INTERCELLULAR POLAR TRANSPORT AND THE ROLE OF THE PLASMODESMATA IN COLEOPTILES AND VALLISNERIA LEAVES*

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SUMMARY

A survey is given of the present state of the theory of symplasmic transport. The symplasmic movement of organic and inorganic substances in *Vallisneria* leaves shows polarity. The polarity is not a stable factor of the tissue, since it can be changed in different ways. Data regarding the movement of auxin in *Vallisneria* leaves have been compared with data about auxin transport in coleoptiles. Attention has been given to the localisation and the function of the plasmodesmata. The conclusion is arrived at that there are two fluxes in coleoptiles, a longitudinal flux which uses the plasmodesmata in the transverse walls and a transverse flux which uses the plasmodesmata in the longitudinal walls.

Movement in the symplasm is caused by concentration differences in combination with electric potential gradients and by gravity. Active transport requires the maintenance of these potential gradients by cell metabolism. The forces presumably influence the transit of the substances through the plasmodesmata, from cell to cell.

At a cut surface cells are opened and substances are released to the exterior. The exit of auxin at the basal wound surface is the consequence of the continuous polar movement of auxin through the plasmodesmata in the transverse walls of the cells and the opening of the adjoining cells by cutting. The movement of endogenous and exogenous auxin are different.

Endogenous auxin is moved by electric potential gradients in the tissue and by gravity, while exogenous auxin is moved by the same factors and moreover by the concentration difference which is the result of the active uptake of auxin in the symplasm.

1 THE SYMPLASMIC SYSTEM

In a plant tissue the protoplasts of adjoining cells are separated by the peripheral plasma membranes and the intermediate cell walls. Cooperation between the cells requires the passage of this barrier.

It has been established anatomically (TANGL 1879) that plasmatic connections between adjoining protoplasts are formed by the plasmodesmata. In this way a coherent symplasm extends throughout the whole plant (MÜNCH 1930, MÜHLDORF 1957, KRULL 1960, KOLLMANN & SCHUMACHER 1963, SPANSWICK & COSTERTON 1967, O 'BRIEN & THIMANN 1967). So it is a fundamental problem as to whether intercellular movement of substances makes use of these plasmodesmata (KLING 1958, SCHUMACHER 1967).

1.1. Significance of the plasmodesmata for transport

Pfeffer assumed that the plasmamembranes are more or less permeable. He

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suggested that a substance which can pass the plasma membrane by diffusion, would not need plasmodesmata for intercellular transport, thus implying that transport through plasmodesmata is less important than diffusion through the walls. It was shown that a great number of non-permeating substances such as sugars, salts, aminoacids and so on actually enter the plasm and are transported in the tissue.

Convinced that diffusion in a solution is a rather slow process, Pfeffer supposed that movement of these substances over a long distance requires special elements which are suited to rapid translocation of solutions.

At that time therefore, a sharp distinction was made between transport in parenchyma cells, which were considered as only fit for short distance movement, and long distance transport which makes use of sieve elements.

1.2. Active membrane passage

Since this time a new concept of membrane passage by means of metabolic processes has been introduced by Hoagland, Osterhout, Steward, Van den Honert, Lundegårdh, and Burström with their coworkers, who mainly studied salt uptake in different plants, by ARISZ & OUDMAN (1937, 1938), who analysed asparagine uptake in *Drosera* tentacles and in *Vallisneria* leaves, and by REINHOLD (1954), who studied auxin uptake by pea epicotyl segments and carrot disks. Little is known about the actual mechanism of membrane passage, but there is no doubt that these processes depend on cell metabolism and therefore on the availability of sufficient amounts of free energy potentials. Active uptake and plasmatic transport have been investigated thoroughly in different tissues.

1.3. Experiments with Vallisneria

Leaves of *Vallisneria spiralis* have been used in Groningen for the study of uptake and transport. They have many advantages such as their uniform structure and their ability for active uptake of substances over the whole surface, since in this water plant the cuticle is not impermeable.

In addition substances that are locally absorbed in the leaf tissue are translocated to the rest of the leaf and to other parts of the plant. Uptake and transport are studied in submerged leaves, without the interference of transpiration. Special water conducting vessels do not occur in the leaves of this plant. Transport of water and substances in the submerged leaves is, therefore, a plasmatic process.

At the beginning of this essay on symplasmic transport it seems appropriate to mention two authors who have given much attention to the study of transport in the symplasm, MÜNCH (1930) and CRAFTS (1939, 1951, 1962). Both have made important use of the symplast concept, to interpret the results of translocation studies in plants.

Münch dealt particularly with the osmotic aspect of transport. He deviated from the view of Pfeffer in considering that the plasma membranes which border the symplast were practically impermeable to the moving substances. He suggested that in parenchyma cells osmotically active substances were formed and the resulting increase in osmotic pressure caused a flow of plasmatic con-

tents through the plasmodesmata to the adjoining cells. In sieve tubes he supposed that an osmotic mass flow of the vacuole sap is produced. Münch assumed that the distance covered by mass flow in leaf parenchyma of the cell contents amounts to some mm per day, the pressure differences being estimated at 0.3–1 atm per cell.

Open pores of the channels of the plasmodesmata are, in his opinion, not necessary for these phenomena to occur.

When Münch was working, active uptake and movement of radioactive substances in the symplasm were unknown. None the less the clear exposition of his views on transport and his appreciation of the significance of the symplasm have been of great value.

1.4. Uptake and passage through the peripheral plasma membrane

The passage of the peripheral plasma membrane in *Vallisneria* leaf cells will not be considered here in detail.

Uptake is a complex of different processes. It consists of the passage through the cellulose wall and that through the peripheral plasma membrane of the absorbing cells. The absorbed substance migrates in the cytoplasm of these cells and is transferred through the plasmodesmata to the adjoining cells. A part of the absorbed substance will be bound or transformed in the cytoplasm with its different organelles (mitochondria, chloroplasts, endoplasmic reticulum) or secreted into the vacuoles. Several substances can only penetrate through the peripheral plasma membrane by means of an active energy consuming process of cell metabolism. The mechanism of this process of active membrane passage is still unsufficiently known. We shall confine us here to consider its influence on the movement in the symplasm.

The process of membrane passage depends as well on the concentration of the substance in the external solution as on its concentration in the cytoplasm at the place of uptake. This internal plasmatic concentration is decreased by the secretion into the vacuole, by the transformation and the binding of the substance in the cytoplasm and by the translocation of the substance to the adjoining cells. Membrane passage will be increased as a result of a greater difference in concentration with the external solution. In this way it can be explained that the total amount of the substance which is absorbed from the medium depends on the length of the leaf strip (ARISZ 1947).

The experiments on uptake in *Vallisneria* leaves have shown that potassium chloride is actively absorbed and accumulated against a concentration gradient. Active uptake is found in the dark and in the light making use of free energy potentials provided by respiration, by photosynthesis and by anoxybiosis (Robertson, van Lookeren Campagne, Mc Robbie, Jeschke, Wilkins).

After the uptake the substance is not released from the cytoplasm to the exterior solution, provided that the cell membranes remain intact. By injury, however, the constitution of the plasma membrane and its permeability may be changed and leakage of cell contents may follow.

It was proved (ARISZ 1963, 1964) that during uptake of labelled chloride no leakage of unlabelled chloride, already present in the tissue, occurred. This means that the peripheral plasma membranes are impermeable to chloride.

In our transport experiments with labelled salts, amino acids and sugars through intact tissue no exit of radioactivity from the transporting tissue into the external solution has occurred, nor an exchange between radioactive ions, in the transporting tissue and identical non radioactive ions, added to the external solution. Therefore it is assumed that the impermeability of the peripheral plasma membrane which was shown for chloride, also holds good for other hydrophilic substances.

Since our transport experiments have mostly been executed with substances which are actively absorbed from an external solution, it has to be emphasized that the concentration of these substances at the place of uptake will have been considerably increased and that therefore in our experiments concentration differences in the symplasm have an important influence on the movement of the substance in the symplasm.

It has been shown that in *Vallisneria* leaves the uptake processes are enhanced by temperature and by application of light and sugar (ARISZ & SOL 1956). SOL (1958) studied the influence of a previous absorption of salts and of sugar on the uptake of chloride. The substances were introduced into the symplasm during a pretreatment. After having been absorbed the salts were shown to regulate the hydration of the cytoplasm at the place of uptake and to influence the active uptake of chloride (SOL 1958).

1.5. Influence of inhibitors of metabolism

The processes of membrane passage, of binding to the cytoplasm and to its organelles and those of secretion into the vacuoles can be influenced by inhibitors of cell metabolism (MACHLIS 1944, ROBERTSON *c.s.* 1951, ORDIN & JACOBSEN 1955). Many data have been gathered from experiments with *Vallisneria* (ARISZ 1953, 1958, 1964 and HELDER 1967) which indicate that several processes in the symplasm can be influenced by specific inhibitors, some inhibitors having an injurious effect at higher concentrations.

When the influence of metabolic inhibitors on the uptake is investigated in isolated leaf segments, it is rather impossible to see which of the different processes, involved in uptake and transport, is influenced by the inhibitor.

In *Vallisneria* leaves a discrimination between the action of the inhibitor on the different processes can be obtained, if the inhibitor is applied to a leaf strip containing two zones, one leaf zone in direct contact with the labelled external solution and an adjoining leaf zone which is surrounded by water. The first zone will absorb the labelled substance, the second zone will transport it in the tissue cells. If the inhibitor is added to the external solution together whith the labelled substance, it will act on the uptake process. If the result is that as well in the uptake zone as in the transport zone the amount of labelled substance is reduced, the inhibitor has acted on the process of membrane passage. If, however, the result is that only in the absorbing zone the uptake is diminished, while in the

transport zone the activity of the labelled substance is not reduced, it must be concluded that the inhibitor has not influenced the membrane passage but the binding in the plasm or the secretion into the vacuole.

In an analogous way it was shown (ARISZ 1953, 1958, 1964) in quantitative experiments that potassium cyanide, sodium arsenate and uranyl nitrate inhibit active membrane passage of salts and of asparagin.

1.6. Evidence for transport through the plasmodesmata

When an inhibitor of membrane passage is applied exclusively to the water surrounding the transporting zone, the migration of the substance from the absorbing zone is continued through the transporting zone.

This result indicates that the movement from cell to cell is not dependent on a process of membrane passage since this process would have been stopped by the added inhibitor. The movement from cell to cell, therefore, is not a process of active membrane passage. Since the plasma membrane is shown to be impermeable to chloride, there is no other possibility for chloride to move from cell to cell than through the plasmodesmata (ARISZ 1957, 1958, 1960).

The data obtained with the above mentioned inhibitors, potassium cyanide, sodium arsenate and uranylnitrate have been corroborated several times.

In the original quantitative experiments short leaf strips of 7.5 cm were used: 2.5 cm for the length of the uptake zone and 5 cm for the transport zone. Recently the experiments have been repeated by making autoradiograms of longer leaf strips which had absorbed labelled asparagine or serine in a middle zone of 5.5 cm in length. Radioactivity moved in the 45 cm leafstrips in acropetal and in basipetal direction. Different inhibitors were applied either to the central zone or to one of the side zones. A preliminary account of some results will be given in Chapter 2 p 38. They support and extend the former quantitative experiments with short leafstrips. Only the influence of 2,4-dinitrophenol used in 1953 in an experiment has not been confirmed, since the results have been variable. They depend on circumstances of light and darkness during and preceding the experiments. In regard of new data published by Mc Robbie, Jeschke and others, these difficulties can be understood at present.

2. MOVEMENT OF SUBSTANCES IN THE SYMPLASM

2.1. Long distance movement through the symplast

The uptake and the transport of labelled potassium and rubidium chloride in *Vallisneria* plants have been examined by means of autoradiograms (ARISZ, Bot. Congress Montreal, 1959; 1960). It was shown that radioactive chloride and rubidium ions, absorbed by the top of one of the leaves, move in the symplasm over distances of 100 cm and more, first to the base of the plant and from there to other leaves (ARISZ & WIERSEMA 1966, HELDER 1967).

2.2. Transport through tissue bridges

By cutting away a part of the tissue over a length of several mm the absorbing

and the transporting part of the leaf remain connected by a tissue bridge containing either parenchyma cells or bundle tissue. In earlier quantitative experiments on transport through such bridges it was shown that both, parenchyma cell bridges and bundle bridges, are able to conduct the absorbed substance along the leaf tissue (ARISZ & SCHREUDER 1956a). The experiments with bridges have been repeated by making autoradiograms after uptake of radioactive chloride ions from a potassium chloride solution or of radioactive rubidium ions from a rubidium chloride solution (ARISZ 1960).

2.3. Symplasmic distribution

After having moved through a parenchyma bridge of 4 mm in length the absorbed radioactive ions appeared not only in the parenchyma cells of the transporting part of the leaf strip but also in the bundles. When the radioactive ions were moved through a bundle bridge, they entered the transporting part in the bundle, but they moved as well to the adjoining parenchyma cells. This picture (ARISZ 1960, fig. 14, ARISZ & WIERSEMA 1966, fig. 1) proved the concept of symplasmic distribution of the absorbed radioactive ions.

2.4. Substances moving in the symplasm

Experiments on movement of radioactive ions in intact plants have been performed also with labelled phosphate and with different organic substances labelled with ^{14}C (ARISZ & WIERSEMA 1966). It has been shown that amino acids, asparagine, sugars and auxin are all absorbed and transported according to the same general pattern. The average velocity of these movements was 2 to 4.5 cm per hour; often over a distance of more than 100 cm in 24 hours. Differences in velocity, depending on the kind of cells used for the transport, will certainly have been present, but they could not be measured, since the differing tissues could not be separated without wounding. As a result of the active uptake a large amount of the absorbed substance is of necessity found in the absorbing zone, though the actual concentration of the mobile molecules and ions in the symplasmic pool remains unknown.

2.5. Movement of molecules and ion pairs by concentration differences

The movement in the symplasm is due to the accumulation of the introduced substance in the symplasm. It is more rapid than can be expected of a diffusion process in a fluid medium. Several authors have put forward this fact as an indication that the movement is not diffusion and does not depend on a concentration difference. Where concentration differences arise, diffusion must follow. It is impossible to conclude from data about its velocity whether the movement is actually diffusion. A departure from the values expected for diffusion is only indicative that there are in addition also other forces which regulate the movement such as protoplasmic streaming and a flow by pressure differences. It will be shown that the movement can be promoted as well as retarded. This suggests that several forces are cooperating (Briggs e.a., Dainty).

2.6. Movement of auxin in the symplasm

An interesting consequence of these experiments is that the behavior of auxin can be compared with that of ordinary nutrients. Since the transport of auxin in coleoptiles is considered to be a strictly polar process, the experiments with *Vallisneria* have been continued with the intention of exploring the polarity of the transport process. The wounds at the cut ends of the leaf strip did not exclude the use of such strips. By putting the end of the leaf strip through a slot in the wall of a short perspex vessel, the exit of substances from the wound can be controlled without influencing the transport. A few essential results, not published before, will be mentioned here.

2.7. Polar transport

Experiments have been carried out with leaf strips of more than 20 cm in length, absorbing substances either at the basal or at the apical end over a zone of 2 cm (*fig. 1*). It was found that the transport of practically all substances was polar, which means the velocity in opposite directions was different. In shorter leaf lengths, however, differences in the distance covered in 24 hours were mostly too small to be measured. Sucrose and serine were apparently more polar than glucose and asparagine. The general result was that basipetal transport covered a larger distance than acropetal transport.

2.8. Polarity of the tissue

Polarity has also been tested in another way, by using a horizontal transport apparatus (*fig. 2*) with three compartments, made of perspex. Here the labelled substance is absorbed in the small middle compartment (5.5 cm in length) by a middle zone of a 40–45 cm long leaf strip. It moves to apex and base of the leaf strip which are in the side compartments containing water. The distances covered by the labelled substance in the acropetal and basipetal directions have been measured. In such experiments the difference in distance cannot be the result of a different power of uptake at the apex and at the base of the leaf, but is the consequence of movement through the tissue in different directions. With the same apparatus the influence of the addition of other substances on the transport can be traced, either on uptake in the middle zone or on the movement in the apical and basal zones.

The results of the experiments on polar transport are not yet sufficiently complete, since the chemical changes of the moving substances have not yet been studied. The pathway of the substances in the tissue may also have had an influence on the polarity of the transport. For these reasons the discussion is confined to the distribution of radioactivity in the autoradiograms.

Fig. 3 is a photograph of four leaf strips of about 20–25 cm length after having been freeze dried. The methods have already been described by ARISZ & WIERSEMA (1966). At the base of the photograph four short leaf pieces can be seen, which are used to check whether there is any leakage of radioactivity into the water of a vessel. Such controls were present in all experiments in the vessels without added radioactivity. When they showed any radioactivity the auto-

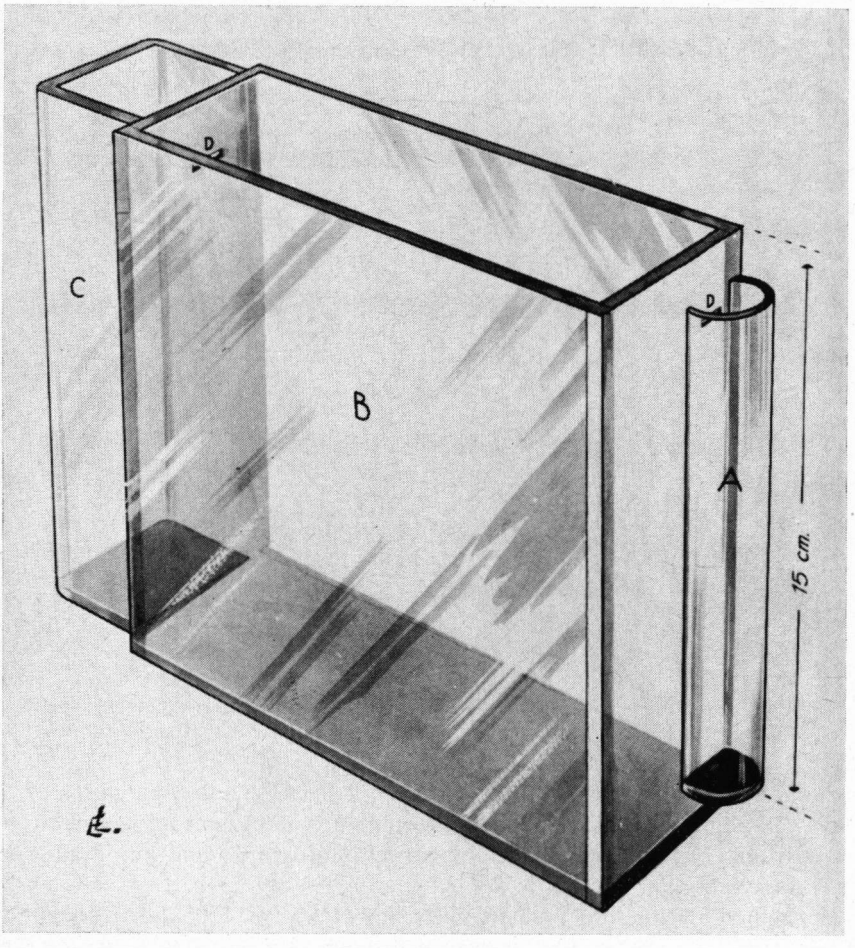

Fig. 1. Perspex vessel consisting of three compartments.

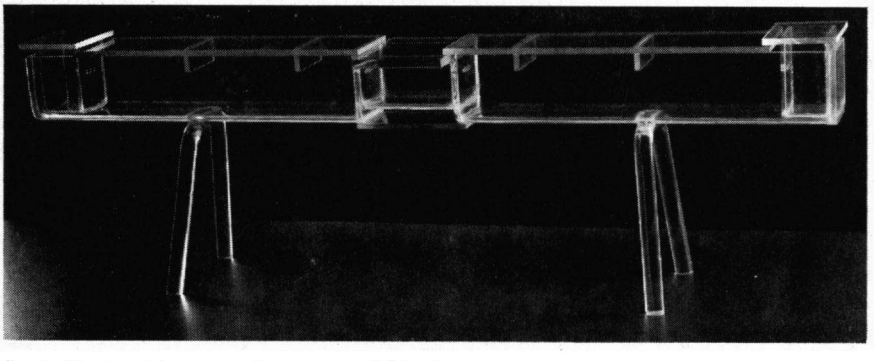

Fig. 2. Horizontal transport apparatus (cf text).

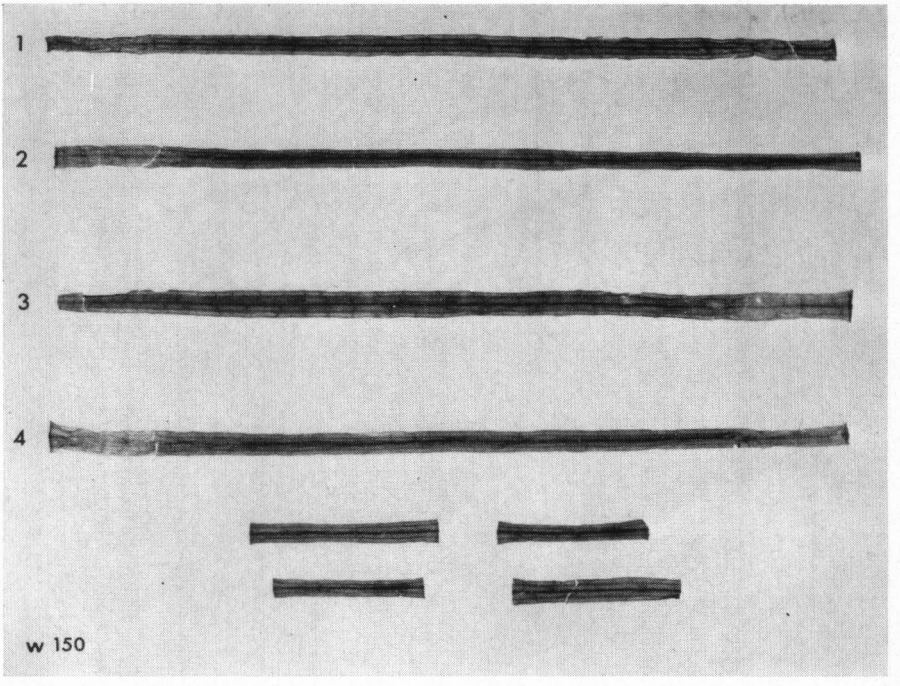

Fig. 3. Photograph of four leaf strips, 20–25 cm in length after freeze-drying. At the base 4 leaf segments used to control contamination with radioactivity. Exp. W 150.

radiogram was considered unfit for use. *Fig. 4* gives the autoradiograms of the leaves photographed in *fig. 3*. The following autoradiograms are given without photographs of the leaves, since comparing photographs and autoradiograms has no special importance.

In the first and the fourth leaf strip of *fig. 4* (exp. W. 150) short zones at opposite ends have absorbed asparagine at the apex and serine at the base respectively, at a concentration of 1 mM during 24 hours. In the first strip the asparagine was labelled with ^{14}C, while in the fourth strip the serine was labelled. The middle part of the leaf strip was in water. Mental combination of the transport of radioactivity in the first and the fourth strip shows both substances moving at the same time in opposite directions. Asparagine moves over a somewhat longer distance (15.5 cm) than serine (8 cm). The two flows pass each other as can be expected from diffusion processes. Radioactivity can be seen in the bundles as well as in the parenchyma between the bundles.

2.9. Unidirectional movement

Leaf strips 2 and 3 show the movement of radioactivity when the substances have been supplied at one end only. In leaf strip 2 labelled serine is applied to the apical part, in 3 labelled serine is applied to the basal end. The rest of the leaf strips are in water.

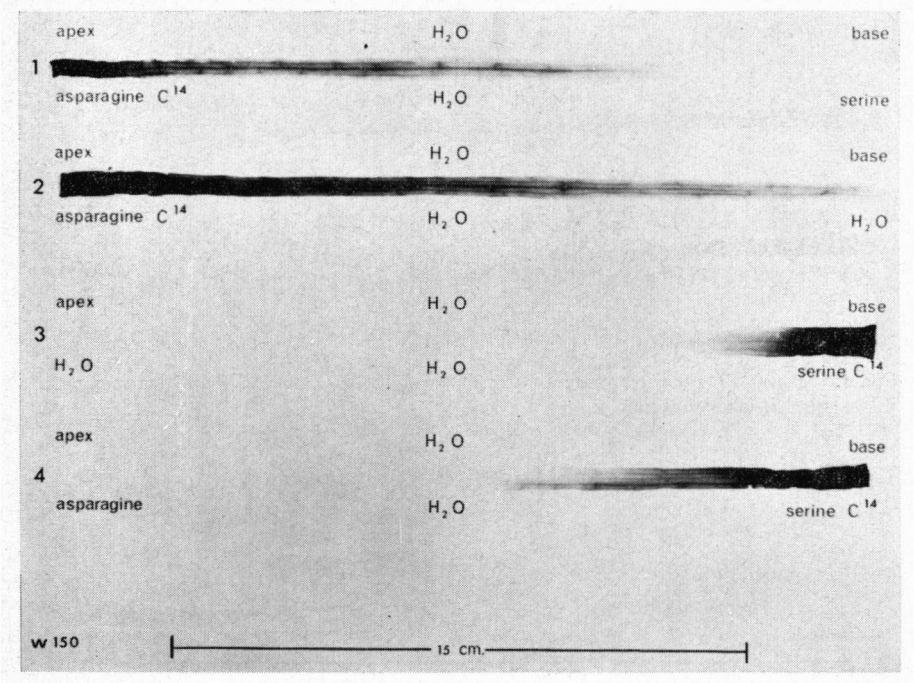

Fig. 4. Autoradiograms of the leaf strips used in exp. W 150. In strip 1 uptake of labelled asparagine (1mMol) in the apical part and of unlabelled serine in the basal end zone. In strip 4 uptake of unlabelled asparagine in the apical part and of labelled serine in the basal endzone. In leaf strips 2 and 3 unidirectional transport; in 2 asparagine is absorbed in the apical endzone, in 3 serine is absorbed in the basal endzone and moves in acropetal direction.

It appears that unidirectional transport is markedly polar. Basipetal transport of asparagine covers a much longer distance than acropetal transport of serine. In leaf strips 1 and 4 the bidirectional movements diminish the polarity of the movement. Basipetal transport takes place over a shorter distance and acropetal transport over a longer distance. The polarity of serine transport in water is shown in exp. W 133 *(fig. 5)*. In both leaf strips transport is unidirectional. In the first the movement is from the apex towards the base (19 cm) and in the second from base towards apex (7 cm). The difference between the basipetal and the acropetal transport is striking.

2.10. Movement from the middle zone in opposite directions

The polarity is also shown in exp. W 161 *(fig. 6)*, in which the horizontal transport apparatus has been used. The middle part of the leaf strip absorbs a labelled serine solution (1 mM).

Movement takes place simultaneously into the apical and the basal part of the leaf strip, which are in the side vessels containing water.

The acropetal movement covers a distance of approximately 6,5 cm and that

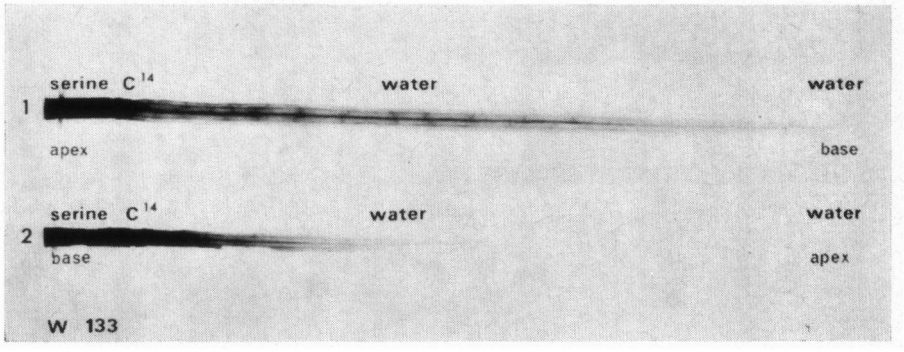

Fig. 5. Exp. W 133. In strip 1 basipetal transport of labelled serine, in strip 2 acropetal transport of labelled serine.

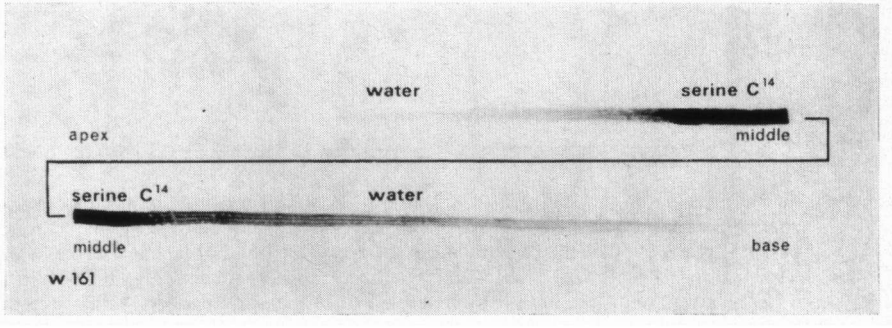

Fig. 6. Exp. W 161. After the freeze-drying the leaf strip was cut in two parts. Uptake of labelled serine in the middle compartment of the horizontal transport apparatus. Acropetal transport is over a shorter distance than basipetal transport.

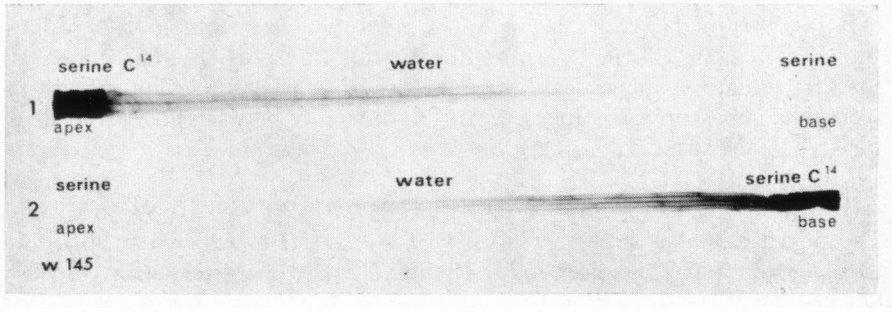

Fig. 7. Exp. W 145. Movement of serine absorbed in both endzones in opposite directions. In 1 the apical endzone absorbs labelled serine, the basal endzone absorbs unlabelled serine. In 2 the serine in the basal endzone is labelled; the apical endzone absorb sunlabelled serine.

in the basipetal direction about 17 cm. While the uptake in the middle vessel is the source for both movements, the fluxes in opposite directions present an important difference.

The autoradiograms of experiment W 145 *(fig. 7)* show a bidirectional transport in opposite directions of serine, absorbed at both ends of the leaf strip. In the first strip the apical end receives labelled serine and the basal end unlabelled serine, while in the second strip the basal end receives labelled serine and the apical end the unlabelled. The result is analogous to that of exp. W 150 *(fig. 4)*, where serine and asparagine have been used. The two fluxes of serine in opposite directions pass each other in the middle part of the leaf. The polarity of the bidirectional serine transport is much weaker than that of the unidirectional movement. Basipetal transport decreases and acropetal transport increases. It appears that polarity is not completely abolished but considerably diminished by the opposite fluxes.

This experiment is in harmony with the conception that the local accumulation of serine at the place of uptake is the cause of a diffusion process in the symplasm. A diffusion potential will arise which may influence the diffusion in the electric field of the tissue. If uptake is accomplished at both ends of a leaf strip, two accumulations take place and their polar effects will be more or less balanced.

It has to be stated that polar transport is not limited to electrolytes. Sucrose also presents a distinct polar movement which is greater in basipetal direction.

In experiments with glucose somewhat more variation of polarity has been found. Changes of polarity may also be the consequence of chemical changes of the substances moved in the symplasm. This problem has not yet been investigated.

An investigation of polar transport in *Vallisneria* leaves requires the discussion of the forces which move the substances in the symplasm.

Münch has pointed out the influence of osmotic potential differences in the symplasm which would be able to move a solution in the symplasm.

In a previous publication (ARISZ & WIERSEMA 1966) with intact plants the rise in concentration in the plasm at the place of uptake has been considered as the consequence of the accumulation of a substance by active uptake. Theoretically, therefore, movement of substances by diffusion and by osmotic forces can be expected. Münch assumed an osmotic pressure flow through the plasmodesmata. He has presumably underestimated the actual velocity of plasmatic movement and the resistance of the plasmodesmata to pressure flow.

2.11. Plasmodesmata

The data in the literature indicate that the channels of the plasmodesmata are not open; they are filled up with cytoplasm. Diffusion of a substance through a plasmodesma can be the result of a concentration difference between the adjoining protoplasts. Whether a pressure flow of a solution also occurs in the symplasm is problematic, but a mass flow of the plasmatic contents is out of the question. The substances move according to the concentration differences in

adjoining protoplasts through the plasmodesmata to all parts of the symplasm where the concentration is lower. It is also evident that forces other than concentration differences can influence the migration of substances in the symplasm. The contents of each cell will be continuously mixed by the protoplasmic streaming, while it is likely that the plasma sap is moved by the action of plasma organelles which work as mixing units. In this way an important acceleration of the transport process in the cytoplasm can be obtained (HUGO DE VRIES 1885, ARISZ & WIERSEMA 1966).

There are not yet sufficient physiological data to decide the significance of the presence of the endoplasmic reticulum (E.R.) in the plasmodesmata. O'BRIEN & THIMANN (1967) consider the plasmodesma and the E.R. as one complex. They suggest that the E.R. may act as a pathway for the intracellular transport of auxin, since the plasmodesma seems to be an intercellular connection between adjoining protoplasts. Through the plasmodesmata large amounts of different substances are moved. It is likely that a large amount of free energy potentials is consumed by these processes. It would be interesting to consider the possibility, whether E.R. contains high energy phosphates which are required for the supply of energy to move substances actively through the plasmodesmata, even when concentration differences are absent. If concentration differences are present, diffusion over the short distances of the plasmodesmata will be efficient. The movement in the cytoplasm can be explained by increased stirring (Curtis).

BRIGGS *c.s.* (1961) have explained that diffusion of electrolytes can be influenced by electric potential differences. They will enhance or reduce the velocity of the movement as a function of the direction of the potential gradient in the tissue. When electrolytes are moved, it can be expected that the polarity of the process will depend on the difference in mobility between the cation and the anion, which move simultaneously.

2.12. Polar transport of salts

The weak polarity of potassiumchloride, which is shown when it moves in *Vallisneria* leaves, may be a consequence of the equal mobility of potassium and chloride ions. At a pH of 8 in the external solution the polarity of Cl transport was weaker than at pH 4. It may be that at a low pH more hydrogen ions and less potassium ions will be introduced together with the chloride ions into the symplasm by the process of active membrane passage. The hydrogen ions will increase the average mobility of the cations moving in the symplasm, and it might then be expected that at pH 4 the polarity of Cl movement would be increased.

2.13. Changing the polar distribution of serine

It is interesting that the polarity of serine movement in a leaf strip can be changed by administration of 1 mM sucrose solution to the compartment of the horizontal transport apparatus, which contains the basal part of the leaf strip.

Table 1. Movement of labelled serine absorbed in the middle part (5.5 cm) of a leaf strip of 45 cm total length in acropetal and basipetal directions. Influence of the addition of non labelled sucrose to one of the three compartments of the horizontal transport apparatus on the polar serine movement. Concentration of serine and sucrose is 1 mM. Duration of exp. 24 hours, temp. 25 °C. In the light.

apical zone	middle zone	basal zone	acropetal transport	basipetal transport
water	serine	water	6 cm	16 cm
water	serine+sucrose	water	7.5	18.5
water	serine	sucrose	17.5	5.–
sucrose	serine	water	3.–	17.–

Table 1 shows the results of adding sucrose (1 mM) to one of the three compartments of the horizontal transport apparatus on the distribution of labelled serine.

Without sugar addition the movement of labelled serine (1 mM) is strongly polar: 6 cm in acropetal and 16 cm in basipetal direction.

Addition of sucrose to the middle compartment has no visible influence on the uptake and the distribution of serine in the leaf strip is 7.5 cm in acropetal and 18.5 cm in basipetal direction.

If sucrose is added to the compartment which contains the apical part of the leaf strip, a similar polar distribution is obtained as without sucrose addition: 3 cm in acropetal and 17 cm in basipetal direction. When, however, sucrose is added to the compartment with the basal part of the leaf strip the distribution of serine is changed. Acropetal transport is increased to 17.5 cm while basipetal transport is reduced to 5 cm. The normal polarity of the serine fluxes is overcome.

Other substances such as mannitol have an analogous effect. If more or less injurious sugars (Stenlid, Loughman) were used, deviating results were obtained.

The phenomenon is complicated and more data are needed to give an impression of the factors which were changed by the addition of sucrose. The experiment corroborates at all events that polarity is not fixed in the tissue and can be influenced in different ways. The different results obtained by addition of mannose and galactose indicate that also chemical data concerning the metabolic changes of the substances involved will be needed.

2.14. Influence of inhibitors of membrane passage on the polarity of the symplasmic transport

Some results of recent experiments with inhibitors of membrane passage have been mentioned on page 29. An inhibitor of membrane passage, such as potassium cyanide, sodium arsenate or uranyl nitrate was added in a concentration of 10^{-5}M to the solution in one of the three compartments of the horizontal transport apparatus *(fig. 2)*.

In the middle compartment (5.5 cm in length) a labelled amino acid (serine or asparagine lmM) was absorbed from the external solution. It moved in the leaf strip partly in basipetal, partly in acropetal direction. The length of a side compartment was ca 20 cm. If the inhibitor was added to the middle compartment, membrane passage was inhibited and the uptake of the labelled amino acid was markedly reduced. As a consequence the movement in basipetal and in acropetal direction through the transporting parts of the leaf strip was likewise diminished.

If the inhibitor was added exclusively to the water in a side vessel, two phenomena occurred: 1) The movement through the transporting part of the leaf strip was continued; 2) The distances covered in 24 hours were, however, changed to such an extent that polarity was reduced.

It was expounded on page 29 that the continuation of the movement through the leaf strip, although it was surrounded by a solution containing an inhibitor of membrane passage, was a conclusive proof that the movement from cell to cell is brought about without active passage of the peripheral plasma membrane. Since the membrane cannot be passed by an amino acid by means of passive diffusion, the transport of the amino acid has to follow the plasmodesmata.

Here the second point has to be considered that the polarity of the movement is reduced by the addition of the inhibitor of membrane passage to the solution in the side vessel.

The normal polarity of the symplasmic movement appears from the different distance which is covered by the movement in acropetal and that in basipetal direction through the transporting parts of the leaf strip. Normally an amino acid such as serine moves in 24 hours in basipetal direction over a longer distance than in acropetal direction. If the inhibitor is applied to the basal part of the leaf strip, an increase of the velocity of the movement in basipetal direction will be hardly visible, since the movement takes place over practically the whole length of the leaf strip. If, however, the inhibitor is added to the apical compartment, the amino acid transport in acropetal direction which is normally rather feeble, is found to be markedly increased.

Recapitulating, it has been shown that by adding the inhibitor to the apical compartment the movement of the amino acid in acropetal direction is increased. This result shows that the polar influence which reduces under normal circumstances the movement in acropetal direction was decreased by the influence of the inhibitor.

By adding the inhibitor to the basal compartment a retardation of the movement in basipetal direction can be expected, when the promotion of the movement by the normal polarity disappears. As we have seen this effect is less striking than the opposite effect on the acropetal movement.

2.14. Transport of auxin

In order to obtain a better understanding of polar transport it seemed appropriate to take full account of the data known about the behavior of auxin in coleoptiles. Strictly polar transport of auxin is known since the discovery of the

growth substance by Went and the investigations of Went and Van der Wey on transport in coleoptiles. Polar processes can better be studied in coleoptiles than in *Vallisneria* leaves.

A coleoptile contains besides parenchyma only two bundles, while *Vallisneria* leaves contain about five. A remarkable number of investigations has been carried out on transport of auxin in coleoptiles with modern equipment and conscientious care. It is my intention to discuss here only a few points and to compare the results on transport of auxin in *Vallisneria* leaves with those on transport in coleoptiles. The way in which transport in coleoptiles has been studied after decapitation and cutting in sections differs considerably from that followed in the recent work with intact *Vallisneria* plants.

2.15. Auxin transport in Vallisneria leaves

In intact *Vallisneria* leaves and plants auxin was actively absorbed from a concentration of the external solution of 6 μM (ARISZ & WIERSEMA 1966). After uptake in an apical zone auxin moved to adjoining zones over a long distance. There is no loss to the external solution of the auxin which is moving in the symplasm. Neither can any exchange be reported with unlabelled auxin which is added to the external solution. On the contrary the unlabelled auxin is absorbed without exchange for labelled auxin. These data indicate that auxin does not pass passively through the peripheral plasma membrane in any considerable amount. The same behavior has been obtained for several other substances which move in the symplasm after absorption, In leaves with a freshly wounded surface, however, exosmosis of labelled auxin can be shown by placing the cut surface in a small perspex vessel containing water. After some time the wound must be renewed for exosmosis to continue. It seems justified to accept the view that auxin is absorbed into the symplasm of *Vallisneria* leaves by an active process as REINHOLD (1954) found for pea epicotyls. Auxin moves through bridges of parenchyma cells and of bundles like other substances. A strict polarity of auxin transport, as is usual in coleoptiles, has not been found in *Vallisneria* leaves.

2.16. Auxin transport in coleoptiles

In intact coleoptiles auxin is produced in the tip and moves through the parenchyma cells to the base. No auxin is released to the external solution. An intact coleoptile will absorb auxin, if it is administered in a paste with lanoline. Auxin in water or agar does not penetrate easily through the cuticle. When the tip of the coleoptile is cut off, and sections of the stump are used, the cells of the tissue show a remarkable resistance to wounding. They are still capable of active uptake and movement in the symplasm. The auxin is given off at the cut basal surface.

Since absorption occurs at a wound surface, the auxin penetrates freely into the free space of the tissue, where it will also be adsorbed. When later on the tissue is placed in water, a large amount of auxin which has been passively absorbed in the wall free space, will be given off again partly after oxidation.

Auxin which is introduced into the symplasm, seems to be protected there against oxidation. It is not so easily transformed as auxin in the free space (*cf.* POOLE & THIMANN 1964).

Many experiments on transport have been performed with wounded tissue. Uptake and exit of auxin in these circumstances are artificial processes which do not occur in the same way in intact seedlings.

2.17. Active uptake of auxin

REINHOLD (1954) has shown that auxin uptake into the cytoplasm is an active process. The strong uptake into the cytoplasm from extremely low concentrations indicates that there is besides absorption a considerable active accumulation of auxin in the tissue at the place of uptake. A free exit of auxin from wounded tissue can be expected, when the cuticle is removed, since much auxin is absorbed in the cell wall free space. Exit of auxin from the symplasm demands the passage of the peripheral plasma membrane. When the cells are openend by the cutting, leakage occurs through the plasmodesmata from the adjoining cells. WILKINS & MARTIN (1967) have shown in experiments with corn coleoptiles that I.A.A. can be absorbed as well from apical as from basal donor blocks containing I.A.A. The amount of basal absorption is 63% of that of apical absorption. This result indicates that, after cutting, the cells at both ends of a section have an equal capacity to take up I.A.A. The difference has to be ascribed to the polar action of the plasmodesmata. In the discussion of the function of the plasmodesmata we return to this problem (page 44).

There is a widely held opinion that high concentrations of auxin have undesired effects. Such concentrations are considered to be unphysiologically high. It is, however, questionable, whether using lower external concentrations, one could guarantee that after uptake and accumulation the concentration in the symplasm had not increased indeed to an unphysiological level.

Several experiments have been performed in regard to the influence of oxygen on auxin uptake and transport. Under strictly anaerobic conditions, when the tissue with donor and receiving blocks is evacuated and oxygen replaced by nitrogen gas, it is difficult to separate the influence of oxygen withdrawal on 1° uptake into the apoplast *i.e.* the cell wall free space, 2° active uptake through the peripheral plasma membrane, 3° active transport through the plasmodesmata.

According to a note from Thimann, transport becomes like diffusion in absence of oxygen; polarity (almost) disappears. This indicates that polarity depends on a metabolic process.

2.18. Movement of endogenous auxin

Endogenous auxin is presumably present in a low concentration (*cf.* ANKER 1962). Therefore its movement will be less dependent on a concentration gradient and more on other forces. This makes it attractive to study the difference between exogenous and endogenous auxin transport.

GREGORY & HANCOCK (1955) studying endogenous auxin transport in woody

shoots of crab apple have shown that the translocation in this object is not of the type of a diffusion process, but of a movement against a concentration difference. After arriving at the base, auxin leaves the tissue and moves to a block of agar, in a process which seems to be ordinary diffusion. A second artificial wounding does not enhance the exit of auxin. If a blank agar block is applied, the auxin moves from tissue to block. These experiments indicate that endogenous auxin is moved mainly by forces other than a concentration difference, presumably by electric potential differences in the tissue.

The transport of exogenous auxin deviates, since by the active uptake a concentration gradient arises in the tissue, which cooperates with the electric potential differences which regulate the movement of endogenous auxin.

BROWN & WETMORE (1959) showed that exogenous auxin transport in the shoots of pine is more sensitive to the presence and absence of oxygen in the medium than endogenous auxin transport, since it depends on the active uptake of auxin, a process which is sensitive to the amount of oxygen present.

3. THE FUNCTION OF THE PLASMODESMATA

3.1. Longitudinal transport of auxin

It has been known for a long time (TAMMES 1931) that the movement of auxin in coleoptiles follows the longitudinal rows of cells from apex to base. When a permanent torsion is brought about in the upper part of an *Avena* coleoptile, the phototropic and geotropic stimuli are no longer transmitted longitudinally, but follow the direction of the twisted cells *(fig. 8)*. This is in harmony with the present view that the movement from cell to cell makes use of the plasmodesmata.

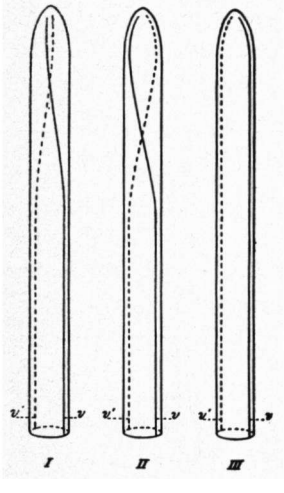

Fig. 8. Experiment of TAMMES (1931). By giving coleoptiles an artificial torsion of the upper part, the curvature moves downwards in the direction of the twisted cells.

3.2. Transverse transport of auxin

In 1960 BRAUNER & APPEL published an interesting experiment on the transverse transport of auxin in coleoptiles. They used *Avena* coleoptiles. At the top of the coleoptile a vertical cut was made between the bundles over a length of 2 mm.

By a second cut one of the halves was discarded. In its place a cubic agar block was set with sides of 2 mm in length. When the coleoptile was placed in a normal vertical direction no auxin entered the agar block, but when the coleoptile was put in a horizontal position with the agarblock below, auxin entered the block *(fig. 9)*. This experiment indicates that in a coleoptile, placed in vertical position, auxin is not moved in a transverse direction through the cells of the apex. When, however, the coleoptile is set in a horizontal direction a movement of auxin into the block occurs *(fig. 9)*. That means that under the influence of gravity, auxin is moved in the coleoptile in transverse direction. At the wound surface it is delivered to the agar block. It may be assumed that gravity influences the movement of auxin in the tissue in transverse direction, when the coleoptile is placed in horizontal position. Analogous results have been obtained by HERTEL & LEOPOLD (1963) and GILLESPIE & THIMANN (1963).

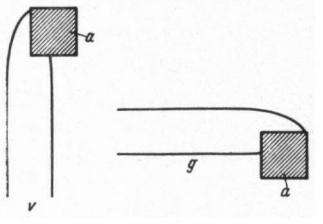

Fig. 9. Experiment of BRAUNER & APPEL (1960). After having made a vertical and a horizontal cut, one half of the apex of a coleoptile is discarded, and replaced by an agar block. Auxin enters the agar block when the coleoptile is placed in horizontal direction.

The latter workers have compared the amount of transverse transport going upwards and downwards in horizontally halved coleoptiles; the downward movement is about 3 times the upward.

In Tammes' experiment transport of auxin occurred in a longitudinal direction. Auxin followed the plasmodesmata in the transverse walls, which have channels in longitudinal direction and moved from cell to cell.

In the experiment of Brauner & Appel a transverse transport was obtained. It used the plasmodesmata in the longitudinal walls which have channels in transverse direction.

3.3. Longitudinal and transverse fluxes

In this trend of thought the following view is obtained. There are in a tissue two fluxes of auxin at right angles: the longitudinal flux and the transverse flux. They pass the intercellular walls by means of plasmodesmata. The first makes use of the plasmodesmata in the transverse walls with channels in the longitudinal direction, the second uses the plasmodesmata in the longitudinal walls with channels in the transverse direction.

The longitudinal flux of auxin is dependent on the concentration difference between apex and base, which is repeated in every cell of a row of cells between top and base of the plasmodesmata. At the same time the flux is influenced by

electric potential gradients in the tissue. The resulting polar transport indicates that a polar influence promotes basipetal transport and reduces acropetal transport. There is also an influence of gravity on the longitudinal transport, promoting the basipetal transport in the normal, vertical position of the coleoptile but reducing it in the inverse position (LITTLE & GOLDSMITH 1967).

3.4. Influence of gravity on transverse auxin transport

In intact coleoptiles, electric potential gradients in the transverse direction are presumably absent. By the influence of gravity curvatures are induced which are proportional to the sine of the angle between the axis of the coleoptile and the direction of gravity in the tissue. The effect is greatest when the coleoptile is in horizontal position. Gravity works then in the direction of the transverse flux through the plasmodesmata. Since every stimulation has a lasting effect on auxin transport a curvature can be obtained by summation of a number of very weak stimulations of short duration, such as arise by irregular rotation of the plant on the horizontal axis of a klinostat.

3.5. Exit of auxin at wounded cells

If the idea that the plasmodesmata are organs which regulate the intercellular flux of substances can be accepted, the exit of substances from the cut wounded cells at the base of a coleoptile section can be considered to be a consequence of the polar flux of substances through the plasmodesmata in the transverse walls. In this way the results of HERTEL (1962) and HERTEL & LEOPOLD (1963) can be interpreted. They stress the significance of secretion of auxin at the base of the cells. WILKINS & MARTIN (1967), however, have shown that active passage of the peripheral plasma membrane even at the base of the cells is always from outside to inside of the cells. Therefore, an exit of substances from a cell which is not a leakage through the peripheral plasma membrane must be a movement through the plasmodesmata. The term secretion for this process may be deceptive since in intact cells it is active transport through a plasmodesma. In cut cells it produces a leakage or an excretion from the tissue.

The conclusion of the discussion on auxin transport is, that different forces can influence the flux of auxin, while it moves through the plasmodesmata. This opens the possibility that symplasmic transport is influenced by electrokinetic potentials and that streaming potentials arise as a consequence of the movement of auxin through the plasmodesmata, *cf* GRAHM (1964), GRAHM & HERTZ (1964), JOHNSON (1965, 1967), KONINGS (1967), NEWMAN (1963), WILKINS (1966).

3.6. Electrokinetic potentials

We shall not consider here the interesting literature which has appeared on electro-phoretic and on electro-osmotic transport in the last few years (DAINTY *c.s.* 1963, WILKINS 1966). Only one point may be mentioned.

SPANNER (1958) has made an interesting study on the translocation of sugar in sieve tubes. He has given an electro-osmotic theory of the function of the sieve plates in sieve tubes. Suffice it to say that the function of the plasmodes-

mata proposed in this paper, has points of similarity with that of the sieve plates in Spanners electro-osmotic theory. The sieve plates may be compared with a complex of plasmodesmata situated side by side. (EVERT & MURMANNIS 1965, ENGELMAN 1965). A conformity of the mechanism of these specialised transport organs with that of simple parenchyma cells would be remarkable. When the sieve tubes age, they seem to change the way in which they move substances and presumably pressure flows arise, but in their youth they follow the same principle as ordinary cells (ARSIZ 1952, KOLLMANN & SCHUMACHER 1962).

3.7. Perception of gravitation

It must be acknowledged that the data are insufficient to discuss here in detail how the movement through the plasmodesmata could be influenced by electric potential and concentration gradients and by gravity.

It is likely that the particles in the plasmatic channels are more or less constrained to files by the orientating action of the walls of the plasmodesmata and the oblong shape of the moving particles.

In this way the moving particles may be more susceptible to the influence of gravity. The data found by Brauner in a long series of interesting experiments on the geoelectric effect, point also to a more direct influence of gravity on transport (BRAUNER 1959, BRAUNER & DIEMER 1967).

3.8. Active transport

Transport in the symplasm consists of two consecutive steps. The first is the movement of substances within the cytoplasm of each cell and the second is the movement from one cell to the other through the plasmodesmata. It is presumably the movement through the plasmodesmata which is influenced by different forces due to concentration differences, pressure differences, electric potential gradients and in addition to the influence of gravity.

It must be emphasized that since transport is the result of the movement of molecules and ions through the symplasm, an essential condition for a continuous transport is to maintain the osmotic and electric potential gradients which regulate the movement.

The concentration differences which produce osmotic flow and movement of molecules and ions and the electric potential gradients in the tissue can only be maintained thanks to the availability of metabolic free energy potentials.

Therefore the process of symplasmic transport is a vital or active process, which continuously needs free energy potentials produced by metabolism. A concentration difference arises as the result of active uptake. The presence of electric potential gradients is known. The mechanism, however, which sustains them, is not known. The electric potentials are not destroyed by low temperature (VAN DER WEY 1934). They disappear on narcosis and are restored afterwards by metabolism, or by external influences.

The mechanism of the movement through the plasmodesmata, of the active uptake through the peripheral plasma membrane and that of the recovery of the acting electric potentials have still to be explained.

3.9. In conclusion

In the preceding survey it was shown that the movement in the symplasm is composed of two parts, intracellular movement through the cytoplasm and intercellular transport through the plasmodesmata which connect the cytoplasm of adjoining cells. The movement in specialized elements such as sieve tubes has been left out of consideration.

Many substances such as salts, sugars, amino acids and auxin move in the symplasm over fairly long distances. If labelled substances are used, autoradiograms display a general pattern of the distribution of radioactivity over a great part of the plant. In this paper particularly the forces which regulate the movement of the substances in the symplasm have been studied. It appeared that it is clarifying to separate their influence on intercellular and on intracellular transport.

In further research the path of transport, the limits of the symplasm over which the substances are distributed and the cooperation of the symplasm with the sieve tubes will have to be investigated. It can be expected that during their stay in the cytoplasm the moving substances will be involved in metabolic processes. They may be changed in different ways. According as more data are obtained about these metabolic influences, greater differences may be found in the pattern of their movement.

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