides, it is known from the experiments of HOAGLAND and others that small excised pieces of root are quite capable of showing normal uptake; and this result has also been confirmed by PREVOT and STEWARD, among others, with Vicia Faba. In spite of the possibility of the tissue retaining its normal condition for a longer time, the experiments usually lasted 16 to 24 hours. After the experiments were ended the appearance and turgescence of the material were studied. After being subjected to an experiment, the root pieces could be kept alive for a few more days. Therefore we may safely assume that the material will in all essentials behave as if it formed part of the intact plant.

§ 2. *Do the xylem-tracts form the sole pathway for the liquid flowing through the root pieces?*

Before we pass on to mentioning and discussing the results obtained from this method, it is desirable that we should study an essential item of the method more closely. We have already tried to justify most of the items concerning the method to which objection might be taken.

The fact that this method makes it possible for a current to flow through the wood-vessels, and that we can then study the transport of substances in a transverse direction through the tissue, in which

among others the endodermis must be passed, is essentially important to the whole construction.

Since the whole root segment is pushed into the tubes when fixing the connection in our apparatus, and not the central cylinder only, one can bring forward in opposition, that it is just as well possible for the supply liquid to penetrate into the intercellular spaces of the cortex. Perhaps lengthwise transportation along these air-channels would even be possible. If we cannot satisfactorily refute this criticism the value of all our data would be very doubtful indeed, since in the transverse transfer it would be the distance from cortex to surroundings only, that would have to be covered. On this stretch, however, there is not a single peculiarity of structure that would force the substances to pass through the protoplasm and the results could at most be important as a confirmation of the possibility of intermicellar cellwall transportation. Now this was not intended at all.

Our purpose will now be to mention a number of observations that will clearly show that there is no question of penetration of the liquids into the cortical intercellular spaces.

The first phenomenon that would be noticed, if air were actually replaced by liquid through suction, is that the root would become glassy, for we see that this always happens in cases of infiltration. When we survey our results, however, it appears that in all the experiments in which the root pieces remained in a good condition, the colour always remained a good dull whitish one. There was no question of glassiness, although this might happen at the place of junction as a consequence of the pressure caused by the squeezing. Apart from this absence of glassiness it also appeared in microscopical sections that the intercellular spaces were always filled with air.

The fact that the air-channels in the cortex are many times smaller than the vessels, and besides, follow winding lines, must make the resistance very strong indeed. Since they were filled with air from the beginning-it will be understood that the suction used is not sufficient to remove the air from them and thus cause infiltration.

More facts, however, can be brought forward to show that the lengthwise transportation must have passed through the vessels in the stele. It is of great importance to know if there is not, by any chance, transfer through the cortex, in the short stretch under the rubber tubes only. This could be very well investigated, since very often some dye was added to the liquid caused to flow through. In sections through the extreme end we should have observed the

diffusion of eventually penetrated dye. It is very important here that, since transportation would have to take place through the intercellular spaces, the diffusion of the dye cannot be reduced by difficulties of permeation.

It appears, however, e.g. that after allowing a current to flow through for 18 hours, fluoresceine can be seen only at the surface of the cut next to the supplied liquid, which is under slight pressure, and a bit further on only in the pericycle. At the supply-side dye can often be observed in the wood-vessels only. Thus it appears that even at the junction, where occasional glassiness of the root may occur, the liquid in the supply-vessel penetrates at most as far as 1 or 2 mm into all the tissues. Since the piece of root is fixed in the rubber tube over a length of about 8 m, no mistakes can arise from this; in 3 to 4 mm from the extreme end of the pieces colouring can only be observed in the xylem-vessels.

The fact that Ca is given off to the surroundings and that K does not exosmose from out of the xylem, is a proof that transmission must take place through the plasm, not through intercellular spaces.

The microscopical observations made in many sections are the best indication, however, that the wood-vessels in the stele conducted the passing liquid-current. The section was often studied in liquid paraffin in order to prevent the dye from being washed away. The sections were taken from the free part of the root pieces often from about the middle.

The results are:

a. Experiments with flowing through by overpressure only

dye	colouring noticed
eosine	in vessels + cells stele to endodermis.
indigo-carmine	in vessels + some cells of the central cylinder.
uranin	in cells of stele and sometimes as far as inner cortical cells.

b. Experiments with flowing through mainly by suction, at an underpressure of 50 to 60 cm Hg.

dye	colouring noticed
Na-fluoresceinate	in vessels, cells of stele and sometimes in inner cortical cells.
K-fluoresceinate	in cells of stele and sometimes in inner cortical cells.

Rhodamin-B, when added to the outer medium, appeared to penetrate into the vessel-walls via the cortex and the endodermis. This too points to transverse transportation, not to lengthwise conduction, in the cortex. No fluorescence was visible in the receiving

vessels, so no lengthwise transportation through the cortex has occurred.

In by far the greatest number of cases the root remained white when Na- or K-fluoresceinate was caused to flow through it. This too points to the fact that no dye has come into the cortex. In the few cases where the root pieces did become a bit yellowish the colouring-matter could at most be observed in sections in the inner corticalcells, adjoining the endodermis. This indicates that the fluorescein came here from the stele via the endodermis, which in the case of this substance is not surprising, for fluorescein is an easily permeating substance and can certainly penetrate via the plasm.

It is important that in an experiment where K-ferrocyanid was pressed through by an overpressure of 30 to 50 cm H_2O, this oozed out at the other end of the root into $FeCl_3$-solution as a little blue jet (fig. 2, pag. 9). The root piece itself, however, remained quite white inside so that neither of the two substances reacted in the cortex. If the K-ferrocyanid had penetrated between the corticalcells a reaction with $FeCl_3$ would have taken place. $FeCl_3$ would certainly have been able to penetrate into the cortex for some distance via the intermicellar ducts in the cellwalls.

After mentioning these observations we hope to have shown with sufficient certainty that the liquid could only get through from the supply-chamber via the wood-vessels, and perhaps, partly through the stele tissue. Lengthwise transportation and supply of substances through the cortex did not take place. Thus we have in fact been able to observe transverse transfer of substances from stele to surroundings and vice versa. Thus in this process the substance concerned must pass the protoplasm, even if it were only in the endodermis-cells. We may therefore be allowed to draw conclusions from our experiments about the permeability of the symplasm.

CHAPTER III

EXTENT OF THE ZONE CAPABLE FOR INTAKE OF SOLUTES.

§ 1. *Anatomical considerations.*

The question as to what parts of the root take part in the process of absorption from the surroundings, has given rise to many investigations. The views obtained are based both on morphological and

experimental considerations. A sound knowledge of the anatomy of the root is indispensable, as the specific structure of the root will exclude beforehand a number of hypotheses and must guide us when considering the possibilities. These, however, will finally be settled by experiment.

We shall therefore preface the further discussion with a brief summary of the essentials of anatomy, as far as this is concerned with our problems.

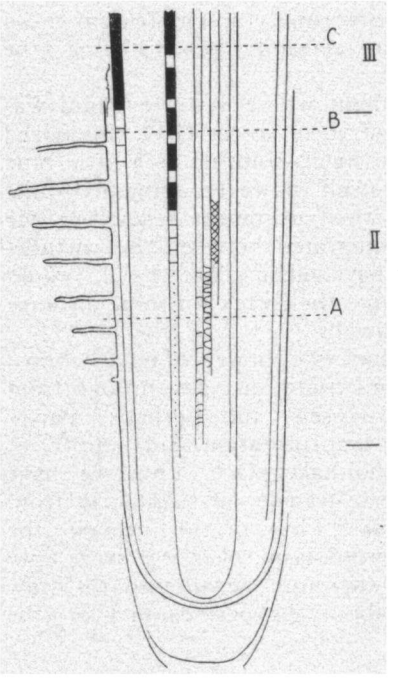

Fig. 7. Longitudinal cut through a young root showing the main anatomical characteristics important to transport problems.
I. Zone of root cap, meristem and parts where cell-elongation starts.
II. Zone containing more fully differentiated tissue, root hairs and an endodermis for the greater part still in its primary or secondary stage.
III. Basal zone of the root with isolating cork layers covering it.

In connection with some structural peculiarities it is best to divide the root into three zones (fig. 7, pag. 20). Beginning at the tip we distinguish as the first zone (1) the whole root cap, the meristem and part of the area in which the cell-elongation starts. The whole of this area is characterized by having exclusively parenchymatic tissue and usually some slight vacuolization. According to SCOTT and PRIESTLEY the cellwall, made of cellulose is partially strongly impregnated with protein. DE RUFZ DE LAVISON says that the central cylinder cells generally have slightly thicker cell walls, which have a sort of mother-of-pearl-like gloss. Here the cells usually form a compact whole, although the first intercellular spaces may occur in the cortex close behind the meristem. The rhizodermis covers the root at the outside and does not yet have root hairs. The root cap, directly connected at the root tip with the root tissue, is of no practical importance to the problem discussed here.

Zone II, which can be distinguished next, is the area of the roothairs. Here the coherent layer of rhizodermis cells is in most

plants potentially capable of forming root hairs in suitable circumstances. The cell-walls of the rhizodermis are made of cellulose.

In the lower area a certain cell-elongation may occur and the differentiation of most elements is performed here. Vacuolization is universal here. At about the same level we notice the first indications of the primary endodermis, phloem and xylem.

Usually another layer of coherent cells, the exodermis, lies beneath the rhizodermis. This exodermis may, in the more basal area, show the first signs of the deposition of suberin. The root cortex in this region consists of long stretched parenchyma-cells with cellulose walls and large vacuoles. Intercellular spaces have been regularly developed here.

In the larger part of this zone the central cylinder is surrounded by a coherent layer of cells: the endodermis in its most primary stage. Here all the radial walls have a Casparian band which shows wood-reactions and also reacts on fatty matter (VAN WISSELINGH). The endodermis can pass into its secondary stage nearer the base, where there is a layer of suberin all over the wall. Many individual cells, however, remain in the original primary stage, forming the so-called "passage-cells".

Finally the central cylinder forms in this area a compact whole of coherent cells, including the wood-vessels. Phloem and xylem become more differentiated as they come nearer the base. The first ring-vessels start at about the same level as where the endodermis begins to have Casparian points. A bit higher up, the spiral-vessels and at last the net-vessels appear. With the exception of the woody xylem the other cellwalls are chiefly made of cellulose. There are no intercellular spaces.

The last zone (III) to be distinguished is the area towards the base where the rhizodermis begins to die off and usually disappears completely. In many roots this zone is by far the largest part. Together with the disappearance of the root hairs, the ordinary rhizodermis-cells die off and are finally cast away. The exodermis now becomes the actual boundary layer between the outer medium and the other root tissues. The exodermis usually becomes corky in all the cells on the whole wall, thus forming a kind of separation from the surroundings. Separate non-suberized cells of a special structure may be left in it, the so-called "Kurz-Zellen der Intercutis" according to KROEMER.

Further inward comes the parenchymatic root cortex with the endodermis in its secondary or tertiary stage with the "passage-cells" lying in it. The whole cortex may also finally be cast off, and in this case the endodermis, in its tertiary stage, acquires the

function of an isolating layer against the outside. Now the central cylinder has been sufficiently differentiated. Of the xylem the larger net-vessels situated more centrally are the most important transportation vessels for water and substances dissolved therein.

Though this scheme of construction is pretty well realized in the Vicia Faba root, numerous, sometimes even very profound, variations may be found in other species. Thus in some plants the contact with the surroundings may be almost completely lost in certain circumstances, by the exodermis becoming suberized about as far as the root cap. The scheme of construction sketched above is essentially quite general however, and we can refer to it in our following discussions. For further data about anatomy we can refer to von GUTTENBERG's article in "Handbuch der Pflanzenanatomie".

It is necessary to give here a few definitions of some concepts in order to prevent misunderstanding later on. Since we can generally distinguish three phases in the total process of uptake of matter through the root, viz. absorption, transport and secretion, we should like the fact to be taken into account that all the processes need not take place in exactly the same area.

The first phase in the process of normal absorption of matter will be the passing of the molecules or ions concerned from the solution to the surface-layer of the plant (a in 3 fig. 9, pag. 62). Although the cell wall can play a certain part, the decisive thing here is getting into contact with the boundary layer of the protoplasm. This first process can be defined as the entrance of the substance into the protoplasm. In accordance with this we shall call the zones in which this process happens in the young root: *the area of intake.*

The intake having taken place, the molecules or ions can be subject to various processes. For part of the introduced substances chemical binding or use in synthetizing various substances will be possible. It is especially for the mineral elements that vacuole-accumulation may be important, in which, by expenditure of energy, matter is polarly secreted into the vacuole (8 in fig. 9, pag. 62). This process, which will not occur in a root saturated with salts to any extent, except in the parts still growing, will not be further considered since it is not to the point in our problem. Another possibility is the transmission of the absorbed solutes towards the base of the root through the living parenchymatic tissue of cortex or stele. We consider this to be of secondary importance for the supply of the shoot.

The principal supply-current to the aerial parts goes by the ascending sap in the xylem. In order to get into the xylem, the matter taken in must be transported through the protoplasm of the cortical cells via the endodermis up to the stele. There is thus

transverse conduction from the cells bordering on the surroundings to the cells around the wood-vessels. The zone where this transverse transportation (2—6 in fig. 9, pag. 62) is predominant will be called the "*transit-zone*". This transverse transmission can be of two kinds. "Active" processes will be predominant as long as the concentration in the xylem-sap is maintained at a higher level than that in the surroundings. It must be taken into account, however, that passive penetration of substances by diffusion is also possible. At any rate the absorbed substances will be freed in the xylem (3 in fig. 9, pag. 62). Thus there they leave the living tissue; we shall not as yet consider the nature of this process. The area for which this is possible under normal conditions is called the "*secretion-zone*".

In part of the root the three processes may occur simultaneously This area, consequently most important for the normal supply of ions from the surrounding solution, will from now onward be called the "*supply-zone*". This can never be larger than the area of intake; it is usually a bit smaller.

On account of the structure of the root the area of intake must be limited to zones I and II (fig. 7, pag. 20). Nearer the base the corky exodermis prevents any contact of the plasm with the outside. In these zones the cell-wall is permeable and the matter can reach the protoplasm. Theoretically speaking, both these zones must therefore be considered as capable of performing the first phase of the intake. As to the *transit-zone* the following must be considered. The process of lateral transfer will probably be restricted to zone II. For in zone I the compact, largely undifferentiated cell-mass will strongly resist transportation. Up to the xylem very many cells have to be passed, as this is rather a long way. We can also expect many substances, especially the salts, to be fixed in the protoplasm or to be subject to vacuole accumulation. Theoretically speaking, one can imagine zone III is supplied with substances by means of longitudinal transfer towards the base via the parenchyma, giving off substances later on to the xylem. In this way the path of transport becomes very long and since we know that conduction over a great distance through parenchyma-cells is usually very slow, this possibility is considered to be of secondary importance. Experiments made by STOUT and HOAGLAND with radioactive ions clearly show that in the stem-cortex of willow and Pelargonium transverse transportation is of paramount importance in an analogous situation. Finally secretion is only possible in zones II and III, while zone III can be considered to be unimportant on account of the expected lack of supply.

So anatomical considerations would show that zone II is pretty well the only *supply-zone*. Studying the writings on this subject we see that many experiments confirm this.

§ 2. *Literature.*

With regard to the absorption zone the following data might be mentioned. SIERP and BREWIG, referring to the same object we experimented with — although it was about uptake of water — stated that the extreme root tip (± 5 mm) does not take in water and that the rest of the root, elongation zone and root hair zone, is capable of absorption. PREVOT and STEWARD found, among others, with barley and Vicia Faba, that salt-accumulation is greatest at the tip, especially in the elongation-zone. Measured from the tip the whole absorption zone is well over 5 cm long. There appeared to be hardly any lengthwise transportation, i.e. the absorption was local. As to the intake of water, ROSENE (1937) obtains to all intents and purposes the same results as SIERP and BREWIG.

One of the most recent investigations in this field is that of GREGORY and WOODFORD. They used the primary main-root of Vicia Faba and by putting this in a number of adjoining chambers the uptake in each zone could be analyzed. The NO_3-intake appeared to be greatest in the tip-zone and became less the nearer the base was approached.

Thus we see that the data mentioned above agree with our conclusions based on anatomical structure. The area capable of absorption is indeed potentially defined by zones I and II, where very permeable cellwalls are found. Owing to the fact that we distinguish three phases in the absorption process, it will be clear that all these data can only be used regarding the first mentioned phase, and do not allow of any conclusions with regard to the area, where transverse transportation to the stele takes place.

As to the third phase of the whole process, we must refer to old experiments made by KRAUS (1887) and repeated by WIELER (1893). Thus KRAUS found that bleeding from the leaves could occur in maize when 5 mm, even when 40 or 50 mm, of the root were cut away from the root tip. WIELER gives similar results for the roots of Vitis, Salix, Richardia, Hyacinthus, Narcissus and Zea. Though it strikes us as rather astonishing that bleeding can occur in wood-vessels cut at the lower end, this points to the fact that giving off of solutes to the xylem is also possible outside the zone taken away — at least the whole of the first zone. The fact that thicker pieces of root too can show bleeding indicates, that in zone III, too, salts can enter the xylem-vessels from the surrounding tissue.

These data fully contradict PRIESTLEY's ideas. This investigator supposed that the contents of the vacuole are disengaged during the differentiation-process of the young xylem-cells and the dying of the protoplasts in the course of this process. The solutes would only get into the xylem in this way. The secretion-zone would have to restrict itself to zone I. At any rate the experiments just mentioned are sufficient proof to contradict the supposition that, during the dying, disengaged vacuole-sap would be the only source of mineral-supply to the xylem.

§ 3. *Own observations.*

Though the experiments were not exactly aimed at a closer investigation of this problem, they did give some data concerning it. Generally speaking about 2 cm were cut away from the root tip, the pieces of root being 6 to 7 cm long. Occasionally, the zone experimented with was slightly different. Part of the piece of the root did not come into consideration since this, when put in the apparatus was covered by the rubber tube for some distance at both ends. The free distance in the apparatus can be said to be about 5 cm. Some observations were also made in the preliminary experiments and in these the extent and situation of the investigated zone have been different.

In the experiments made it appears that transportation of matter through the root tissue was possible from surroundings to xylem and vice versa. Not always could certain conclusions be drawn about the nature of the transportation process, but in many cases

TABLE I

Survey of the results obtained in investigating the lateral transport of solutes through living root tissue. Root pieces of different lengths and cut away at different distances from the apex have been used in separate experiments.

	limits of investigated zones in cm	accumulation in the tissue	inward bound transfer	outward bound transfer
K	1—7	O	+	O
	3—7	O	O	—
Ca	3—8½	O	O	+
Cl	1—7½	O	+	O
NO_3	1½—7	O	+	O
	3—7½	O	O	+
H_2PO_4	3—8	+	+	O
urea	3—8	O	O	+
glucose	1—5½	O	+	O
sucrose	3½—8½	O	O	+

+ conduction does occur. — no transfer O not investigated.

we had to conclude that this was a passive one, i.e. a consequence of diffusion phenomena.

In the table I, pag. 25, a survey is given of the observations, made on transport in opposite directions. The numbers indicate the limits of the root zones, measured in cm from the tip.

By inward bound transportation is meant that a substance can be shown in a liquid caused to flow through, when this substance has been added to the outer solution. By outward bound transfer is meant the reverse process: the substance is added to the liquid caused to flow through the xylem and can afterwards be shown in the surrounding medium after it has passed the root tissue.

So it appears that in the whole area between 1 cm behind the root tip and $8^1/_2$ cm transverse conduction of various substances through the living root tissue is possible. We can therefore say that all the zones of 1 to $8^1/_2$ cm after the tip act as *transit-zones*, and that the whole of this area is important as a *supply-zone*. Though it also appeared that at any rate the 3 to 8 cm zone can accumulate, we shall as yet not consider the nature of the process which effects the transportation.

Surveying all this we see that the prevalent opinion is correct. So the intake of appropriate substances is possible in the whole area suitable for this, i.e. zones I and II. It has not yet been decided upon whether zone I also plays as important a part as an absorption zone for further transportation, but this is very improbable. It has been shown experimentally, however, that zone II actually acts as an area from which substances are given off to the vessels. PRIESTLEY's view will undoubtedly be correct but is probably of secondary importance in the supply of substances to the ascending xylem-sap. Thus it is zone II especially that acts as the *supply-zone* proper.

It is very probable that the *secretion-zone* can also continue in zone III, as a longitudinal conduction of substances in the tissue is possible. This tissue can also serve as a storehouse, giving off substances to the xylem in due time (JAMES and BAKER). This view is supported by WIELER, who tells us that even pieces of thick, consequently of old roots of some kinds of trees can show bleeding, although it takes some days before the process starts. In this case the surrounding tissue must have given off substances to the xylem, if the observations actually refer to bleeding from the xylem-vessels.

Thus the experiment confirms the speculation based on anatomy.

CHAPTER IV

PERMEABILITY OF THE SYMPLAST.

§ 1. *General considerations and literature.*

The process through which the plant can take up substances from its surroundings can, theoretically speaking, be of two kinds. One can imagine active absorption, usually attended with accumulation processes, as well as passive permeation, merely based on diffusion processes. If one wishes to see how far the latter plays a part in the nutrition of the plant, one must have experimental data about the permeability of the root tissue. The further investigation of this was the main purpose of the experiments made.

Again it seems to be desirable to point out a number of facts about the anatomical structure of the root zone concerned. Section A in fig. 8, pag. 27, is characteristic of the structure in zone II (see fig. 7, pag. 20). An uninterrupted cell wall net, consisting chiefly of cellulose, is found in rhizodermis and cortex. Only the Casparian points in the primary endodermis interrupt this pathway, which must, on account of STRUGGER's results, be looked upon as a zone extremely suitable for intermicellar transportation in the cell walls. Here the root hairs are a means to obtain more and better connection with the medium. The presence of the Casparian points makes everything passing the endodermis enter the protoplasm. At this point, everything has been brought under the control of the living protoplasm and is consequently dependent upon the specificity and activity of these cells. Inside the endodermis a compact tissue is found without intercellular spaces, with cell walls mainly consisting of cellulose. Here again movement through the cell walls up till the xylem would be possible.

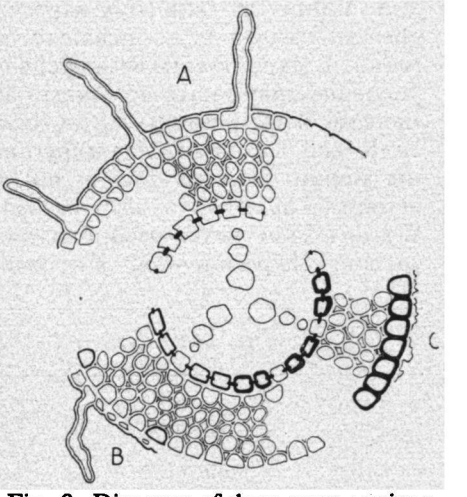

Fig. 8. Diagrams of three cross-sections through the root at various distances from the apex. (see A, B and C in fig. 7, pag. 20).

In the transitional area between zones II and III, the section at B, we only see a change in the endodermis, which is in

its secondary stage here. Anyway it may be assumed that the cells letting substances pass, behave essentially in the same manner as the endodermis in its primary stage. Thus all the substances passing the protoplasm can also be absorbed here. Possible absence of the rhizodermis does not cause any changes, since an exodermis with cellulose walls would most probably take the function of the rhizodermis.

It is only in zone III, the section at C, that the suberized exodermis will hinder contact with the surroundings. As long as no other isolating layers occur and there is a cortex, reversible transverse transportation from the vessels to the cortex and vice versa must be considered as a possibility, because the passage-cells form a pathway in the endodermis.

Now that the results obtained by STRUGGER have shown once again that considerable transfer of dissolved substances over a distance of some cells is possible in a cell wall, mainly consisting of cellulose, we can assume that all the cells of the cortex are directly connected with the outer solution. Thus even the innermost cortexcells might perhaps directly absorb substances from the surroundings, a view already found to a degree in PREVOT and STEWARD. PRIESTLEY, too, assumed that transportation would take place via the net of the cortical cell walls as far as the endodermis.

A number of data from papers on the subject made us expect the root tissue to be permeable to various substances. Thus FREELAND found in experiments with Helianthus, Coleus and Solanum lycopersicum that increased transpiration was attended with a slight increase of the amount of absorbed ions. The influence is strongest on K and Ca. In similar experiments by MUENSCHER increase of the amount of water used is not attended with increase of uptake of salts. The effect is also marked in more recent experiments by WRIGHT. The latter used Phaseolus vulgaris, var. humilis, during 96 hours' experimenting. His results were:

absorption of water	abs. of P	abs. of Ca	abs. of NO_3	abs. of K	
330	13,6	25,0	41,4	35,6	Pot I.
150	8,6	15,0	41,0	27,8	Pot II.
335	11,2	27,0	46,8	56,4	Pot II.
165	9,6	13,0	41,8	52,8	Pot I.

In these experiments there is especially a good correlation between absorption of water and of salts for Ca, which is, however, only slightly noticeable with K and especially with NO_3.

BÖTTICHER and BEHLING, who used maize-plants and barley, also made experiments of short duration. Positive correlation between absorption and transpiration is clearly marked here with Ca, in a slighter degree with K. These phenomena can best be explained by the assumption that the salts are passively carried along with the water-current through the tissue. We need not be surprised at the influence not being exactly the same with all the ions since their physiological importance varies greatly. Yet one could think of another explanation. For it might also be possible that at slight transpiration the salt-supply would be a limiting factor so that only the outer layer of cortex cells would be capable of active uptake. Then increasing transpiration might cause cortex cells situated more inwardly to come into operation via the intermicellar cell wall channels, in case of absorption (ARISZ 1945).

The results obtained by LUTTKUS and BÖTTICHER are a faint indication as to which of the two explanations is most acceptable. In connection with other observations of exosmosis of salts through the root tissue, this process is further analyzed. They used maize-plants in culture-solutions. By alternately putting the plants for two days in the light and two days in the dark it is possible to get first intake of K and after that giving off of K. In experiments with 4 days' darkness 40 % of the K-content of the whole plant, coming from the shoot, may be given off. It is obvious that in such a process one should think of diffusing away from the root tissue One can of course also imagine K being actively secreted outward as long as the contrary has not been proved.

Data can be found in publications, however, which sometimes give almost direct proof of the permeability of the symplast to various substances. Of the older investigations concerning this problem we mention DE RUFZ DE LAVISON's. He put intact roots in a non-poisonous salt-solution for between 24 hours and several days. After this sections were made and chemically analyzed. The substances sometimes penetrating as far as the central cylinder or even the shoot are: acetates, sulphides and chlorides of Li, Na, Rb, Cs, Sr and Ca. Another group of substances, which does not penetrate further than the endodermis, can exclusively be demonstrated in the cell walls of the cortex. These substances, not occurring in the protoplasm, are: $FeSO_4$, Fe-acetate, $FeCl_2$, $FeCl_3$, Fe-tartrate, Pb-acetate, $NiCl_2$, $CuSO_4$, $HgCl_2$, $HgBr_2$, $CrCl_2$ and uranyl-acetate.

Remarkable in this investigation is the behaviour described of roots in 1/4 or 1/3 n NaCl solutions. In these, plasmolysis occurs very soon, which may have got as far as the central cylinder in

5 or 10 minutes. Since the plasm remains fixed to the cork band during plasmolysis of the endodermis-cells, diffusion has to take place through these protoplasts. Later deplasmolysis appeared to be possible.

A number of dyes too is known to pass the endodermis rather easily. PRIESTLEY and NORTH say that eosin and acid-green can easily penetrate from the vessels as far as the cortex.

The results obtained by RENNER are an indirect indication of the possibility of permeation of salts through the root tissue. RENNER used cut off root systems, in which, either the bleeding is measured under various circumstances with the help of a dacryometer connected with the stump, or the absorption from a potetometer containing varying solutions. When water as the medium of the roots is suddenly replaced by KNO_3, bleedingsap is first sucked back into the stump; this is soon stopped and bleeding begins again. Sudden replacement by H_2O around the roots first causes more bleeding which, too, soon decreases. The following data were written down about sucrose — the numbers indicating the shifting of the meniscus over a number of graduations per minute —:

Exudation in water	+1,5	to +2					
Exudation in 3% sucr.	—4,3	—4,5	—4,3	—4,2	—4,4		
Exudation in water	+2,4	+2,3	+2,2	+2 to +1,5 (10 min.)			
Exudation in 3% sucr.	—5,8	—5,5	—5,1	—4,9	—4,7	—4,3	—4,5
	—4,1	—4,1					
Exudation in water	+3	+2,7	+2,6	+2,4	+2,3	+2,2	+2,1
	+2	+1,8					
Exudation in 3% sucr.	—4,8	—5	—4,7	—4,1	—4 to	—3,3	(10 min.)
Exudation in water	+3,2	+2,5	+2,5	+2,5	+2 to	+1,6	(10 min.)

Especially the fact that replacement by water so quickly results in the bleeding returning to its former level, points to the process being passive. SABININ, too, who got the same results in bleeding experiments, explains this by the permeability of the root tissue to the substances concerned.

Finally PERIS proceeded to use the potetometer-method on account of these data in order to investigate the permeability of the root system in an intact plant. It was found that in the experimental plant, Phaseolus multiflorus, the permeability would always be far greater in giving off than in inward movement. The result obtained by PERIS was that the permeability was greater for K than for NH_4; for NO_3 greater than for Cl, and for Mg greater than for Ca. Permeability was also found for glucose, sucrose and urea. We shall

not consider how far the method justifies the conclusions drawn, but we do believe that the results are essentially correct.

The preceeding results, which at any rate refer to plasm-permeability, conflict with the data, from which it appears that the same salts can often take water from the rootsystem. Such an osmotic process can only occur when there is perfect, or at least a high degree of permeability for the substance concerned. Comparing the results mentioned, we are at once struck by the complete lack of agreement with data obtained in the general permeability investigations. But this need not astonish us since, as ARISZ (1945) explained, different quantities were investigated in these experiments, viz. penetration into the vacuole. Most of the investigations refer to the transmeability of the protoplasm. The fact, however, that e.g. in GELLHORN's work positive results of salt-permeability are several times given, will support us. Though there will chiefly be transmeation here of the substance investigated, that is permeation of plasm + tonoplast till the vacuole, this implies the plasm being permeable too.

Some results obtained with organic substances, e.g. sugars, cause difficulties in the interpretation as it is not always certain that assimilation or consumption of them, is not partly responsible for the results obtained in absorption-experiments.

§ 2. *The results obtained.*

In the first place we shall now give the data obtained by this investigation as regards the behaviour of some electrolytes. In most of the experiments one-salt solutions were used. In a number of experiments the solutions were more or less balanced. After the experiment, further analysis was usually restricted to one species of ion. We thought we were justified in doing so as numerous results indicate that ions can penetrate into the plant more or less independently of each other. This is only limited by one condition, the necessity to maintain the electric equilibrium. Thus a relatively much greater uptake of one of the components of a salt can only be effected by exchange for another ion of equal charge. Our view, however, is that this does not essentially matter to the independent behaviour of the component ions of one salt.

a. Experiments with K.

One of the ions that was extensively investigated was K. Rather a long series of experiments was made under the following conditions. Six pieces of root were put in the smaller apparatus, next a solution of aqua dest. with 1/100—1/10 Mol K per litre as KNO_3

and a trace of uranin as leakage indicator was sucked through the xylem. In all the vessels the surrounding solutions always consisted all the time of aqua dest. Temp. 20° C. Never could a single particle of K be shown in the outer surroundings, with the help of Na-Co-nitrite, in a series of 14 experiments in all with a total number of 74 observations (for more extensive records of single experiments see page 75).

In a series of 7 experiments with 30 observations a concentration of 1/10 Mol KNO_3 was used, but the outer medium had previously been made as anaerobic as possible by letting N_2 pass through it. A reaction on K was only noticed in two observations. Neither did the addition of NaCN 1/250 Mol to the surrounding liquid bring about loss of K.

Since in this experiment a water-current may be expected from the outer surroundings through the tissue to the xylem — for here there are both suction caused by the underpressure and osmotic suction — we endeavoured to reverse the flow of water. In the above mentioned experimental procedure it might be possible that the diffusion would not be able to overcome the flow of water in opposite direction. Therefore in 16 experiments sucrose was added to the surrounding solution in concentrations of 2/10 or 3/10 Mol, usually 1/4 Molar. By far the best result was obtained with sucrose 3/10 Mol. The purpose of adding sucrose to the outer medium was to achieve an osmotical displacement of water in outward direction. In view of the later obtained results concerning the permeability of this substance it remains highly questionable if it brought about this movement to the desired extent.

It actually appeared that K could pretty regularly be shown in a number of the tubes, although the reaction was often extremely weak. With a concentration of 1/10 Mol flowing through a positive reaction resulted in 20 out of 37 observations. When KNO_3 1/20 Mol was used the results were positive in 4 out of 38 observations. Sucrose + NaCN 1/500 Mol caused freeing of K in 1 out of 9 observations.

Thus this leads us to think that the living root tissue, when not influenced, is not at all or very slightly pervious to K.We must, however, communicate the fact that, apart from the series of experiments already mentioned, four other experiments were made with narrower tubes, so that the surrounding solution was about 1 cm^3 instead of 3 $^1/_2$ cm^3. In this case K was shown in 10 out of 18 observations. This might mean that K was in fact given off in the experiments first mentioned, but in such small quantities that the concentrations obtained could not be detected. The sensi-

tivity of the reagent was as follows: 10^{-3} Mol KNO_3 positive reaction, 2.10^{-4} Mol KNO_3 no reaction.

Before we proceed to analyze the results of our experiments we want to stress one essential item. In the experiments just mentioned the conduction of K given in the xylem towards the surrounding liquid was always followed up. This is of course contrary to the normal direction of uptake. The last mentioned process might be acting as a contrary influence. Thus the results may be the combined outcome of both possibilities. The fact that K hardly proves to diffuse through the tissue at all is thus not fully reliable.

Finally we shall give a table in order to have the results conveniently arranged:

TABLE 2

Results obtained in experiments made to ascertain the penetrability of the root tissue for K.

composition of surroundings	capacity of vessel	K-concentration in xylem	positive result K test in medium
aq. dest.	3½ cm^3	1/100—1/10 Mol	0 out of 74
aq. dest.	1 cm^3	1/100—1/50 Mol	10 out of 18
aq. dest. + N_2	3½ cm^3	1/10 Mol	2 out of 30
aq. dest. + NaCN	3½ cm^3	1/10 Mol	0 out of 9
aq. dest. + sucrose	3½ cm^3	1/10 Mol	20 out of 37
aq. dest. + sucrose	3½ cm^3	1/20 Mol	4 out of 38
aq. dest. + sucr. + NaCN	3½ cm^3	1/20 Mol	1 out of 9

A number of experiments was also made in which it was investigated if K could also penetrate from the outer medium into the vessels. Thus a number of experiments was made in which the large and often the small apparatus was used in a horizontal position. All the little roots were immersed in a medium consisting of a v. d. Crone culture-solution in tapwater of 1/2 or 1/4 of the concentration of normal strength, a trace of carmine serving as indicator for the observation of leaks. Common aqua dest. or twice distilled H_2O was sucked through the xylem. Usually the surroundings were also aerated. In 9 out of 16 observations K could be shown in the receiving vessel. The roots were not always in a good condition after the experiment, however.

In order to decide whether we had to do with active absorption or a diffusion-process, the surroundings were in a number of experiments, made as anaerobic as possible by blowing through N_2; 6 out of 19 observations, in which no leak could be assumed, appeared to be positive in their reaction on K.

Finally it must be mentioned that K could be shown in the exudate in a few bleeding-experiments with intact root system immersed in a balanced culture-solution.

Surveying all the data gathered about K we can conclude the following. The results obtained in the experiments concerning outward transfer point clearly to the fact that, under normal conditions, K does not or at least with great difficulty penetrate the symplast in the direction contrary to that of the normal absorption process. It is only when high sugar concentrations are used, which approach plasmolyzing concentrations and consequently do not improve the condition of the tissue, that satisfactory giving-off occurs.

Though the second group of results relating to the inward transfer must be considered to be slightly less reliable, we do get the impression that the same root pieces — the 2 to 7 cm zone after the tip — are fairly well capable of intake of K so that this ion gets into the vessels.

To be able to judge of the nature of this process, we endeavoured to experiment under anaerobic conditions in order to stop the active metabolism here. The anaerobic condition, obtained by letting N_2 flow through, was sufficient to achieve this. Yet these experiments admit of no conclusions, for, as was said before, in a number of the observations K was found in the receiving vessel. Since it appeared in blank experiments that these "low-salt" roots still contain a sufficient quantity of K to lose some amount under anaerobic conditions, this observation is of no importance. For the K found can either have been disengaged from the root tissue or have come in from the surrounding solution through the tissue. Thus it cannot be decided whether the transverse transportation is due to diffusion or active transfer.

b. Experiments with Ca.

A long series of experiments was made in order to investigate the behaviour of Ca. As regards this ion, experiments were only made in which the salt was taken into the xylem-vessels and the penetration to the outside as far as the surroundings was analyzed. Here too, only a qualitative reaction was applied. A saturated solution of NH_4-oxalate served as reagent.

In a series of experiments there was in the xylem-tracts a solution of $Ca(NO_3)_2$ 1/30 Mol in aqua dest., to which had been added traces of Na- or K-fluoresceinate as indicator of leaks. Aqua dest. was put in the vessels as surrounding liquid. The result was that Ca could be shown in 9 out of 27 observations. In order to be certain

that the roots should not lose Ca already present, blank experiments were made, proving this was out of the question, for the issue of all 18 observations was negative. The result is thus quite reliable (see record IV, pag. 76).

This experiment was varied in several ways to get indications in the question as to whether the tissue plays any active part in the transmission. For this NaCN 1/250 Mol was added — sometimes even to both liquids in order to get better penetration into the tissue —, or a saturated urethane solution was taken as medium. The idea was thus to check the respiration, in this manner lessening the activity of the tissue. The concentration of cyanide we used here has proved to effect a distinct inhibiting influence on the uptake of salts and water in the experiments by HOAGLAND and BROYER, MACHLIS and ROSENE. Surveying the results, collected in the following table, we do not see any appreciable effect in a definite direction.

TABLE 3.

Ca-concentration caused to flow through	composition of surroundings	positive reaction	%result
0	aq.dest.	0 out of 18	0
1/30 Mol	aq.dest.	9 out of 27	33
1/30 Mol	aq.dest.+NaCN 1/250 Mol	3 out of 18	16
1/30 Mol+NaCN 1/250 M	aq.dest.+NaCN 1/250 Mol	4 out of 18	22
1/30 Mol+phenylur.	aq.dest.+ur.	6 out of 16	36
1/30 Mol	aq.dest.+sucr. 1/4 Mol	2 out of 11	18
1/30 Mol+phenylur.	aq.dest.+sucr. 1/4 Mol	4 out of 26	15
1/30 Mol+phenylur.	aq.dest.+sucr. 1/4 M+ur.	4 out of 23	18
1/30 Mol+KCl 1/10 Mol	aq.dest.	2 out of 35	6
1/15 Mol+KCl 1/20 Mol	aq.dest.	6 out of 21	28

As in the experiments with K, we endeavoured to effect a simultaneous transmission of water to the outside, through the symplast, with the help of a sucrose solution of 1/4 Mol. If this plan succeeded at all — which is doubtful though, as sucrose proved to be permeable — at any rate no pronounced effect is noticeable.

Since it had to be taken into account that the Ca-ions, added in a rather high concentration, would influence the condition of the tissue, we tried more or less to balance the solution flowing through the xylem by adding the antagonistic K. Since we must not exceed the osmotic value at which this tissue plasmolyses — $CaCl_2$ 125/1000 Mol, sucrose $\pm$ 4/10 Mol — there were no great possibilities. The result obtained from the combination Ca 1/30 Mol and K 1/10 Mol,

only 2 out of 35 observations giving a positive reaction, might indeed serve as indication. On the other hand a result, analogous to those of most other experiments, is obtained from the combination Ca 1/15 Mol and K 1/10 Mol. Thus a specific influence of the Ca is presumably not of great importance in these experiments.

From the data obtained we must arrive at the conclusion that Ca passes the living root tissue rather easily. The fact that respiratory poisons do not greatly change the results makes us conclude that we have to do with transportation based on passive diffusion.

One might argue against these results that fully positive reactions were nowhere obtained and that the variety of issues is rather great. This may be understood when one considers the difficulties met with when trying to get a sufficient current through each root, as the maintenance of the diffusion-gradient is dependent upon a good regular flow. Since this ideal was never as yet realized, in a great number of experiments the required conditions were not achieved and so their outcome is of no value. It was sometimes observed that there could be sufficient positive correlation between the quantity of Ca-solution sucked through and the strength of the reaction on Ca in the surroundings, which confirms the explanation.

Two data obtained with $Ca(NO_3)_2$ 1/30 Mol will be mentioned now as an observation, which is a confirmation of our assumption of independent behaviour of the ions. Both Ca and nitrate were involved in the analysis. Under normal aerobic conditions there was good Ca-permeation, but NO_3 could not be shown in the outer solution. When, however, phenylurethane was added both to the internally passing $Ca(NO_3)_2$-solution and to the surroundings, it appeared that both ions came out simultaneously.

c. Experiments with the anions, NO_3, Cl and H_2PO_4.

The behaviour of the anions NO_3, Cl and H_2PO_4 was investigated far less in details; the results obtained will be discussed in this order.

In the experiments with NO_3 this was always added to the outer solution and then it was studied whether the substance concerned could penetrate as far as the vessels. A great number of the experiments was made with the second large apparatus. In conformity with a few data obtained beforehand from preliminary experiments with a more primitive apparatus, this ion appeared to penetrate well and could then be shown in the liquid flowed through.

The experiments were mostly made as follows. Either common aqua dest. or distilled tap-water served as liquid caused to flow

through, while in a number of experiments tap-water was used to eliminate an eventual poisonous influence. The surroundings were nearly always a v. d. Crone nutrient-solution, usually made with tap-water. Its concentration often varied from 1/2 to 3/2 times the value indicated, the concentration of half the prescribed one being used most frequently. The surroundings contained indigo-carmine as leak-indicator, very rarely another dye, e.g. rhodamin-B. The O_2-supply of both liquids often varied. Now the surroundings contained no O_2, next the liquid caused to flow through was made anaerobic by passing N_2 through. In some experiments both solutions were made as anaerobic as possible. Since the various combinations do not give any appreciably different issues, we shall only mention that in over 60 % of the data NO_3 was shown in the circulating liquid with the help of diphenylamine-H_2SO_4. It appeared in the blank-experiments that the tissue never gave off NO_3 already present to the passing liquid.

So here we see NO_3-uptake under conditions, that must be considered as physiological. In bleeding-sap too NO_3 could be shown. Nothing can be said about the nature of the process, since it is not sufficient to make the two solutions anaerobic, as NO_3-reduction might maintain the metabolism concerned.

On the other hand quite different results were obtained with Cl. Again this ion was practically always added to the surroundings and the penetration was studied as far as the vessels. Apart from a few exceptions, a balanced solution was always used: sometimes a dilute Brenner-solution, usually a mixture of a van der Crone-solution at 1/2 of the normal strength in combination with a Brenner physiological salt-solution diluted to 1/20. The oxygen-supply of both liquids varied, but several times the outer solution was aerated and the inner fluid freed of oxygen. In this way we tried to get an O_2-potential gradient in conformity with CRAFTS and BROYER's theory.

In a few bleeding-experiments in corresponding solutions it appeared that the normal root tissue was capable of absorption of Cl. It appeared that this ion could very well be shown in the discharged exudate.

Penetration as far as the xylem could not be shown in the experiments with the isolated root pieces. This need not mean that Cl does not penetrate at all, for if the concentration in the circulating liquid should be too low, the reaction with the help of $AgNO_3$ would also be negative. At any rate we must conclude that Cl gets

through the symplast with difficulty. This result gains in importance when compared with the data obtained with NO_3.

In the experiments in which the behaviour of H_2PO_4 was studied, quantitative results were obtained. The phosphate was determined colorimetrically both in the liquid flowed through and in the surroundings, and occasionally the root tissue was analyzed after destruction.

When we survey the whole series of results, it appears that H_2PO_4 can easily penetrate from the surrounding liquid into the vessels. Giving-off experiments have not been made with this substance. A complete Hoagland culture-solution served as medium, so that micro-elements also occurred in it. Usually common aqua dest. served as circulating liquid. The experiments were made in the small apparatus standing vertically, and the salt-solution was given in the six glass tubes with a cubic capacity of $\pm 3^1/_2$ cm^3. Again indigo-carmine served as indicator, for detecting possible leaks.

In the experiments made under normal aerobic conditions H_2PO_4 could be observed in the received liquid in 12 out of 16 observations. The amounts taken in by each root went as high as 17 γ. Not much can be said as yet about the nature of the process, though occasional observations indicate that the tissue must have an active part to play, for in two observations the phosphate concentrations in the liquid after circulation had become $\pm 1^1/_4$ and $\pm 1^1/_2$ times that of the nutrient-solution outside the roots. Thus accumulation must have taken place. In general the concentration in the surrounding salt-solution was not attained (see record V, pag. 77).

In some cases about the same amount of phosphate could be detected in the liquid flowed through, as had disappeared from the salt-solution in the glass tubes. In these experiments we were sure no leakage had occurred, because the total quantity of liquid that had passed the xylem corresponded very well with the amount which had disappeared from the supply-chamber.

It was however more usual that a greater loss of phosphate in the medium occurred than we could detect in all the receiving vessels together. This can be explained by accumulation in the tissue, as could be verified by direct analysis of the root pieces used. This fact at the same time forms an indication of the normal physiological condition of the tissue during the experiment.

Experiments in which NaCN 1/250 Mol was added did not give any results that might be used for further interpretation of the process. Some phosphate was given off to the xylem, in fact, but in the surroundings, too, increase could be determined. Thus

here the tissue loses H_2PO_4 present beforehand under conditions, in which respiration must have been partly hindered.

Surveying all this, we see that it has been shown that H_2PO_4-ions penetrate very well from the surroundings into the vessels. There are indications here that the symplast may be active, but one can also imagine that the tissue meanwhile remains permeable to these ions.

d. Experiments with urea.

Besides the data mentioned already and obtained from experiments with inorganic substances, something must be said about results achieved with some organic substances. Although the choice was rather arbitrary, we were more or less guided by the idea of investigating substances, which are supposed to permeate well or to have very predominant active transportation. We also used as a basis experience obtained in other investigations at the Groningen Laboratory.

We shall first and foremost deal with the results obtained with urea. In these experiments the small apparatus was used under the usual conditions for the experiments. A 2/10 Mol urea-solution in tap-water + a trace of fluoresceine was caused to flow through small pieces of root. The glass tubes contained tap-water. First some experiments were made in which reactions to possibly exosmosing urea took place with the help of paradimethyl-amidobenzaldehyd. When it appeared that urea actually passed the tissue the quantitative determinations were started.

To begin with, some blank experiments were made in which pure water passed through the xylem. It appeared that some kind of N-containing substance diffused out of the root, giving rise to a slight reaction with Nessler's reagent. The colouring equalled that given by 10—17 γ of urea and for this reason values obtained in other experiments lower than 20 γ were not taken into account.

The solutions contained in three glass tubes were tested for urea (urease + Nessler's reagent). To the other three surrounding solutions Nessler's reagent was added without previously administering Arlco urease. In this manner we should have been able to detect a stronger exosmosis of nitrogenous substances on account of a possible influence of urea on the tissue. The possibility of urea partly being converted into ammonium by the metabolism of the root cells also existed. These latter determinations are the direct ones.

The quantities of urea found in the surroundings were: 40, 40, 115, 110, 125, 72 and 160 γ. The other series of direct determinations

gave values of 25, 10, 10 and 17 γ taken as urea. When the latter numbers are deducted, in which the blank value is also included, 20—140 γ of urea must have passed the living tissue. By calculation we find, since the quantity which disappeared from the supply-chamber is known, that in some experiments presumably 7 to 10 % of urea led into the vessels has come to the outer medium unchanged. Finally, it is important that in one of the experiments there are fair indications of simultaneous intake of K from the outer solution. In this case the surroundings consisted of KCl 1/100 Mol. This points to a good physiological condition of the tissue, though it is not decisive. By way of an illustration we insert one of the protocols with some supplementary data (VIII and IX, pag. 79).

On the ground of the data obtained we think the conclusion for urea is justified, viz. that this substance can very easily pass the symplasm. Although there are no experiments in which KCN or urethane was added, it seems to us — on the ground of the direction in which transfer takes place here — that this can with a great deal of certainty be looked upon as diffusion. In this case the root tissue as a whole would be extremely permeable to urea.

e. Experiments with sugars.

Another substance, with which experiments were made, was glucose. Its behaviour, however, was less closely studied so that the interpretation of the data will be a bit uncertain.

In all the experiments glucose was added to the surroundings and it was studied whether, after some time it appeared in the vessels. The very first experiments in pure glucose-solutions of e.g. 15/100 Mol gave indications as to possible presence of glucose in the liquid flowing through. Glucose was reacted upon with the help of Fehling's solution, after a series of blank experiments had shown that giving off of reducing substancs from the tissue hardly ever occurred (also see sucrose experiments).

In order to have more physiological surroundings a v. d. Crone salt-solution of varying concentration was used in later experiments, to which glucose of a concentration of 1/35 to 1/10 Mol had been added. In about 50 % of the analyses the recieved liquid gave a reduction, which was usually slight. We think that from this the conclusion may be drawn that glucose can penetrate. It cannot be decided, whether the symplast is permeable or the living tissue actively transports this substance.

For the sake of comparison a bleeding-experiment was also made with a complete root system, and here too sugar could be shown

in the discharged liquid, with the help of the reaction with α-naphtol and H_2SO_4.

Finally rather numerous experiments with sucrose were made. For these the small apparatus was used and the sugar was added to the liquid caused to flow through, to all intents and purposes always of a concentration of 2/10 Mol. Uranin was always added so that leaks could be discovered. After the experiment, which used to last $\pm$ 18 hours, a sample of the liquid in the surrounding tubes was qualitatively tested for sugar. After inversion with H_2SO_4 the quantity of inverted sugar was fixed according to Hagedorn-Jensen's micro-method.

Twelve blank observations, during which there was tap-water both in and around the root pieces, gave reduction values in accordance with 0 to 12 γ sucrose in the outer medium. In the experiments in which sucrose 2/10 Mol had been added to the tap-water passing the vessels, far higher reduction-values were found in the surroundings. Out of 28 observations 15 reacted positively and the reduction-values were in accordance with amounts of 7->640 γ sucrose (vide table 4, pag. 42). The damage done to the pieces of tissue was slight. Besides, in occasional experiments a rather good correlation pertained between the exosmosed quantity of sugar and the quantity of liquid sucked through, i.e. the supply.

A few experiments in which the 2 cm^3 samples of liquid were analyzed without previous inversion also gave some reduction, viz. a little more than the blank experiments. This might indicate that part of the sucrose at least is converted into monoses during the passing of the symplast. (Vide table 4, pag. 42).

But surveying all the data, we arrive at the conclusion that under normal aerobic conditions, sucrose is quite capable of penetrating to the outside through the living root tissue.

To learn more about the nature of this transportation we made experiments in which aqua dest. + sucrose 2/10 Mol + NaCN 1/250 Mol was caused to flow through. The surroundings were a 1/250 Mol NaCN solution in aqua dest. In general the roots suffered some more damage and would sometimes have a gray colour after the experiment. On an average the obtained reduction-values were about the same. Sometimes good correlation with the supply would also occur here.

The experiments made by adding NaCN to both solutions, do not give a fully correct idea of the ability of sucrose to permeate, for in experiments where only NaCN 1/250 Mol was added and no

sucrose, it was found that on account of the influence of this substance, reducing substances were given off by the tissues (vide table 4). Also direct analyses, without previous inversion of the outer liquid in experiments with sucrose, gave rather high values.

Average value of all observations:

TABLE 4

Experimental results obtained in investigating the outward penetration of sucrose.

tap-water (blank)	4γ taken as glucose
tap-water + sucrose (analyzed without inversion)	15γ taken as glucose
tap-water + sucrose (analyzed after inversion)	94γ taken as glucose
aq.dest. + NaCN (blank)	57γ taken as glucose
aq.dest. + NaCN + sucrose (analyzed without inversion)	63γ taken as glucose
aq.dest. + NaCN + sucrose (analyzed after inversion)	90γ taken as glucose

Considering all this we must yet arrive at the conclusion that sucrose is capable of passing the root tissue rather easily and that this is most probably due to permeation. Active participation of the tissue in the giving-off process will at most have been of very secondary importance.

§ 3. *Discussion of the results.*

In some recent text-books on general physiology it is stated that the cells can be considered as permeable to salts. In the book edited by KONINGSBERGER (1942) we see that BUNGENBERG DE JONG considers both anions and cations as usually being able to penetrate through the protoplasm. MILLER (1938) in his book says that plant-cells of the root, capable of intake, are permeable to most salts. On the other side, however, the conception of active uptake and accumulation has come to the fore in later years, and quite a number of investigations deal only with this aspect of the problem. A consequence of this is, that the latter view makes us assume a high degree of impermeability to the ions concerned, without considering where this impermeability is located, e.g. COLLANDER (1942). The fact that there is no reason for excluding a permeability of the plasm is sometimes overlooked. In a recent publication ARISZ (1945) manages to solve the controversy that may be perceived here. He makes a clear distinction between the conception of *transmeation*

and that of *permeation*. In transmeation passage of the tonoplast is involved. So the tonoplast may be impermeable and yet salts may enter the protoplasm by passive diffusion from out of the medium.

Most of the older investigations only refer to transmeability as ARISZ has pointed out. As transmeability implies a passage of the protoplasm, we may always refer to these older data as a confirmation of our own results, in which we are sure that we have demonstrated at least plasm-permeability. The only question remaining is, whether these older data were always obtained in conditions, which are to be considered physiological, as high unbalanced salt-solutions often were used.

It is first and foremost the experiments by DE RUFZ DE LAVISON discussed before, that make it probable that salts at least in strong concentrations should be able to penetrate passively into the root tissue. Besides this e.g. ECKERSON, who specially investigated the influence of temperature, found permeability to several salts. In this case Phaseolus multiflorus served among others as an experimental plant, of which the roots are used. The results obtained by PERIS also definitely point to permeability of the symplast.

As regards exosmosis, too, several data were found which all point to permeability to the substance concerned. STILES and SKELDING mention exosmosis occurring in carrot tissue. ACHROMEIKO's work on root excretion emphasizes the freeing of P_2O_5. Older investigators thought they had shown more substances in root excretions, among others Ca, K, SO_4, Cl. Although not much can be said about the nature of the process here, usually passive processes are thought of, so plasmpermeability must be presupposed. Quite occasionally transmeability might have to be assumed when the substances appear to come from the vacuoles.

The general conclusion we can draw from our own experiments is that the root tissue, i.e. the protoplasm of the root cells is permeable to quite a number of substances. This means that these can pass through the plasm by means of diffusion phenomena.

These results fit in completely with everything mentioned before in connection with the permeability to salts and indirect indications of this. We also think that this conclusion, i.e. the root being easily permeable to most of the important nutritional ions, will probably be operative to a large extent. We refer to a following chapter for the discussion of the possibilities and difficulties occurring, in order to make these results fit in with what we know about absorption and transportation of matter. But we shall first pass under review the results obtained, and give some comments on these.

The conclusion that many substances can permeate well is demonstrated by the fact, that they can pass outward through the living root, when given into the circulating solution in the xylem. The results obtained with K, however, are a deviation from the general fact, for it appeared that this ion could only penetrate through the symplasm to the outside with great difficulty, if at all. It must be explained why Ca is able to diffuse out and why K, which usually permeates more easily, is not. This is especially so, since LUTTKUS and BÖTTICHER did observe exosmosis of K in a similar object.

In connection with what is known we think it must be assumed, that K really has the power of diffusion through the living root tissue. The fact that this was not determined at all in the experiments must be due to counteracting influences. In connection with this we must draw attention to the fact that our material has been cultivated in tap-water, and would consequently have a relatively low salt status. Since it is known that K can very well be bound by the organism, it would not be at all amazing if the supply from the vessels in our experiments were not sufficient to give a surplus which can exosmose.

In experiments by PULVER and VERZAR with yeast, it is very clearly shown that the great capability of K to be bound can be very well associated with good plasm-permeability. K penetrates into the cells or disappears from them just as quickly, dependent upon the fermentation-metabolism.

It is due to the nature of the material that we do find permeability to Ca in our experiments, for the root cultivated in tap-water will have contained a fair quantity of Ca, whereas it must be exceedingly poor in K. To this must be added that there usually is only slight affinity to Ca. So it will have been easy to supply a surplus of Ca to cause diffusion as far as the outer surroundings, in spite of the slight mobility of this ion. Some lesser data in our experiments (cf. pag. 32) do show that K actually penetrates, though K-accumulation prevails in most experiments.

The relationship of the behaviour of K and Ca-ions normally to be expected can be illustrated by the following example. WEIXL-HOFMANN working with epidermal cells of the stem of Lamium determined permeability to KCl. Put into 5/10 Mol KCl, plasmolysis occurred, followed after some time by deplasmolysis. It appeared that this was not caused by anatonosis for the fact that, when the cell was put into an isotonic Ca-solution, plasmolysis occurred once more. This is only possible when K exosmoses again and Ca penetrates hardly at all or very slowly in the vacuole. These

results tally completely with the theoretic expectations in connection with the velocity of diffusion of the two ions.

There is also agreement between the explanation — based on difference of affinity — and some results of the influence of transpiration on the uptake of salts. Here good correlation is found for Ca. The active K-absorption is so predominant that the intake cannot be directly accelerated. BROYER and HOAGLAND also conclude that, especially in low-salt roots, the polar accumulation process dominates absolutely in the process of intake of salts.

It fits in with this train of thought that LUTTKUS and BÖTTICHER do find giving off of K in the dark. Results of HOAGLAND and DAVIS with Nitella indicate that green organisms have a greater power of absorption of matter in the light. Once the tissue is fairly well saturated, a decrease of "power of absorption" must necessarily result in the diffusing away of the ions now freed. This decreased "power of absorption" was caused by the darkness.

Having considered all this we think we are justified in drawing the following conclusions: K-ions as well as Ca-ions can diffuse through the living root tissue. This tissue is easily permeable to these ions. In the experiments this is less well shown with K on account of the great affinity of the tissue to this ion. The fact, that the diffusion is opposite to the normal direction of transfer of solutes in the plant, must not be lost sight of in our considerations.

The results obtained with NO_3, where absorption was investigated exclusively without further analysis of the process, do not need a long discussion. Several times permeation, usually transmeation, of NO_3 was mentioned in publications, among others by FITTING, TRÖNDLE with root cells of Lupinus albus, KAHO (1921), etc.

On the other hand no penetration of Cl into the xylem could be shown in the experiments where a liquid was caused to flow through. In bleeding-experiments of the whole root system this proved to be in the exudation, however.

The experiments with NO_3 and Cl do not admit of very well outlined conclusions. The only thing that can be said with any great certainty is, that NO_3 can penetrate into the xylem better than Cl. If this penetration should partly be due to diffusion, these results would agree with the sequence of the two ions in observed transmeation-series (GELLHORN) and permeation-series (PERIS). It is not by any means certain that there is a diffusion-process here, though we endeavoured to work in anaerobic circumstances. Probably there was an active absorption-process in the bleeding-experiments.

In spite of the lack of good evidence in our experiments we think, that the two ions can more or less quickly diffuse through cytoplasm and plasmalemma in physiological conditions.

The experiments with H_2PO_4 deviate slightly in this connection. The results point emphatically to the great predominance of the active accumulationprocess. This is also concluded by ARISZ (II 1942) after experimenting with Drosera.

As to the organic substances, urea was found to penetrate through the living root tissue to the outside quite easily. Probably there is no accumulation at all here. Such a result would conform very well with the general data on permeability and transmeability, for urea belongs to a group of substances that showed the power of good diffusion through all sorts of protoplasts. This is again confirmed by investigations by ARISZ (1942) with Drosera. In the latter results it is striking, however, that absence of O_2 strongly influences the transport. Though one might also think here of indirect activity via a change in the properties of the protoplasm, data indicate that permeability and possibilities of active transportation may occur simultaneously here. It is a pity it cannot be found out if this also holds good for our object, on account of the lack of experiments with blocked respiration.

The results obtained with sucrose and glucose are far more important. Although the results achieved with glucose do not by any means admit of a definite conclusion, we can distinctly observe a diffusion-process with sucrose. It may in many cases be assumed that sugar is simultaneously involved in active processes. In connection with the results obtained for sucrose, we consider the same conclusion for glucose to be justified.

This is partly a surprising result, viz. that the two substances are able to penetrate passively through the living tissue, during which at any rate the protoplasm must be passed. Sucrose especially is counted among the good plasmolyzing solutes, which is due to its very weak power of penetration into the vacuole. Several investigators pointed to the difficulty of explaining transportation of sugar when the permeability is so slight. But since this very weak power of diffusion refers to transmeability in many experiments, this is not really an objection (WEEVERS). Slight transmeability may very well attend good plasm-permeability. Besides this, the active processes can also play a part in the transportation of sugar over a somewhat greater distance in parenchymatic tissue, and these processes need not have any connection with permeability. Thus

the behaviour of sucrose is much more comprehensible than the two forementioned apparently conflicting aspects.

It is striking, that so specific an absorption-tissue as that of the young root, appears to be permeable to these substances. Yet fair transmeability to sucrose has been determined several times (vide GELLHORN). Besides, PERIS too, arrived at last at the conclusion that the root system of Phaseolus multiflorus is permeable to this substance, in rather a high degree. It is a pity, however, that these results were obtained rather indirectly. Though the interpretation may not always be quite correct — e.g. a possible influence of the substances investigated upon water-permeability was not considered at all — we believe that the conclusions essentially agree with the truth.

HÖFLER's experiments with diatoms prove that great transmeability can occasionally exist indeed with sugars. We see that in order to avoid a leaking away, it is converted into fat for the storage as reserve-food. In many other experiments on sugar-metabolism, e.g. with yeast, kidneys, a strong plasm-permeability of these substances has long been known.

Our own results being quite clear and obtained directly, they will have to be considered the more, as some other investigations had already led to the same conclusion with similar objects. Thus one should be very careful in the use of sugars on roots, as a medium to withdraw water, and then found one's conclusions on this, as BREWIG did (vide WIERSUM).

The fact that some monose was often found in the surroundings in sucrose experiments need not astonish us, as it has been known since BURSTRÖM's investigations that inversion can be brought about by the root surface.

Surveying all this, we cannot but state that this investigation has made it very probable, and has in some cases even clearly shown, that the young root tissue is either very well or at least satisfactorily permeable to several substances. Though these results may at first sight be somewhat unexpected, considering the views of intake of matter at present demanding most attention, we must accept them: the more so as the conclusions drawn by no means clash with all the other results in the domain of permeability-investigations, and as they make it possible to explain many phenomena satisfactorily. The remaining difficulty, however, is to use these facts in order to get a good explanation of the process in which the plant takes in dissolved substances.

While preparing this paper for printing we have been able to get an insight into some of the recent Anglo-American reports of investigators in this field, though not without difficulty, as many of them are not yet available. In giving a short review of this literature we conceive that our conclusions are, to a great extent, in accordance with views expressed by many of these investigators.

Results achieved by MAZIA stress the displacement of ions in the protoplasm by others, which are given in the surrounding medium. Nevertheless only a certain part of the cations is susceptible to interchange. It is thought that the fraction subject to interchange occurs in the surface of the protoplasm. The data given, from which it appears that K-citrate is able to remove nearly all of the Ca out of yeast and Arbacia eggs, while Elodea cells retain a much larger percentage of their Ca-ions, might allow a somewhat different explanation (c.f. ARISZ 1945). The vacuole is of far more importance in Elodea than in yeast and Arbacia, so we might just as well suggest that the fraction not removable is contained in the cell-sap. This would imply removement of ions inside the protoplasm and this cannot be achieved without permeation of particles concerned in the process.

DEAN (Symposia, pag. 202) says it is unwise to assume impermeability of cell or tissue for salts, only because no loss occurs against aqua dest., while ion-interchange indeed exists. LEVITT (Symposia, pag. 202) calls attention to the fact that exosmosis experiments have indeed given positive results some times. HOAGLAND (1940) also states, that during anaerobic conditions he has found a loss of salt. He also suggests that some metabolic activity is necessary to retain salt in the cell. This loss can only be thought of as occurring by means of diffusion. Also it is stated that transpiration might effect a passive transfer of salts to the shoot especially through damaged or metabolic inactive roots.

Most authors in the field of research on permeability come to accept the possibility of movement according to given gradients. There does not exist unanimous agreement as to whether ions can penetrate as such or as undissociated molecules. COLE considers only a small fraction of the membrane ion-permeable. BLINKS also interpretes his results of electrical measurement as that the separate ions carry the current through the protoplasm. OSTERHOUT is able to show that even KCl can diffuse across a guaiacol layer without entering into chemical combination with it. STEINBACH, in investigating muscle-cells, comes to the conclusion that K can penetrate almost as fast as it would move by diffusion in pure water. That the penetration into and through the protoplasm does not necessarily

imply a free diffusion in a liquid phase, but may be brought about by phenomena of surface migration along protoplasmatic macromolecules, is brought forward by BROOKS. He also states that the amount of absorbed ions by "induced accumulation" is far too great to be contained in the cell surface only.

DEAN's remark (Symposia, pag. 266) in the discussion of the paper agrees very closely with our own concept. He states that "the cell really has the ability to keep its concentration of potassium in spite of the slow leak". This leakage must involve a passive permeation according to our ideas.

Thus we see that these authors all conceive of the protoplasm as being permeable, although this is not always extended to the tonoplast. Besides this they are also in full agreement on the part of active metabolic processes in accumulation.

CHAPTER V

POLARITY OF THE ROOT TISSUE.

The normal function of the root system is absorption of water and salts. In the process the transfer is directed inwardly across the living tissue from surroundings to xylem-tracts, as it is chiefly this system of vessels that serves for further conduction to the shoot. Since the movement of water can pretty certainly be fully explained by suction caused by transpiration and osmotical forces we shall only consider the transportation of salts.

The transmission of salts is in nearly all cases directed inwardly. The transfer is also brought about by active processes, leading up to a secretion of ions into the xylem. Both facts lead us to the assumption of a polarity of the symplasm in the young root. We are induced to assume this the more, as many data concerning the transport of growth-substances have been published. Here we have become familiar with the idea of tissues that show polar behaviour and in which substances are transported in one direction only.

For a long time it seemed as if the same conditions availed in the root. Yet data have become known that make us doubt, whether the root tissue has an identical polarity for salts as e.g. an Avena-coleoptile with regard to auxin.

In these data it has been stated that giving off of substances from the roots, especially of salts, can occur. Further discussion of this is found in the publications mentioned before, among others

by LUTTKUS and BÖTTICHER and also ACHROMEIKO. In some ways comparable to these facts, are the results obtained in recent work on the intake of minerals by living cells. Many researches have now made us realize that absorbed ions can be replaced by others, by means of interchange processes. This interchange takes place between one species of ion as well as between more or less similar ions, for example Cl for Br. In some researches a more or less state of equilibrium between the interchanging particles can be attained, which dynamic equilibrium can only be accounted for by considering a free movement of the ions. This is indeed the view taken by the investigators in this field (BROOKS, STEINBACH, OVERSTREET and BROYER, HOAGLAND, KENT).

We should also like to mention the fact, that researches in the permeability of living cells, which all possess the capacity for accumulation of specific ions, have led to the insight that the outer plasma-membrane must be permeable (COLE, BLINKS, OSTERHOUT).

All these facts are indeed contradictory to the idea of an absolute polarity of the root tissue, since the course of diffusion — even surface-migration processes — is solely regulated by concentration- or activation-gradients.

Further experiments as to the extent of the possibility of diffusion phenomena can contribute to a better insight.

Some explanatory hypotheses have been given for the observed polar behaviour of the root. Thus PRIESTLEY wants to effect secretion of salts by the assumption of unequal permeability of the symplasm. The outer cortical-cells would absorb salts, while the stele cells lose them on account of their high degree of permeability. All this is based on PFEFFER's first scheme. PRIESTLEY and ARMSTEAD think they have shown this greater permeability by having 1/10 % dye solution absorbed by Vicia Faba roots and then studying the colouring of the cells in the stele.

WEIS has shown by experiments that PFEFFER's first scheme can actually lead to secretion. For this long internodal cells of Nitella were half put in water. The upper half was in an atmosphere with alcohol vapour. By this reaction a certain secretion was obtained, caused by the increased permeability.

Another explanatory hypothesis has been given by CRAFTS and BROYER. These investigators assume that the corticalcells actively take in salts and can maintain a high concentration owing to good O_2-supply. They evidently assume, though this is not clearly indicated, that there is a tendency to level the concentrations inside the symplast. The xylem-parenchyma would not be able to maintain the high salt concentration, owing to less O_2-supply in the cells

of the central cylinder and to a greater CO_2-concentration: the surplus oozes away into the xylem. So transportation in one general direction is brought about in this way.

This theory, in which transport is brought about by a gradient of the O_2-potential, can essentially be tested by experiments. We intended, among others, to make experiments for this purpose with the help of the designed "flowing-method". For the time being this appeared to be impossible, however, since the quantity of liquid caused to flow through was as yet too small to effect the required O_2-supply to the stele. The method can be used for this purpose, however, when the suitable materials are available.

LUNDEGÅRDH thinks he can explain the general direction for anions especially by a difference of redox-potential between cortex and stele as a consequence of a difference of tension for O_2. This would cause accumulation of salts in the xylem-vessels by active transportation processes.

Finally, there is the technical possibility of accumulation of the ions in the xylem under the influence of an electric difference of potential. Then the whole symplast acts as a membrane. Referring to LUNDEGÅRDH's investigations one could also assume the existence of a potential-gradient required for this process. LUNDEGÅRDH (1940, 2) conducted many experiments which demonstrated that the root was negative in regard to the surrounding solution. The potential inside the root tissue is measured by placing the cut and the top piece of an excised root in a suitable solution connected with an electrode. The root tip is placed in the surrounding solution. Experiments, however, must afford a sound basis for the conception of salt transport through the symplasm by means of a potential gradient.

The hypothesis mentioned last has another great drawback, viz. that according to the nature of the electric potential-difference, exclusive transportation of one of the two categories of ions would be possible. The difficulty of calling in the help of electric potential-differences found by experiments in order to explain polar transport, appeared very clearly in the analysis of the growth hormones. Thus HELLINGA in his experiments, comes to the conclusion that no direct connection can exist between potential-gradient and transportation of auxin in Coleus cuttings. Growth substances were always transported to the morphologically basal end, independently of the way a longitudinal potential difference, brought about experimentally, was directed. Also two reverse electric currents flowing through the cuttings had no influence. There is thus no kataphoresis of the growth substances.

Considering our own experiments we must first mention that most of our transportation experiments were made in a direction opposite to the normal one. Usually the substance to be investigated was brought into the xylem, and the transportation to the surroundings was analyzed. This had an advantage, viz. that generally speaking, more conclusions could be drawn about the nature of the process.

The result obtained was that to all intents and purposes all the substances investigated could pass the tissue. But from this we concluded that this is passive transportation, i.e. diffusion according to concentration-gradient or activation-gradient.

It appeared, however, that especially with the K-ion this movement was largely counteracted by the activity of the tissue.

With regard to the polarity of root tissue we consider the following conclusion justifiable. The first point we wish to stress is that two quite separate possibilities for transport through the symplasm can be distinguished.

One of these processes is of an essentially passive nature. This means that the movement of the particles in solution is only guided by either activity- or concentration-gradients. As far as this passive process is concerned no real polarity exists and the transfer of particles is not under full control of the living organism. Many facts, revealed in the papers in the Cold Spring Harbor Symposia on Quantitative Biology vol. VIII, are in accordance with this idea and these authors also consider the protoplasm as permeable.

As far as the other process is concerned, this is a transfer of solutes under full control of the living cell and taking place in one direction only. This polar transport bringing about accumulation in plasm, cell-sap and xylem-sap, only takes place by expenditure of energy derived from aerobic metabolism of the cell. It is strictly one-sided and cannot be changed by simply altering the composition of the medium outside the root. As to this active process we can only consider the root tissue as a strictly polar symplasm. This idea is in full accordance with views stressed by quite a number of investigators (a.o. HOAGLAND et al., ARISZ (1945), LUNDEGÅRDH).

The conclusion to which we are thus led — polarity of active absorption, absence of a general direction of the diffusion-processes — demands further consideration with the help of some data from publications.

Thus we find in an investigation by WENT that Avena-coleoptiles are extremely polar with regard to auxin. There appeared to be no polarity, however, for salts that were simultaneously investigated,

and these salts passed short coleoptile-cylinders with equally great ease in either direction.

In experiments made by KOK it is clearly shown that, in a very specific absorbing organ, which has clearly marked polarity for many substances, this polarity need not apply to other permeable solutes. KOK stated in Drosera-tentacles that caffeine was transported equally quickly both acropetally and basipetally. The transportation is also based on diffusion. Here polar transportation applies only to substances that are subject to the process of active absorption.

Therefore we shall go a bit further as regards the transportation of salts through the root tissue. We now assume for one and the same substance that it can both diffuse freely and be subject to the active absorption process, which has a polar nature.

CHAPTER VI

MECHANISM OF THE TRANSPORTATION PROCESS.

§ 1. *Introduction.*

In concluding this paper we intend to summarize and point out again a few things connected with the whole process of intake and transfer. Without entering into all details, we shall bring some points to the fore, especially in connection with results obtained in this investigation.

The main facts which we have to consider in discussing the function of the root as an absorbing organ, are the following:

a. the root system has proved to be permeable to quite a number of substances.

b. many investigations have now clearly demonstrated that the living tissue is capable of accumulating substances and of keeping up a strictly unilateral transportation-process under aerobic conditions.

c. the possibility of discriminating between different ions.

Next to these three main points we might also take into consideration the velocity of the process of uptake of solutes. Literature provides us with ample data.

BIDDULPH, who made his investigations with the help of radioactive phosphorus, thus showed a high transmission-velocity. Thus it appeared that after 1 hour absorption, P could only be shown in the root system, after 2 hours already in the hypocotyl. Now it is quite well possible that in these two observations we have to

do first and foremost with mutual interchange-processes of the two P-isotopes. But all the same these data demonstrate that ions can cover a certain distance in the plant in a certain time, and this power of moving is part of the normal transportation-process. Finally after 4 hours radio-active P could be shown everywhere in the bean-plant. Since transportation via the xylem has taken place, interchange cannot have been the only factor, and the normal transportation process must also have been operating. Transportation was even faster in CRAFTS and BROYER's experiments, where Br could be shown in the exudate of a cut-off root system after half an hour.

It is precisely this last point that demonstrates the inadequacy of an older hypothesis as proposed by SACHS (1863), who looks upon the transport through living cells as a diffusion-process regulated by consumption and formation of the substance concerned. A normal diffusion-process would be far too slow to get a measurable quantity of the solute into the xylem in such a short time. We must not overlook the resistance in the cortical-, endodermis- and pericycle-cells, especially in the protoplasm.

Considering the fact that diffusion in solution is not sufficient to explain normal transfer over somewhat longer distance, we see new hypotheses being created. As early as 1885 DE VRIES advances the idea that acceleration of the conduction must occur. This would be effected by the streaming of the protoplasm, which implies that the protoplasm must be the route of transportation.

However at that time, general attention as regards this process was not yet drawn to the activity of the protoplasm. It was far more frequently assumed that the salts entered passively and that the transpiration-current would facilitate this. In this connection it is important that PFEFFER as early as 1876 considered transportation through the cell wall to be possible.

The fact that most transmeation experiments have revealed a low degree of permeability, did not make it likely that salts would pass the root passively, and it was often stated that in balanced media the possibility of penetration would be even less. Since HÖFLER managed to obtain deplasmolysis of green algae placed in twice concentrated sea water, and BIEBL refers to similar results of salts permeating in balanced solution of a species of brown alga, there is a possibility of maintaining the forementioned view. Nowadays indeed the permeability of root cells is taken into account.

STILES and SKELDING definitely consider the cells of the root tissue of carrot permeable to ions. They have been able to detect exosmosis of some ions, which process can hardly be interpreted on any other

basis. Also their conception of ion-exchange, occurring during uptake, implies a free movement of the particles concerned. KENT also in discussing his results obtained on uptake and translocation of Lithium in wheat regards displacement of this ion regulated by the existing gradient. As Li can also migrate back into the medium this process must be a kind of diffusion, thus inferring permeable cells. HOAGLAND and BROYER (1942) mention a small efflux of K under anaerobic conditions. The decreased uptake of water and Br in experiments with tomato roots, subjected to suction at the place where the shoot had been cut away, when the medium was deprived of O_2 or supplied with CO_2, is accounted for as being a result of decreased permeability to both substances. BIDDULPH and MARKLE conducted experiments on translocation in the phloem. This movement is to a great extent directed by existing gradients. They also remark that phosphorus after having been transferred downward, may again diffuse quite readily into the xylem. In conclusion OVERSTREET and JACOBSON communicate experiments in which they were able to replace all radio-active Rb absorbed at o° C by exchange for the inert isotope. This replacement can only occur when the cell surface to be passed is permeable.

The fact that salts permeate the root tissue, which was for the first time clearly demonstrated in our experiments by exact analyses, forms an excellent basis for regarding the transverse translocation in the root as a passive process.

Afterwards many data experimentally obtained, however, pointed to the fact that salt-concentrations higher than in the surroundings occurred in the vacuole of the cells as well as in the xylem-sap. As this was the case for both anions and cations at the same time it could not be accounted for by assuming Donnan-equilibria. Also the older hypotheses could not explain these facts, at any rate not for the root tissue. Thus we see that HOAGLAND and BROYER (1942) suggest that the tonoplast is almost impermeable, stressing the "pumping-action" of the living protoplasm (also see ARISZ 1945).

It appeared more and more that this accumulation only takes action if the metabolism of the cell were capable of providing the energy required for the concentration of the dissolved substance (ARISZ 1942, 1943, ARNON and HOAGLAND, GRAHAM and ALBRECHT, HOAGLAND, LUNDEGÅRDH, STEWARD 1937). The metabolism concerned has proved to be of an aerobic nature and recently MACHLIS has been able to determine a more close relationship with certain metal-catalyzed respiratory cycles, by means of diverse inhibiting substances.

COLLANDER (1937), when discussing the results of the permeability

investigations, says that the results obtained are of subordinate importance for the problem of translocation and that, as regards the latter point, the "adenoide Tätigkeit" according to OVERTON is certainly paramount. This refers only to organic substances, it is true, but in our opinion a number of investigators apply the same principles to electrolytes.

§ 2. *Simultaneous occurrence of active transfer and permeability in regard to the same substance.*

ARISZ has, by distinguishing permeation and transmeation, been able to found a basis for reconciling both aspects in translocation. In discussing the polarity of the root tissue in chapter V we held the view that ions, and possibly other substances too, may be subject to a polarly active uptake- and transport-process and can also diffuse freely in the same tissue. This same idea can very well be illustrated by investigations made by ARISZ (1942, 2).

In Drosera-tentacles ARISZ finds a gradual difference of behaviour with respect to asparagine, which is actively transported, and to caffeine and methylurea. The latter substances predominantly permeate, but the inhibiting influence of the withdrawal of O_2 points to a partial liability to vital processes. Thus KCN 1/300 Mol appears to check about 77 % of the asparagine-transportation in the tentacle, that is about the same as the effect of the withdrawing of O_2.

We shall now give a table from ARISZ' investigations (1942) in which the ratio of transport in anaerobic conditions to that in normal conditions is given:

leucin	1,00	methylurea	0,58
glycocoll	0,97	thiourea	0,45
alanine	0,96	phenylurea	0,35
asparagine	0,88	urotropine	0,35
urea	0,76	caffeine	0,31

Caffeine chiefly diffuses. Yet we see that in case of absorption from the lowest surroundings-concentration (1/320 Mol) the ratio becomes almost 1, viz. 0,96, which was not at all to be expected on account of the diffusion-gradient, for the route of transportation through the tentacles is too long and too narrow for this.

The ratio for KH_2PO_4 is about 0,78, perhaps a little more. So the possibility of diffusion remains here.

The common rule is: the worse the transmeation, the stronger the active working of the protoplasm. But it appeared at any rate that the two possibilities of transportation, passive and active, may

exist simultaneously for one and the same substance. HOAGLAND and BROYER (1942) also observe accumulation of Br and permeation of the same ion in experiments with tomato-roots.

If we assume that, in order to effect active absorption, the substance concerned must at least temporarily be bound to parts of the plasm, it is quite plausible that the two possibilities can occur simultaneously. Even MANGHAM also suggests for the transportation of sugar an equilibrium between absorption to the surface of the protoplasm components and the free concentration in the aqueous-phase of cytoplasm and in the vacuole. According to our view diffusion must be possible for that part which occurs in a free state in the liquid-phase.

Such a view may also be applied to results obtained by JENNY, OVERSTREET and AYERS, OVERSTREET and BROYER, SCHUFFELEN and LOOSJES. Here exchange for radio-active K, taken in previously, appeared to happen simultaneously with active absorption of K. We can very well imagine that this exchange or getting-out does not only occur in the border-layer, but considerably deeper in the cells or tissue for ions disengaged from the adsorption points of the protoplasm. Outward bound movement through diffusion ensues.

With the help of what is at present known about the very intense synthetizing and decomposition of plasm-components, it can be understood how ions are freed and bound all the time. It can be seen that a change of metabolism makes more or fewer points of binding available, thus influencing the power of absorption.

It might appear from the results obtained by TIEDJENS that the protoplasmatic binding is very important and involves a great number of the ions. TIEDJENS found in tomato tissue that 1 to 3 times as many nitrate ions were bound to the protoplasm as there were free ones. The bound ions were determined with the help of electro-dialysis, and the free ones by water-extraction. The NO_3-ions would be bound to proteins and soluble ampholytes, the binding being dependent upon pH and I.E.P. of the ampholytes.

§ 3. *The concept "accumulation-level".*

If active accumulation takes place in a cell or tissue that is at the same time permeable for the substance concerned we cannot but arrive at the view, that a constant leaking away of eventually absorbed and free substances will have to occur. The metabolism of the cell will all the time have to provide energy in order to maintain the gradients created. The idea of a dynamic equilibrium, which can only be maintained at the expense of energy provided by the cell or the tissue itself, has been emphasized by others before (a.o.

DEAN, HOAGLAND, ROBERTSON). In this connection it is striking that we never see cells in a "state of rest" unless, at the same time a communication between protoplasm and medium is lacking (seeds, spores and formation of cysts), for then no soluble substances can leak away and no processes providing energy are required.

The idea of absorption or *accumulation-level* of the plasm must be brought to the fore in connection with the dynamic nature of the accumulation-equilibria. By this we mean the changing power of the protoplasm of a cell, of a tissue, or even of an organism to effect a certain increase of concentration of substances with regard to the surroundings. It does not essentially matter whether the substances occur in a free or a bound state. A difference of accumulation-level, or changes of this, may cause transportation phenomena of a passive nature. This idea, which does not explain anything for that matter, makes it possible for us to see clearly the variable powers of cell or tissue.

Since the aerobic metabolism of the cell is — usually — closely connected with absorption of matter, one might expect any check of this metabolism to result in the freeing e.g. of salts. Thus we see HÖBER conclude that it has been proved unequivocally with plants by HOAGLAND, and by STEWARD, with animals by KROGH and others, that during oxygen withdrawal or by poisoning with narcotics, cyanide, azide, jodo-acetic acid, the uphill movement is stopped, or the level reached beforehand falls rapidly, by simultaneous diffusion of cations and anions (HÖBER 1940, pag. 40). This did not occur, however, in many other experiments. As long as we are occupied with objects with large vacuoles, where most of the salts are located, we can explain this when developing ARISZ' latest publication. For he points out that, though a cell may have great permeability, the tonoplast may very well be only slightly permeable. There is à priori no question of leaking away when transmeability is virtually or actually nil, because there is no possibility of exosmosis from the vacuole. In referring to results published by BLINKS we may also conceive of the plasm becoming far less pervious in anaerobic conditions.

Guided by the data taken from publications, we shall try to illustrate this idea further and to elaborate it. Next we shall consider how far it can be used to give a better description of the way in which we must imagine the process of absorption and transportation of salt through the root tissue.

A good example may be derived from data published by BRANDT. This investigator mentions experiments made with washed "Trockenhefe". By drying beforehand the permeability of the cell

membrane had greatly increased. This yeast was suspended in a succinate-succinic acid buffer and next the amount of free phosphate given off is determined in the medium.

The quantity of phosphate in the surroundings increases regularly, by aerobic shaking, up to 19 % of all the phosphate after 21 hours. But in the cells too the quantity of free phosphate increases, evidently as a consequence of decomposition-processes.

When glucose is added a fermentation-process starts and as long as this lasts the quantity of free phosphate in the solution and in the cell decreases. An increase occurs after the fermentation. During the fermentation the total quantity of phosphorus of the cells even increased, in other words absorption took place.

Here we see that the accumulation level, i.e. the power of the plasm to bind substances is subject to rapid changes according to the intensity of the metabolism. According to the accumulation-level we find intake or giving off of P.

In a publication by HEVESY and NIELSEN some results obtained by PULVER and VERZAR are mentioned. PULVER and VERZAR used yeast and could determine in this object a close relationship between absorption of K and the intensity of the carbohydrate-metabolism. When glucose was added to the surroundings, much K entered with it. When afterwards fermentation started much K came out again. They assume this to be the freeing of this ion from intermediate products in the shape of poly-saccharids and the diffusing away to the solution. Thus here too we have an example of varying accumulation-level, accompanied by permeability to the substance concerned.

When discussing their experiments, HOAGLAND and BROYER (1942) say that loss of K might occur after 24 hours in accumulation-experiments with barley-roots. They consider simultaneous active absorption of K in young cells and loss of the same substance in older ones to be possible. It was also shown with this object that the quantity of sugar in the cells is important to the level attained by the accumulation. Again the substance concerned leaks away when the power of accumulation decreases.

The essentials of this idea are used by CRAFTS and BROYER in their theory in which they see the better aeration of the cortex-cells — consequently more intense respiration with regard to the stele-cells — as the cause of the transportation of the ions to the inside. That is in our terminology, there would be a difference of *accumulation-level* between the two sides of the symplast. When we assume a tendency to level the concentrations within the symplasm by passive processes, it cannot be otherwise than that the most inward

cells are incapable of retaining all the salts, so that these enter into the xylem. These passive processes, corresponding to diffusion are evidently tacitly assumed.

In using the concept "*accumulation level*", we want to make clear, that we restrict its use to the protoplasm itself only. Of course the idea could easily be extended to the cell and tissue as a whole, but this would include the vacuole. For our use it will be better to exclude the cell-sap. Also in the previously discussed experiments we are concerned with cells largely consisting of plasm and with only relatively small vacuoles. The rather high impermeability of the tonoplast would also impede easy movements of free ions through this boundary. This fact is clearly demonstrated by the phenomenon that in most cases ions in the cell-sap are not liable to be susceptible to ion exchange. The recent results of OVERSTREET and JACOBSEN also point to this fact. At 0° C radio-active Rb was absorbed in a form easily removed by exchange for inert isotopes. At this low temperature there will have been no active uptake and so most likely no Rb-ions will have been secreted into the cell-sap.

This accumulation-level of the plasm may be dependent upon many factors. The most important one is often the intensity of the aerobic respiration. It appears from the experiments by BÖTTICHER and BEHLING that other influences too can be active. In experiments with maize stronger illumination of the shoot immediately caused increased uptake of K. Influence by change of transpiration was not possible, as the plants were in an atmosphere saturated with water-vapour. It also appeared that Ca did not to all intents and purposes react to the illumination. The authors say that K is better bound to the plasm under the influence of the light. The same was found by SCHMIDT for K and Mg.

§ 4. *Function of the root as a whole, also in regard to its polarity.*

After these speculations and the necessary elucidations we can pass on to the consideration of the problem of transportation of matter as a whole, as this is manifested in the absorption by the root. We must now ask ourselves if we can give a good description of what happens in the plant from the fact that the root tissue is permeable and from the concept of *accumulation-level.* If this is not so, we must ask ourselves in what respect this description is insufficient and try to bring other principles to the fore.

The two ideas are sufficient for our own experiments, in which it is especially the permeability to the substances investigated, that is emphasized. As far as we could say anything definite about the nature of the transportation-process, it was mainly concerned with

diffusion. The fact that very little outward diffusion was found with K, can be explained by saying that there the power of accumulation was relatively speaking too strong to allow the disengagement of the ions, for as soon as we interfered and blocked part of the aerobic metabolism, diffusion appeared to be possible. But here we also interfered directly with factors that govern the *accumulation-level.*

The scheme given here can also explain the experiments on bleeding-pressure made by GROSSENBACHER. It can be explained by a difference of affinity to the salts — the passive behaviour towards Ca — that more pressure is developed in a complete nutrient-solution than in an osmotic equally strong $CaSO_4$-solution. This small affinity to Ca is evidently a phenomenon that occurs far more frequently, as we find when we study COLLANDER's data concerning Characeae (1941).

Since we showed the existence of plasm-permeability to salts through the root tissue, it can be perfectly well understood (although with another object, viz. Helianthus) that in case of lack of O_2 the bleeding-pressure decreases. GROSSENBACHER himself says it should be assumed that a salt-solution disappears from the vessels, but evidently does not much believe the idea of diffusion outwards or re-absorption e.g. through the parenchyma. But now the former assumption cannot be objected to.

A normal HOAGLAND culture-solution is apparently the most favourable one for the root. In a 4-times concentrated solution, we see the increased osmotic value of the surroundings manifest itself in a slight exudation-pressure. The roots are thus not able to maintain a proportionately higher salt-concentration in the vessels in these circumstances.

We must also ask ourselves to what extent further conduction through the tissue remains active after the first intake at the surface. Many theories are only based on accumulation, assuming a more passive spreading-process, which is what we did just now, more or less based on CRAFTS and BROYER's hypothesis (5 in fig. 9, pag. 62). One of the difficulties to be taken into account with all these explanations, is the rapid movement of matter. This can never be effected by passive diffusion in a liquid phase. It might be achieved by surface migration however. KIDD in a criticism of MANGHAM's ideas, denies accelerated diffusion of adsorbed ions or molecules. Besides, this process could never lead to accumulation.

Although we have been able to demonstrate that the root tissue is permeable for solutes, there still exists another method for ac-

celeration of movement. The free ions could be carried along by the transpiration current. But, true as this may be, this explanation fails when considering results obtained in bleeding experiments.

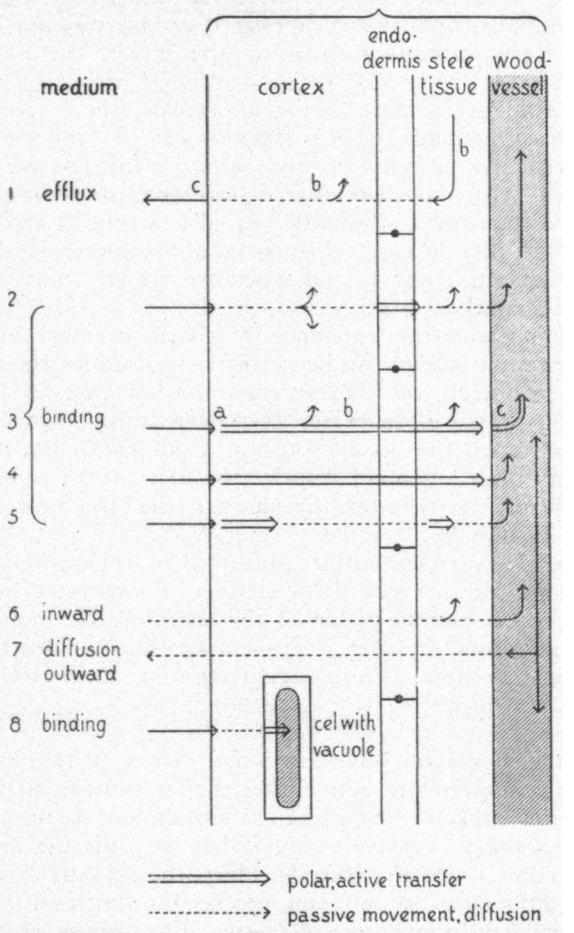

Fig. 9. Scheme illustrating the theoretical considerations concerning lateral transport through the living root tissues.

In the considerations just mentioned we have regarded each cell in the tissue working more or less as an entity and having its own accumulation-level. The lower accumulation-level of cells inside the endodermis gives rise to a gradient in concentration.

Passive movement would be the result and the freeing of ions into the xylem.

According to our idea the process of accumulation into the vacuole and that of accumulation into the wood-vessels is of the same nature in its principal aspects. Regarding one cell it seems difficult to understand how a difference in accumulation-level could exist between the outer and the inner cytoplasm. HOAGLAND, BROYER and STOUT have reported experiments, which also clearly demonstrate the inadequacy of the ideas proposed up till now. They found that the concentration of the xylem exudate may contain higher concentrations of ions than the root tissue. As it is quite well possible to assume that vacuole-accumulation is much slower than xylem-accumulation, the low concentration of the tissue (calculated from expressed sap) need not imply a low concentration in the plasm. Thus we might still be able to maintain our proposition, as the plasm could still contain more salts than the exudate, so making efflux into the xylem possible. BROYER and HOAGLAND (1943) however have now demonstrated with Nitella-cells that Br can be more concentrated in the vacuole than in the cell-plasm.

This is why others came to look upon the whole transportation-process, which is attended with accumulation, as an active one, that is to say the collaboration of protoplasm and of energy provided by the metabolism are required for it (b in 3 fig. 9, pag. 62).

LUNDEGÅRDH specially pays attention to anions and tries to explain the presence of a directed field of forces on the ground of a difference of redox-potentials. The difference of redox-potential is a consequence of lack of O_2 in the xylem with regard to the surroundings. In other respects too this investigator tries to analyse intake and adsorption as much as possible with the help of known physico-chemical principles.

VAN DEN HONERT suggests the idea of a protoplasmatical conveyor-belt mechanism from medium to vacuole after the particles to be transported have been adsorbed. This only relates to conditions in one single cell and not to tissues such as the root.

ARISZ' plasm-binding-theory (1944) is based on many more experimental data. Here the view is stressed that active transport, accumulation and acceleration of the movement of matter can only occur, if the substances concerned are subject to the activities of the protoplasm in a bound state.

In a previous chapter we have already concluded that the active transfer of solutes in the root is of a strictly polar nature in contra-distinction to the passive diffusion. We might now put the question as to where this polarity is located. In regarding one single cell we

must look to the cytoplasm. Here ARISZ would like to locate the polar "pumping activity" in the cytoplasm layers bordering the cell-sap (8 in fig. 9, pag. 62). For the root as a whole, he suggests the vital importance of the endodermis-cells in forcing the solutes into the stele. See 2, in fig. 9, for a simplified diagram of this concept.

We should like to suggest the following picture of uptake. As soon as solutes, especially dissociated ones, come into contact with the solid structural phase of the protoplasm, which need not necessarily occur at the root surface, but can also happen deeper in the cortex, they are bound in some way. These bound ions are transported inward only, through a series of cells of cortex, endodermis and stele. This is possible as the protoplasm forms a united entity — the symplasm. This polar and active transfer brings the particles up to the cells bordering the xylem. On their way inward these bound particles may be released or interchanged for others in the liquid phase of protoplasm. The particles in the liquid phase can diffuse freely and are directed in their movements by existing gradients of concentration or activation. The amount of bound particles depends on the *accumulation-level* and in this manner is dependent on O_2-supply. A sudden lowering of the accumulation-level may release numbers of particles, also in more inward located cells and these particles will then tend to diffuse away, mostly back to the medium. This concept is illustrated by combining 3 and 7 in fig. 9.

Regarding the route of transportation we can make the following remarks. The passive permeation-process is not confined to one fixed route, if there is a liquid phase present. Since the transmeability is usually extremely slight, however, the vacuoles will only rarely be suitable for transportation-route. But movement through the cell walls will be possible.

Active transfer, being caused according to LUNDEGÅRDH's view by collaboration of potentials of an electric nature, would be possible along all components of the tissue — cell wall, plasm and vacuole. In the endodermis the protoplasm will have to be passed anyway and can exert control.

If active transmission and acceleration of movement are brought about together with a binding of the substance to components of the protoplasm, the symplasm is the only route. We agree with HOAGLAND (1940) who remarks, that the absorbed salts are immediately transferred to the xylem in some cases, without first undergoing accumulation in the vacuoles of the cells that are passed. This can

be concluded from the fact, that several times the exudate had a higher concentration of a certain ion than the expressed sap of the root tissue.

The next point we want to discuss is the manner in which the solutes are given off to the xylem. As the plasm has proved to be permeable, we might conceive this to be a passive efflux into the vessels. The surplus of free solute in the stele-cells could be brought about by the lower accumulation-level. This loss of particles into the xylem is illustrated in 4 in fig. 9. The scheme suggested shows some likeness to a scheme for secretion by PFEFFER, based on a difference of permeability at two sides of the cell. A realization of this idea is more or less found in the fungus Pilobolus.According to LEPESCHKIN (1906) the guttation-sap here contains as much as 0,5 % of minerals. The loss of salts into the xylem appears to be possible, according to data given by LUNDEGÅRDH (1940). The latter observed the following concentrations in conducting bleeding experiments: medium 0,002—0,005 m Mol, tissue 0,050 m Mol and bleeding-sap 0,030 m Mol. Thus the concentration in the tissue is higher than in the vessels.

But we have already pointed out that in considering vacuole-accumulation, we might be forced to accept an active polar transfer into it, i.e. secretion, if sap concentration exceeds that of the plasm. Although as yet no data of the same kind have been given for the root as a whole, we consider it wise not to overlook such a possibility. That is why we prefer the suggestion of an active secretion of solutes into the xylem. Some authors have also expressed the opinion that water is secreted actively into the vessels (ROSENE 1944, GROSSENBACHER).

All through the problem of uptake of matter by the root, we are struck by the specificity which this tissue may have with regard to some substances. This pronounced power of selection of the protoplasm contrasts sharply with its behaviour towards diffusing substances. Numerous substances appear to be permeable, both organic and inorganic ones, behaving according to certain principles that can easily be physically understood. The capacity for uptake, however, is one that may be highly selective. Many data are known about this (vide e.g. KROGH, COLLANDER, etc.) and lately we came across another typical example, viz., in the different reactions of K and Ca. Even less comprehensible is the relatively great distinction often made by the plant between K and Na. The power of accumulation with its selectivity is a process, the principles of which were

never grasped clearly by analysis, although suggestions have been made. Here we quote STEINBACH (pag. 251): "In those cases where extensive studies have been made, cells appear to be quite permeable to all ions. The reason that certain ions are present in protoplasm in excess of others, must then depend on the activity and make-up of the whole protoplasm. Protoplasmic make-up could operate in two general ways to cause special distribution of ions: first, by contributing diffusing factors which in turn give rise to forces, electrical probably, that can selectively move certain other constituents; and secondly, by contributing certain organic constituents that form compounds of different physico-chemical properties with the various inorganic ions of the environment. A non-diffusible, slightly ionized, potassium compound, for example, should lead to potassium accumulation, while if other organic anions formed diffusible, slightly dissociated sodium compounds, sodium would tend to be removed from the cell."

It appeared many times, however, that the selectivity is not unrestricted. In numerous experiments on the mineral food of the plant it appeared that an altered composition of the surroundings is always manifested by the proportions of ions in the plant. From this it is apparent that the plant does not possess a constant ion-concentration. Another good example is found in ARISZ' investigations (1944) with Drosera. With this object the plasm in the process of transportation appears not to be able to distinguish between asparagine and some amino-acids, although it discriminates between these substances and phosphates.

The preceding paper is a report of an investigation undertaken at the Botanical Laboratory at Groningen on a suggestion of Prof. Dr. W. H. ARISZ. It is here that we wish to express our sincere gratitude for the very stimulating advice and criticism that was given to us and the great amount of interest taken in our work.

SUMMARY.

In considering the results obtained in the investigations concerning the intake of solutes by the roots of the normal higher plant, two principles come to the fore, which, in the first instance, might seem to be contradictory. The process that nowadays attracts the main attention of research workers in this field is that of accumulation, which can only be brought about by the active participation of aerobic metabolism. Besides this fact we cannot neglect the statements in which a passive method of intake is suggested. We have to accept the fact that the plasm of cells has proved to be permeable and besides this, there have been several results published which are explained on the basis of permeability of the root tissue. As yet, exact measurements of the permeability of the whole root tissue did not exist. It has been the main purpose of this investigation to examine the importance of passive transfer in the symplasm of the root.

Our first object has been to devise a method in which we could use the total living tissues between the xylem-vessels and the medium as a membrane between two solutions, which could be varied at will. This was attained by building an apparatus in which we could place pieces of root of about 6½ cm length, cut from the young secondary roots of Vicia Faba. The xylem-tracts having been opened at both ends, the living tissues formed a membrane enclosing the pathways through which we could pass a solution by means of a suction-tension. In this way we could examine lateral transport of solutes by dissolving them in the outer surroundings and analyzing for them in the water we had caused to flow through the wood-vessels. Also the set up could be reversed by flowing through a solution containing the solute to be examined and then following up its outward transport across the stele cells, endodermis and cortex by analyzing the outer medium. Presence of the Casparian strips in the endodermis cells or a suberin layer in the cell wall necessitates that all transport passes through the protoplasm, in this place at least.

By means of this new technique we have been able to ascertain that a number of ions can pass through the root symplasm. This is valid for Cl, NO_3 and H_2PO_4, although we are not absolutely certain that no active processes have partaken in the transfer. For K and Ca we have been able to demonstrate that these substances can penetrate the root tissue from the xylem to the surroundings. By

applying anaerobic conditions and also respiration-poisons we were able to establish the passive nature of this transport. The root tissue showed even a far higher degree of permeability for Ca in its outward transfer than for K. This can be accounted for by the low-salt condition of the roots, which would contain Ca to a fair extent, as they were grown in tap-water.

Also three organic substances were investigated. As could be expected, urea proved to permeate well. The other substances examined were glucose and sucrose. The latter especially was again extensively analyzed in its transfer from the vessels to the outer medium. Conforming with our expectations, although it may be somewhat surprising, sucrose was able to penetrate through the tissue easily.

Finally, we have come to regard the root tissue as easily permeable to a number of solutes and we suggest that this conclusion will be valid to a much wider extent. This conclusion may seem to be somewhat doubtful in view of the many statements on the impermeability of the protoplasm, obtained by plasmolysis experiments. But following the example of ARISZ, we wish to make a clear distinction between permeability sensu stricto, where only plasmalemma and the cytoplasm are penetrated, and transmeability, where also the tonoplast has to be passed. Our conclusions only refer to permeability of the protoplasm.

As the pieces of root were cut at different distances from the root apex, we could confirm the opinion that the whole zone of the root, where there are no suberized cell walls, is capable of uptake. But we were also able to demonstrate that a crosstransfer and a secretion into the xylem can occur throughout a zone corresponding to the length of the surface, where root haircells are able te develop.

Concerning the function of the process of intake and transfer we arrive at the following point of view. Transportation across the root tissue may be of two different types. Passive transport is made possible by permeability of the tissue, and here the direction can be controlled by changing the driving forces, i.e. concentration- or activation-gradients. This passive transport is not subject to polarity. Far more important for the normal supply of the shoot with nutrients is the active transport of solutes. This can only be brought about by energy delivered in aerobic respiration and is of a strictly polar character. This accumulation process only operates an inward bound transfer from surface to vacuole or xylem-vessel.

In the last chapter some theoretical considerations are discussed. We propose to consider the amount of salts absorbed by a cell or tissue the result of a dynamic equilibrium. The "*accumulation-*

level" a cell attains is the result both of leakage of solutes, as a consequence of the possibility for permeation, and the intensity of active accumulation. It seems to us that in agreement with the hypothesis of CRAFTS and BROYER we might arrive at a good description of absorption by accepting a different "accumulation-level" between cortex and stele. As the latter cells have the lower capacity for retaining salts, they ooze away into the xylem, where they can give rise to the phenomena of exudation or are carried off by the transpiration stream.

This scheme has its limitations, however, and so we propound the following hypothesis. The whole root tissue has the capability for an active inwardly directed transfer of solutes — probably in bound state — and secretes them into the xylem. Loss of particles can occur at any place, also by interchange, and the free particles may leak away to the medium again, as the tissue is permeable.

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EXPERIMENTS WITH POTASSIUM AND CALCIUM.

I. 30-6-42.

14.25 plants 20 days old, sprout cut off while kept in tapwater to prevent air getting into the vessels.
side roots cut off and sorted, placed in tapwater.

14.45 root tip cut away for 2 cm, root pieces cut to the length of 6½ cm, placed in aqua dest.
side roots were 11 cm long, mostly rather thick, colour slightly brown, smooth, sometimes with the beginnings of the secondary side roots, rather straight.

15.15 pieces of root put into the smaller, first apparatus, liquid flowing through the xylem consists of aqua dest. + KNO_3 1/10 Mol + trace of uranin.
in the $3^1/_2$ cm^3 glas vessels as an outer medium aqua dest., a small over-pressure on the liquid in the supply chamber.

15.25 apparatus was put in the thermostat, temp. 20° C; experiment started, suction 55—65 cm mercury.

1-7-42

9.10 finished the experiment, no leakages have occurred at the uppermost connexion at the side of suction chamber; outer medium analyzed for K by adding Na-Co-nitrite.
tube 1, amount of liquid sucked through the xylem moderate, medium clear, root whitish, no reaction.
tube 2, amount of liquid sucked through the xylem large, medium clear, root yellowish, no reaction.
tube 3, amount of liquid sucked through the xylem large, medium clear, root yellowish, no reaction.
tube 4, amount of liquid sucked through the xylem large, medium clear, root yellowish, no reaction.
tube 5, amount of liquid sucked through the xylem large, medium clear, root yellowish, no reaction.
tube 6, amount of liquid sucked through the xylem moderate, medium shows fluorescence as result of leakage, root whitish, positive reaction.
root pieces still in good condition when the experiment is ended, still a good turgidity.
root ends only slightly damaged at the ends where they have been covered up by the rubber connexion tubes, only somewhat blackish at the side towards supply chamber.
root pieces of tubes 3, 4 and 5 were cut into sections; slides made with paraffinum liquidum.
in all pieces a clearly yellow colouring of the wood-vessels and whole of the stele, sometimes even some colour in the innermost cortex cells; this demonstrates a good passage of supply liquid with the uranin.

II. 23-4-42 (abbreviated)
plants 22 days old
root tip removed for $1^1/_2$ cm, pieces 7 cm long
liquid that flows through aqua dest. + 1/50 Mol KNO_3 + uranin
in small tubes, ± 3/4 cm^3, a medium consisting of aqua dest.

24-4-42 experiment conducted for ± 23 hours
analysis for K gives the following results
tube 1, no fluorescence in the medium, very small positive reaction
tube 2, no fluorescence in the medium, no reaction
tube 3, no fluorescence in the medium, small positive reaction
tube 4, no fluorescence in the medium, positive reaction
tube 5, leakage has occurred
tube 6, medium shows fluorescence caused by leakage, positive reaction, only slightly damaged root ends.

III. 10-9-42 (abbreviated)
plants 22 days old
root tip cut away for 2 cm, length of root pieces $6\,/_2$ cm
liquid that flows through aqua dest. + KNO_3 1/20 Mol + uranin
in wide tubes, ± $3\,/_2$ cm^3, a medium consisting of aqua dest. + sucrose $^1/_4$ Mol.

11-9-42 experiment has lasted 25 hours
no leakages have occurred
1 cm^3 has disappeared out of supply chamber
analysis for K in the outer medium has the following result
tube 1, large amount of liquid has flowed through, medium clear, root white, positive reaction
tube 3, large amount of liquid has flowed through, medium clear, root white, positive reaction
tube 4, large amount of liquid has flowed through, medium clear, root white, no reaction
tube 5, large amount of liquid has flowed through, medium clear, root white, positive reaction
tube 6, a good amount of liquid has flowed through, medium clear, root white, positive reaction
roots still in moderate good condition after finishing the experiment, some loss of turgidity as a rule, slight damage of root ends.

IV. 25-1-43 (abbreviated).
plants 19 days old
root tip cut off for 3 cm, length of root pieces $6\,/_2$ cm
liquid that flows through aqua dest. + $Ca(NO_3)2$ 1/30 Mol + fluoresceine
in wide tubes, $3\,/_2$ cm^3 aqua dest.

26-1-43 experiment conducted for 23 hours
$1^1/_4$ cm^3 has disappeared out of the supply-chamber, thus probably passed through the xylem-tracts
analysis for Ca gives the following results
tube 1, flowing through none, medium clear, root yellow, Ca positive in medium
tube 2, flowing through good, medium clear, root white, Ca positive

tube 3, flowing through good, medium clear, root white, Ca negative
tube 4, flowing through none, medium clear, root slightly greyish, Ca positive
tube 6, flowing through good, medium clear, root grey, Ca positive
roots still in good condition at the end of the experiment, still possessing their turgidity, only slight damage at the root ends (no flowing through means we have no proof for passage of liquid through the xylem when the experiment has ended but nevertheless liquid may have passed and disappeared later by air getting in by leakage and pushing the water out of the receiving tube).

EXPERIMENT WITH PHOSPHATE.

V. 16-9-43 (abbreviated).
plants 22 days old
root tip cut away for 2 cm, pieces 7 cm long
circulating liquid in xylem consists of aqua dest.
in the $3^1/_2$ cm³ glass vessels a KH_2PO_4-solution
3 pieces of root were put in 10 cm of the same solution in a test-tube
6 pieces of root were immediately taken for analysis and another 6 pieces were put in a tray with aqua dest. to act as a blank.
16.00 experiment started, temp. 20° C, suction 55—65 cm Hg.

17-9-43
10.40 end of the experiment, only very little leakage has occurred, suction still maintained.
about $^3/_4$ cm³ aqua dest. has disappeared out of the supply-chamber
tube 1, moderate circulation, root tissue white, moderately turgescent, no damage,
in the liquid flowed through 7½ γ H_2PO
tube 2, minute circulation, root tissue white, very turgescent, some damage of root at the ends,
in the liquid flowed through 5½ γ H_2PO_4
tube 3, small circulation, root tissue white, very turgescent, no damage,
in the liquid flowed through 5½ γ H_2PO_4
tube 4, minute circulation, root tissue white, moderately turgescent, no damage,
in the liquid flowed through 7½ γ H_2PO_4
tube 5. minute circulation, root tissue white, very turgescent, no damage,
in the liquid flowed through 5½ γ H_2PO_4
tube 6, small circulation, root tissue white, moderately turgescent very small damage,
in the liquid flowed through 5½ γ H_2PO_4
the 3 pieces of root still in good condition, very turgescent, white and undamaged
samples (2 cm³) of used KH_2PO_4-solution contained
115 γ? 127 γ and 127 γ H_2PO_4
2 cm³ samples of solution in the small glass vessels contain
tube 1: 112 γ; tube 2: 120 γ; tube 3: 117 γ; tube 4: 117 γ; tube 5: 114 γ; tube 6: 115 γ H_2PO_4
the 6 control pieces of root contain 140 γ H_2PO_4

the 6 pieces of root in blank experiment contain 163 γ H_2PO_4
the 3 root pieces in 10 cm^3 solution contain 110 γ H_2PO_4
the 6 root pieces used in experiment contain 190 γ H_2PO_4
calculated estimation of results of this experiment

received in liquid flowed through	37 γ
amount accumulated by roots	$\pm$40 γ
	77 γ
amount disappeared from medium	118 γ
concentration in medium	63 γ/cm^3
concentration in liquid flowed through	$\pm$ 48 γ/cm^3.

EXPERIMENTS WITH SUCROSE AND UREA.

VI. 23-9-42
14.50 plants 21 days old, roots cut off in tap-water and sorted, length of roots 14 cm, rather thick (0,6—1 mm), colour slightly yellow, smooth, sometimes already with secondary side roots, straight
15.00 roottip cut away for 2 cm, length of root pieces 6 1/2 cm, in aqua dest. liquid to flow through consisting of tap-water + sucrose 1/10 Mol + uranin in wide tubes; tap-water as outer medium
15.50 experiment started, temperature 20° C., vacuum 55—65 cm Hg.

24-9-42
17.00 everything still in good order, no leakages

25-9-42
9.20 finished the experiment, no more vacuum on account of leakages which have occurred
about 1/3 cm^3 liquid must have flowed through the six root pieces; sucrose determinations in the medium yield the following results:
tube 1, flowing through good, medium clear, root yellowish, 90 γ sucrose
tube 3, flowing through moderate, medium clear, root white, 29 γ sucrose
tube 4, flowing through very good, medium clear, root yellowish, 62 γ sucrose
tube 5, flowing through moderate, medium clear, root white, 29 γ sucrose
leakage has made what remains of water in tube 2 and 6 unfit for sucrose determinations.
roots still in good condition at the end of the experiment, no loss of turgidity, only slightly damaged at the end.

VII. 12-10-42 (abbreviated)
plants 19 days old
root tip cut off for 2 cm, length of pieces 6 1/2 cm
liquid to flow through tap-water + trace of uranin
medium tap-water, blank experiment

13-10-42 experiment has lasted 23 hours
sucrose determinations give the following results:
tube 1, flowing through very good, medium clear, root white,

tube 1, flowing through very good, medium clear, root white, 0 γ taken as sucrose
tube 2, flowing through very good, medium clear, root white, 11 γ taken as sucrose
tube 3, flowing through very good, medium clear, root white, 11 γ taken as sucrose
tube 5, flowing through none, medium clear, root white, 11 γ taken as sucrose
tube 6, flowing through none, medium clear, root white, 11 γ taken as sucrose

VIII. 28-6-43 (abbreviated).
plants 18 days old
root tip cut off for 2 cm, length of root pieces 7 cm
liquid to flow through tapwater + urea 2/10 Mol + fluoresceine, medium consisting of tap-water.

29-6-43 experiment has lasted 18 hours
about 2/5 cm^3 of liquid must have flowed through
urea determinations yield
tube 1, flowing through very good, root yellow, medium green, more than 200 γ
tube 2, flowing through moderate, root yellow, medium clear, 130 γ
tube 3, flowing through very good, root white, medium clear, 110 γ
tube 4, flowing through none, root yellow, medium clear, 17 γ
tube 5, flowing through moderate, root white, medium clear, 10 γ
tube 6, flowing through very good, root white, medium clear, 15 γ
determinations of liquid in tube 1, 2 and 3 have been made by adding urease
determinations of liquid in tube 4, 5 and 6 were done without adding urease to be able to control the probability of the plant forming NH_4 we can conclude urea is not desintegrated
roots still in good condition, no loss of turgidity
about 15/100 cm^3 of liquid must have passed through roots 2 and 3 containing 800 γ urea.
total amount of urea detected in outer medium 2 and 3 is 200 γ urea.

IX. 21-6-43 (abbreviated).
blank experiment lasting 18 hours.

22-6-43 about 1/3 cm^3 tapwater must have flowed through the xylem, urea determinations yield
tube 1, flowing through good, root yellowish, medium greenish, 12 γ taken as urea
tube 2, flowing through none, root white, medium clear, 11 γ
tube 3, flowing through none, root white, medium clear, 11 γ
tube 4, flowing through very good, root yellowish, medium clear, 10 γ
tube 5, flowing through none, root white, medium green, 11 γ
tube 6, flowing through good, root white, medium clear, 11 γ
roots still in good condition.