STUDIES IN TRANSLOCATION

III. THE CYTOPHYSIOLOGY OF THE PHLOEM OF CUCURBITA PEPO

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Summary

The phloem of Cucurbita pepo (Duchesne) consists of respiratorily inert conduit-like sieve tubes of low resistivity to flow, respiratorily active companion cells, possibly specialized for secretion or absorption, and highly vacuolated parenchyma. The sieve pores are open and slime not cytoplasm is present in the lumen. This division of function is interpreted as evidence for the mass flow theory. Active theories are considered improbable.

I. INTRODUCTION

The cytophysiology of the phloem is of fundamental importance to our understanding of the mechanism of translocation. The various active hypotheses assume that cytoplasm is present in the sieve elements, and has an essential role in the translocation mechanism. Thus translocation should be dependent upon the metabolism of the sieve tubes, and possibly the companion cells along the pathway of transport.

In contrast, if mass flow occurs along a hydrostatic gradient, translocation is a passive process, i.e. it is not directly dependent upon metabolic processes in the conducting elements along the pathway of transport. Moreover the sieve-tube elements must be freely permeable in the longitudinal direction.

The electroosmosis hypothesis postulates a mass flow of solution through longitudinally permeable sieve tubes under a potential gradient. If the potential is maintained by H⁺ and HCO₃⁻ ions the respiration of the sieve elements along the pathway of translocation is essential for translocation, whereas the respiration of the companion cells but not that of the sieve tubes is essential if the potential is maintained by K⁺ ions.

These divergent views, which have been described in recent review articles by Arisz (1952), Esau, Currier, and Cheadle (1957), Swanson (1959), and Zimmerman (1960), stress the need for a better understanding of the interrelationship of structure and function in the phloem. The present paper discusses the significance to current hypotheses of translocation of the findings of the authors on the submicroscopic structure of the phloem of Cucurbita pepo (Duchesne) (Duloy, Mercer, and Rathgeber 1961b) and on its respiration (Duloy and Mercer 1961a).

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II. Results

The main structural features and the respiratory characteristics of the phloem of *C. pepo* as determined by the authors are summarized below. Qualitatively, phloem respiration is identical with the respiration of the ground tissue, and appears to proceed via the normal glycolytic pathway and tricarboxylic acid cycle, and not by a peculiar type of respiration unique to the phloem. Quantitatively the respiration rate of the phloem tissue on a fresh weight basis is on the average seven times higher than that of the ground tissue. The highest rates calculated were 1200 µl O₂/hr/g fresh weight for phloem tissue, as compared with rates of 240 µl O₂/hr/g fresh weight for ground tissue. On a protein nitrogen basis the respiration rates are approximately equal.

The electron-microscope studies showed that at maturity the structure of the sieve-element protoplast is very much simpler than that of a normal protoplast. It is enucleate with a conspicuous parietal layer, but contains no ground cytoplasm and only a very few mitochondria. The membranous parietal layer is continuous from element to element lining each sieve pore. The pores are open. Slime occurs in the lumen, and is frequently concentrated at the plate and in the pores. This pattern of distribution we believe to be a fixation artefact. *In vivo* the slime is considered to be distributed more or less uniformly throughout the lumen. The sieve elements are not discrete entities, but form segments of a conduit, the sieve tube, which contains the slime dispersed in a solution phase enclosed by the membranous parietal layer. In contrast the companion cells are richly cytoplasmic with a conspicuous nucleus, numerous mitochondria, ribonucleic acid granules, organelles, endoplasmic reticulum, and vacuoles. The phloem parenchyma cells are highly vacuolated, with only a thin lining layer of protoplasm.

III. Discussion

From a consideration of the respiratory characteristics and submicroscopic structure of the phloem of *C. pepo* a number of conclusions can be reached about the mechanism of translocation.

Cytologically the sieve tube appears incapable of a high level of metabolism. Since a protoplast as usually understood (Mercer 1960) does not occur, the existence of those enzymes located on the endoplasmic reticulum, in mitochondria, and in the ground cytoplasm seems highly improbable. In particular, since ground cytoplasm may be entirely absent, and the number of mitochondria is very low, the sieve tubes of *C. pepo* must be virtually inert as far as oxidative respiration is concerned, and therefore the high respiratory rates observed for *C. pepo* phloem tissue cannot be attributed to the sieve tubes, but must be due to the activity of the companion cells and phloem parenchyma. Since the companion cells contain a very high concentration of mitochondria, and are rich in cytoplasm as compared with the highly vacuolated parenchyma, it seems likely that much of the respiration of the phloem can be attributed to the companion cells. As the sieve tubes occupy about 40% of the phloem (Duloy 1960), the respiration rate of the companion cells may be as high as 2000–3000 µl O₂/hr/g fresh weight of tissue.
Further, the high density of protoplasm which is characteristic of the companion cells suggests that they are metabolically highly active cells. The endoplasmic reticulum and cytoplasmic vacuoles are well developed. Little is known about the function of these structures in plant cells. But they are well developed in animal cells which are specialized for secretion, e.g. in the pancreas; and Rathgeber and Mercer (unpublished data) have found that the endoplasmic reticulum is well developed in nectary tissue which is specialized for the secretion of sugars. It is possible, therefore, that the companion cells are specialized for the secretion or absorption of substances from neighbouring cells or for both of these functions.

Thus, a characteristic feature of the phloem in *C. pepo* is the association of respiratorily highly active companion cells, possibly specialized in secretion or absorption, with respiratorily inert conduit-like sieve tubes. This division of function within the phloem has considerable significance to our understanding of the process of translocation, and will be considered in relation to current theories.

(a) *Active Theories*

Active theories require the direct participation of the sieve-tube cytoplasm, and assume that the mechanism is directly dependent on the respiration of the sieve tubes. The various active theories are based on three main lines of evidence:

1. Certain light- and electron-microscope observations which have led some workers, e.g. Curtis (1935), Kursanov and Turkina (1952), and Hepton, Preston, and Ripley (1955), to the view that the mature sieve element contains a complete layer of "normal" cytoplasm, i.e. that the element is essentially similar in structure to other living plant cells.

2. The calculations of Kursanov and Turkina (1952) of very high rates of respiration in the phloem tissue which led these workers to suggest the possibility of a direct active transfer mechanism operative in the conducting cells.

3. The experiments showing that low temperatures and respiratory inhibitors applied to a point along the path of movement inhibit translocation (Esau, Currier, and Cheadle 1957; Swanson 1959; Zimmerman 1960). Inhibition is explained as resulting from the action of the inhibitor on either the enzymes of the active transport system, or on the respiratory enzymes of the sieve tubes and surrounding cells. Discussion of the inhibitor and temperature effects will be deferred until Section III(c).

The findings of our electron-microscope studies (1961b) are at variance with those of most previous workers, and indicate that the mature sieve elements of *C. pepo* are devoid of "normal" cytoplasm, and are therefore respiratorily inert. Clearly these findings raise serious objections to the first two lines of evidence listed below. The possibility remains that the parietal layer or the slime could be the site of an active transport system which is linked by an energy-supplying process to the respiration of the companion cells. The temperature and inhibitor effects could then be explained as resulting from the action of the inhibitor on the active sites of the slime or parietal layer, or on the respiration of the companion cells.
Such an active transfer system could be either of two general types: (1) a specific transfer system such as envisaged by Arisz (1952) or Kursanov and Turkina (1952), and possibly similar to those involved in sucrose accumulation in parenchyma cells; or (2) a non-specific system, such as streaming involving the slime or parietal layer, "activated" or surface diffusion along the slime and parietal layer, or electroosmosis. Apart from electroosmosis, which will be considered later, any of these mechanisms would require enzymes within the sieve tube linked in some way with the companion cells, possibly by the movement of ATP–ADP or TPN–TPNH between the companion cells and the sieve tubes. Assuming a yield of 36 molecules of ATP per molecule of glucose oxidized, Duloy (1960) calculated for C. pepo that $2 \times 10^{-3}$ mole ATP was produced for each mole of sucrose translocated along the length of one sieve tube. These values are some thousand-fold smaller than those estimated for sucrose-uptake systems in other plant cells, e.g. 5 moles ATP per mole sucrose absorbed by sugar-cane tissue (Bieleski 1959), suggesting that a specific transfer system, similar to a sucrose-uptake system, is not involved in translocation in C. pepo.

For a non-specific system the ratio of the amount of sugar translocated to the energy yield of the respiration of the companion cells might be expected to vary with the concentration of sucrose in the sieve tubes. It is possible that the energy yield of the respiration of the phloem and the amount of slime in C. pepo are great enough to provide an activated surface for transport. In contrast slime is almost entirely absent from many species of sieve tube (Esau 1950) suggesting that it is not essential for translocation. The small volume of the parietal layer relative to the large volume of the solution phase in the sieve tube suggests that it could not provide a large enough surface or the quantity of enzymes necessary for translocation. Therefore translocation is not likely to involve a non-specific mechanism in which slime or the parietal layer provides the actual surface for activated diffusion or the enzymatic machinery for the translocation process.

It seems likely therefore that translocation does not involve either specific or non-specific active transport mechanisms located in the sieve tubes.

(b) Electroosmosis

Electroosmosis is consistent with the general structure of the phloem, namely metabolically inert sieve tubes of low resistivity in contact with metabolically active companion cells and parenchyma. Despite this general agreement several features of phloem structure are difficult to reconcile with electroosmosis. The electroosmosis system proposed by Fensom (1957), in which the potential is assumed to be maintained by the respiratory activity of the sieve tubes, can be dismissed for C. pepo since the sieve tubes are respiratorily inert.

If the sieve pores are open the maintenance of a diffusion potential with a steep gradient across the plates may be impossible. The concentration gradient of K+ responsible for the potential arises, according to Spanner (1958), from the secretion of potassium on one side of the plate followed by its absorption on the other side of the plate. Known rates of flow would prevent the development of
steep diffusion gradients across plates with open pores unless the rates of secretion and absorption are extraordinarily high, and limited to the extreme ends of the companion cells next to the plates. On the other hand the destruction of the diffusion potential by mass flow would not occur if the movement of the K\(^+\) ions is retarded in some way. In *C. pepo* there is abundant slime dispersed in the open pores which, because of its proteinaceous composition (Mercer and Withie (unpublished data) have detected a number of amino acids in the hydrolysate of the slime of *C. pepo*), may absorb the K\(^+\) ions, and allow the necessary potential gradients to develop. Despite this possibility, the fact that each sieve element in *C. pepo* is in contact with a linear series of four to six companion cells and not with a single companion cell along its entire length is against electroosmosis. The middle cells of the series would appear to have no role in the process, and presumably the circulation of K\(^+\) would be limited to the end cells of the series. As the volume of the end cells is only about 1/1000th that of the sieve tube these cells would have to possess an extraordinarily high rate of uptake of K\(^+\) in order to maintain a steep concentration gradient for K\(^+\) across the plate. Also Crafts (cited by Spanner 1958) has already pointed out that the cyclic movement of potassium requires the juxtaposition of the sieve plates and walls of the companion cells—this structural arrangement is not found in all plants, and, as admitted by Spanner (1958), an alternative structure is needed in many plants.

As a general mechanism for translocation electroosmosis seems improbable because of (1) the incorrect structural arrangement of the transverse walls of the companion cells and sieve plates in many plants, (2) the small amount of slime in the sieve tubes of many species of phloem, and (3) because of the open sieve pores.

*(c) Mass Flow*

The mass flow hypothesis, in its present form, requires freely permeable sieve plates, and a semipermeable barrier along the side walls of the sieve tubes to prevent the lateral leakage of solutes from the sieve tubes. Bauer (1953) suggests that sucrose is transferred across the semipermeable barrier by an active process located in the cells surrounding the sieve tubes.

Our observations on the submicroscopic structure of the phloem in *C. pepo* are consistent with this modified mass flow hypothesis. The sieve tube has a conduit-like structure with open pores which presumably would offer little resistance to free flow, and the sieve tubes are lined by a membranous parietal layer which could be the site of the permeability barrier to the lateral leakage of solutes.

The undoubted plasmolysability of sieve tubes (Schumacher 1939; Rouschal 1941; Currier, Esau, and Cheadle 1955) (which indicates that the parietal layer is impermeable to sucrose) has been interpreted as demonstrating that the sieve tubes are completely enclosed by a semipermeable barrier, and therefore mass flow is excluded. Currier, Esau, and Cheadle (1955) have noted the difficulty of reconciling this interpretation with the phenomenon of surge along the sieve tubes during plasmolysis and deplasmolysis. These difficulties, as well as the long-standing
confusion centred around the plasmolysis of sieve tubes, can be explained on the basis of the submicroscopic structure of the sieve elements.

Ignoring, for the moment, the diffusion of solutes through the cut ends of sieve tubes in a section of phloem tissue immersed in a hypertonic solution of sucrose, the form of plasmolysis should be determined by the continuity of the parietal layer. Water will diffuse outwards from the sieve tubes through the parietal layer, and sucrose will diffuse inwards through the lateral walls allowing the parietal layer to shrink from the walls, but because of the continuity of the parietal layer from element to element through the pores, it should remain attached at the plates. That is, the plasmolysis forms, as observed with the light-microscope, should appear concave along the lateral walls and attached at the plate, as is observed in plasmolysis studies (Currier, Esau, and Cheadle 1955).

Open (i.e. freely permeable) sieve pores, are not likely to prevent plasmolysis or allow deplasmolysis. The plasmolysis of any cell depends upon the rate of loss of water, the rate of penetration of the plamelyzing solution into the protoplast, and the rate of penetration of the plasmolyzing solution through the cell walls to allow the collapse of the protoplast away from the walls. If the parietal layer is semipermeable the penetration of the plasmolyzing solute (sucrose) is limited to the cut ends of the sieve tubes. This is likely to be slow. The half-time for the diffusion through the ends to the centre of a cylinder 1 cm long (the length of sections frequently used in plasmolysis studies) is about $2\frac{1}{2}$ hr. In sieve tubes the half-time would be much longer as the area available for diffusion is reduced by the sieve plates, and also the pores may be blocked by slime following the surging movement of cell contents during the isolation of the phloem tissue. In contrast, the loss of water from the sieve tubes is likely to be rapid since diffusion occurs through the lateral wall surfaces, and the diffusion path is short. Under these conditions the loss of water (diffusion over entire surface) is likely to be considerably greater than the penetration of sucrose (diffusion through cut ends), and plasmolysis of the sieve tube must result. Deplasmolysis will not follow unless the osmotically active contents of the sieve tubes increase. Since there will be a simultaneous diffusion of cell solutes from the sieve tubes, through the cut ends, an increase in the osmotic concentration may take some considerable time. Indeed deplasmolysis may never occur if all the osmotically active constituents of the sieve tube are freely diffusible. Thus the fact that deplasmolysis is not observed in short-term experiments of a few hours should not be taken as evidence for the presence of a semipermeable barrier across the sieve plates.

Finally the continuity of the parietal layer and the open sieve pores would account for the surging movement of cell contents observed in sieve tubes transferred from hypotonic to hypertonic solutions.

As far as C. pepo is concerned, therefore, the anatomy of the sieve plate and sieve pores is not a weak point in the mass flow hypothesis. However, several difficulties remain: first the presence of slime in the sieve tube and sieve pores; and secondly, the classical objection that the blockage of translocation by metabolic inhibitors applied to a point along the path of movement is contrary to mass flow. These problems are discussed below.
The objection that the mass flow hypothesis does not explain the inhibition of translocation by respiratory inhibitors applied to a point along the pathway of movement requires rigorous examination before it can be accepted. The degree of inhibition produced by respiratory inhibitors ranges from zero to about 50% with very few observations of greater inhibition (Willenbrink 1957). Yet one might expect a much larger percentage inhibition with the concentrations used since active processes such as salt uptake, sugar uptake, and protoplasmic streaming appear to be extremely sensitive to respiratory inhibitors. Lack of more significant inhibitions is usually attributed to a lack of penetration of the inhibitors into the phloem, but this seems an improbable explanation for those experiments where inhibitors were applied directly to exposed bundles. Swanson (1959) has already pointed out that the interpretation of the effects of petiole-applied inhibitors is complicated by the possibility of the inhibitors being transported via the xylem to the lamina where they may inhibit translocation by affecting the primary uptake processes. Such a possibility is consistent with the highly variable results obtained with petiole-applied inhibitors and by Bauer’s (1953) observations that translocation is more effectively blocked by inhibitors applied to the lamina rather than to the petiole. Also inhibitors applied to the petiole may enter the phloem, be translocated to the sink, and inhibit translocation by inhibiting the movement of solute from the sieve tubes to the living cells of the sink.

Another possibility is that inhibitors may reduce translocation by increasing the viscosity of the sieve-tube sap, and the resistance of the pores by affecting the colloidal state of the slime.

Apart from these possibilities it may be argued that an inhibition of translocation might be expected following the application of an inhibitor, if the maintenance of the semipermeable membrane of the sieve tubes is dependent on the metabolism of the companion cells. Zimmerman (1958) has obtained evidence for a metabolic maintenance of sieve-tube semipermeability.

The inhibition of translocation by low temperatures has also been used as evidence for active transport, but for reasons similar to those already discussed this explanation seems unlikely. An inhibition of translocation is to be expected from viscosity changes alone, although the magnitude of this effect is difficult to assess. If flow through sieve tubes obeys Poiseuille’s Law the volume transported should vary inversely with the viscosity. As the viscosity of a 20% sucrose solution ranges from 1·5 cP at 30°C to 3·15 cP at 5°C, 50% inhibitions may be expected from temperature alone. The situation is more complicated as an increase in resistance to flow should increase the hydrostatic pressure and the two would compensate to an unknown degree. It is suggested, therefore, that temperature could act by affecting the viscosity of the sieve-tube sap, and the inhibitors by affecting the primary-uptake processes at the source or at the sink or by affecting the semipermeability of the parietal layer, but not by affecting an active translocation process in the sieve tubes.

It is difficult to imagine the role of slime in mass flow. The quantity of slime in the exudate of C. pepo is far greater than the amount held in the layer of cut elements, demonstrating that it can move from element to element through the
sieve pores over considerable distances. That is, under conditions of exudation, slime appears mobile. As it does not appear to accumulate at the sink end of the sieve tubes in the intact plant, slime may form a more or less immobile colloidal phase in vivo. Under these conditions mass flow would require the movement of the solution phase between the micellar framework of the slime phase. But since slime is almost absent from some species of sieve tube its presence cannot be essential for the translocation process. It may, if present, because of colloidal properties, assist in maintaining the turgor of the sieve tubes.

It is stressed that the present analysis of the translocation problem centres around the state and role of the slime in vivo. For reasons discussed earlier by the authors (Duloy, Mercer, and Rathgeber 1961b) we are of the opinion that the slime is dispersed more or less uniformly throughout the lumen in vivo, and that the concentration observed on the sieve plates and in the sieve pores in electron-micrographs is an artefact of preparation. Even if this view proves incorrect any explanation of translocation would have to account for the fact that slime not cytoplasm is present in the sieve tubes of C. pepo. The conclusions are based on observations made on highly specialized phloem. Similar studies should be undertaken on phloem in which the sieve pores are known from light-microscope studies to be small.

(d) Conclusions

From the discussion it is concluded that translocation in mature sieve tubes is by mass flow, and not by active transfer mechanisms. This being so, the role of the companion cells and phloem parenchyma requires an explanation. At present we can only speculate. It seems possible that the companion cells, which appear to be metabolically highly active, are involved in the lateral movement of sugar and other solutes, in either direction between the sieve tubes and the surrounding tissue. In addition, the companion cells may be involved in maintaining the structure of the respiratorily inert sieve tubes. The highly vacuolated structure of the phloem parenchyma suggests that these cells may be concerned with the temporary storage of solutes during their lateral transport between sieve tubes and other ground tissues.

Thus on the basis of the structure and respiratory characteristics of the phloem of C. pepo it is concluded that the mechanism of translocation in mature sieve tubes is osmotic mass flow: with the companion cells involved in the active lateral transfer of solutes between sieve tubes and the surrounding cells and possibly with the maintenance of sieve-tube structure, and with the parenchyma cells functioning in the temporary storage of solutes.

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V. References


