THE METABOLISM OF PHLOEM ISOLATED FROM GRAPEVINE

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Summary

Small slices of phloem tissue have been isolated from grapevine canes and cultured in sugar–buffer solutions, and measurements made of the respiration rate and of the changes of sugar concentration in the tissue. Calculations from these measurements of the permeability of the tissue to sucrose, and of the rate of sucrose synthesis from glucose and fructose, show that the phloem is not very different from other plant tissue in either activity. The bearing of these findings on our knowledge of the translocation of carbohydrates by the phloem is discussed.

I. Introduction

The unique structure of phloem has not been shown to be related to its unique capacity for rapid translocation of sugars, nor, in spite of various attempts, have unusual features been found in its physiology which might be related to its capacity for transport. There was a period when plant physiologists believed that sieve tubes were completely permeable to solutes and therefore could not be plasmolysed, but it has been shown recently (Currier, Esau, and Cheadle 1955) that if sufficient care is taken in the preparation of phloem slices, the sieve tubes can be plasmolysed and deplasmolysed repeatedly. Their permeability to solutes cannot therefore be as high as was thought. Very high respiration rates for excised vascular bundles of *Plantago* have been reported by Kursanov and his colleagues (Kursanov 1956) who claimed that this was related to their capacity for transport. Other workers have found difficulty in repeating this work, and no one else has recorded rates of anything like the maximum value given by Kursanov (5000 μl CO_2/g fresh wt./hr). For a review of the early work on this aspect of the problem, see Esau, Currier, and Cheadle (1957). Willenbrink (1957) has measured the respiration of excised vascular bundles of *Pelargonium*, and Ziegler (1958) those of *Heracleum mantegazzianum*, both recording rates of CO_2 production around 220 μl/g fresh wt./hr.

A principal difficulty in all studies of phloem has been that it is very easily damaged, and most statements about its anatomy and physiology have been criticized on the grounds that cutting the tissue upsets its organization by the release of tensions and pressures. The preparation (by Currier, Esau, and Cheadle 1955) of phloem sections sufficiently undamaged to permit the first observations of plasmolysis seemed to us to offer the possiblity of making physiological measurements on phloem that had suffered a minimum of damage. The respiration rate of the isolated tissue is of interest in view both of the discrepancy between the results of Kursanov and those of Willenbrink and Ziegler, and also of the general uncertainty about

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the energy supply to the process of translocation. The permeability of the phloem cells to sugars and particularly to sucrose, the translocated sugar, is of interest in any consideration of movement of sugars through the phloem or between phloem and surrounding tissue. Finally, any inferences that can be drawn about enzymes present in the tissue and their activities will provide facts that may help in constructing hypotheses to explain the translocation process.

Another consequence of this sensitivity of the phloem to damage is that the systems on which we may study the translocation process are always complicated by other processes. Nothing simpler than an intact plant with some defining cut or excision has been found to carry on translocation unimpeded and yield data of rates and quantities and paths of movement. The experimental investigation of the process would be much simplified if some simple system could be found that would translocate sugar, and, with this in mind, attempts were made to measure translocation along the short pieces of phloem prepared like those of Currier and his colleagues.

II. Methods

(*a*) *Material*

Pieces of phloem were obtained from canes of *Vitis vinifera* (L.) in a way very like that used by Currier, Esau, and Cheadle (1955) in isolating tissue for plasmolysis studies. A 1-ft length from the middle of a current season's cane was placed immediately on cutting in the 0·25M sucrose–buffer (Currier, Esau, and Cheadle 1955). From the middle internodes of this piece, short pieces were cut under buffer solution with a jeweller's saw; and from these short pieces tangential sections were cut, still under buffer solution, at a thickness of 100 μ on a small horizontal microtome. These sections comprised phloem with a strip of periderm on either side. The strips of periderm were easily detached, leaving a piece of phloem (sieve tubes, parenchyma, and fibres) about 2 by 0·5 cm, and 100 μ thick, weighing about 14 mg. The bands of sieve tubes were separated by bands of phloem rays (Fig. 1). Up to six sections containing phloem tissue could be cut along one radius of the cane, and of these only the third and fourth were used.

Ten to 30 of these slices were cultured in 10 ml of a solution consisting of the sugar–phosphate buffer to which was added sulphanilamide (0·001M). All buffer solutions had an osmotic pressure equivalent to 0·25M sugar, and when the metabolic sugar concentration was varied, the remaining osmotic pressure was made up with mannitol. Buffers containing mannitol as the only sugar were used in some experiments in which the behaviour of the tissue was studied in the absence of external substrates.

(*b*) *Estimation of Sugars*

Sugars in the tissue slices were estimated thus: a sample of slices was withdrawn from the culture, rinsed in mannitol buffer and in distilled water, and placed in hot 70 per cent. ethanol. Two further extractions were made with 70 per cent. ethanol, and the pooled alcohol solutions evaporated and chromatographed on acid-washed Whatman No. 3 paper with the descending eluent ethyl acetate–pyridine–water (80 : 20 : 10 v/v) for about 20 hr. The spots corresponding to suc-

rose, glucose, and fructose were eluted from the chromatogram and determined by the copper-reduction method of Wager (1954). The sucrose was hydrolysed before estimation by holding it at 100°C for 20 min in 0·01N HCl.

(c) *Respiration*

Two types of respiration measurements were made on the phloem slices. In one set of experiments, the slices were aerated in small glass tubes and the carbon dioxide content of the effluent gas measured in an infra-red gas analyser (IRGA) by comparison with an identical stream which was passing through a similar tube

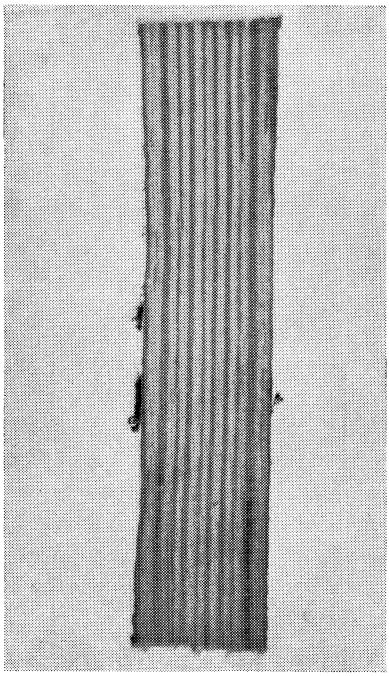

Fig. 1.—Slice of phloem tissue like those used for the experiments. The pale bands consist of sieve tubes and fibres, the dark bands of ray parenchyma. The strips of periderm on either side have been removed.

with no tissue. In the other set of experiments, the carbon dioxide output was measured in a conventional Warburg manometer. Values obtained in the two systems for the same set of slices agreed to within 5 per cent.

III. Results

(a) *Respiration*

In 0·25M sucrose, glucose, or fructose buffer, the rate of evolution of carbon dioxide, as measured in either the IRGA or the Warburg manometer, was 220–230 μl/g fresh wt./hr. These values are not extraordinarily high for excised plant

tissue, and are consistent with those of Willenbrink (1957) and Ziegler (1958). Both these workers found the carbon dioxide production of the vascular bundles to be much higher than that of the neighbouring parenchyma, but in the vine cane there is, of course, no neighbouring parenchyma for comparison. The xylem, cut in similar sections on the same radius, gives a carbon dioxide output of the same order as that of the phloem. This also agrees with Ziegler's work on *Heracleum*, where the xylem and phloem from a bundle hardly differed in their respiration rates.

(*b*) *Changes in the Sugar Content of the Phloem after Cutting*

The changes in the sugar contents of the phloem slices in aerated mannitol buffer are shown in Figure 2. There is present on cutting about 2·5 mg/g fresh wt. of sucrose and about 0·5 mg/g fresh wt. of each of fructose and glucose, a point to which we shall return in Section IV. The pattern shown in Figure 2 of falling

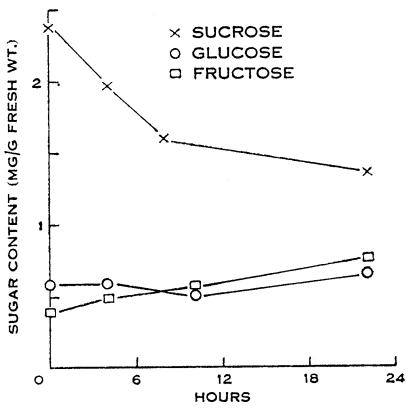

Fig. 2

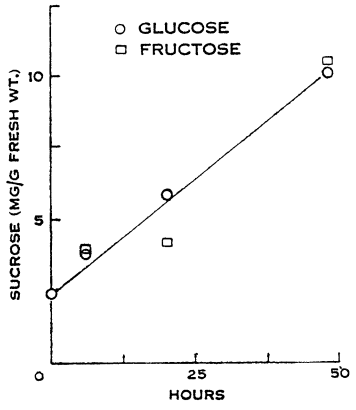

Fig. 3

Fig. 2.—Changes in the sugar content of the phloem during culture in mannitol buffer. The initial rate of sucrose loss corresponds to an output of respiratory carbon dioxide of 82 μl/g fresh wt./hr.

Fig. 3.—Synthesis of sucrose: the rise of sucrose content of the phloem in glucose and fructose buffer solutions. The slope of the line corresponds to a rate of synthesis of 0·2 mg/g fresh wt./hr.

sucrose, and slightly rising hexose concentration, is typical of other excised plant tissue in the first hours (James 1953). The initial slope of the sucrose curve in this graph corresponds to a carbon dioxide output of about 82 μl/g fresh wt./hr. Direct measurement of this in the experiment quoted above gave about 90 of the same units.

(*c*) *Synthesis of Sucrose*

If the cultured phloem tissue is supplied with either glucose or fructose in the buffer solution, sucrose is rapidly synthesized in the tissue. The sucrose content rises from the initial value of about 2·5 mg/g fresh wt. as shown in Figure 3. The rate of increase is about 0·2 mg/g fresh wt./hr.

(*d*) *Permeability of the Phloem to Sucrose*

From the measurements of increase in sugar content of the tissue cultured in sucrose buffer, we can estimate the permeability of the phloem to sucrose. Figure 4 shows the changes in the three sugars with time in four different concentrations

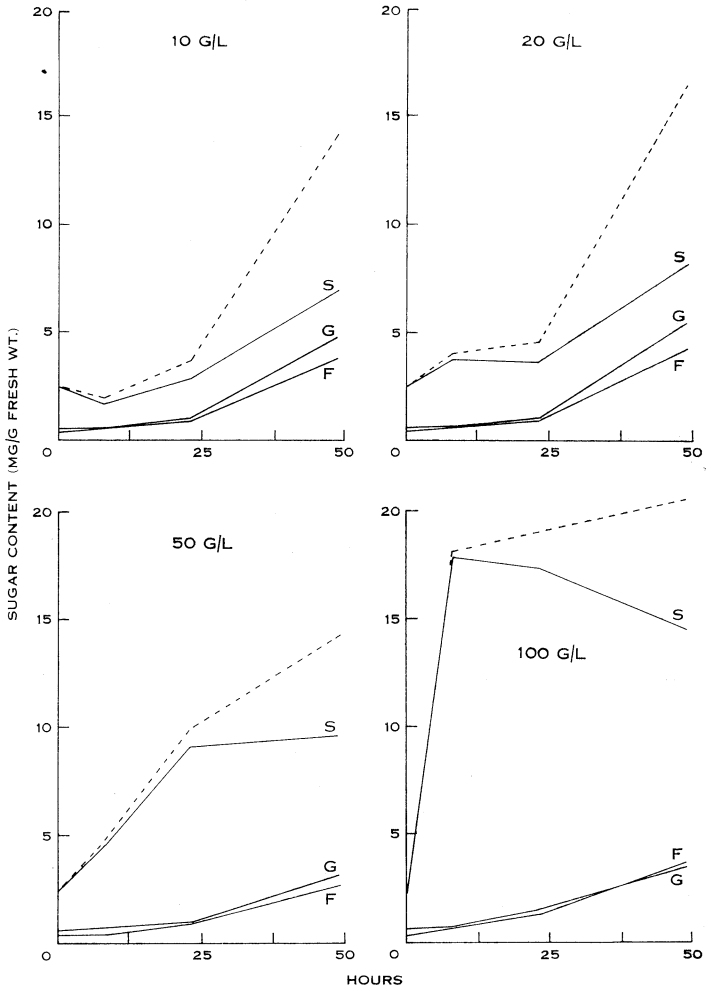

Fig. 4.—Permeability of phloem tissue to sucrose. The changes are shown of the sucrose, glucose, and fructose contents of the phloem when cultured in four different concentrations of sucrose buffer: 10, 20, 50, and 100 g/l. The extra glucose and fructose produced with time are presumably products of sucrose breakdown, and the dotted curves represent the measured sucrose content plus the additional sucrose which would produce the measured increases in glucose and fructose.

of sucrose buffer. The increases in glucose and fructose are presumably due to breakdown of sucrose in the tissue, and the dotted curves are the sucrose values obtained by adding at each estimation the extra glucose and fructose. The initial

slopes give the rates of increase: that in 10 per cent. sucrose buffer represents a permeability of 2 mg/g fresh wt./hr.

(e) *Translocation along Pieces of Phloem*

Attempts were made to reveal transport of sucrose along isolated slices of phloem by feeding ^{14}C-sucrose to one end of a slice while the other end was supported over a metal slit under which was a Geiger tube. If rates of movement anything like those found for sucrose in intact phloem could be realized in the isolated system, radioactivity should be readily detected at the far end of the slice in a few minutes. We have to record our failure to observe any transport of radioactivity in the slices, just as Ziegler failed to observe any transport along isolated vascular bundles of *Heracleum*.

IV. Discussion

The tissue isolated by the technique described and used for the experiments has been referred to as "phloem", and in the morphological sense it is so, but from the physiological point of view it is a heterogeneous tissue not all of which may be concerned with sugar transport. As will be seen in Figure 1, the paler bands of elongated cells are separated by darker bands of isodiametric ray cells which are mostly filled with starch. The paler bands contain many sieve tubes, but also many fibres. The observed reactions may therefore be reasonably described as characteristic of phloem in its broad sense, but not of sieve tubes only since these comprise probably less than half of the bulk of each slice. The observations may therefore be interpreted as related to translocation only in so far as the whole phloem is concerned in translocation, or if the reactions of the sieve tubes alone are important, we must realize that these have been diluted by other cells.

The results presented above are unremarkable. The physiology of slices of phloem is little different from that of other isolated plant tissue in that each of the values obtained can be paralleled by work on other plant parts. A respiration rate of 220 μl CO_2/g fresh wt./hr is perhaps a little higher than would be expected of most tissues in sugar buffers, but small compared with values like those recorded for some flower parts (800 μl/g fresh wt./hr). A rate of sucrose synthesis of 0·2 mg/g fresh wt./hr is comparable with those recorded by Nelson and Auchincloss (1933) for potato tuber slices, 0·38 of these units; by McCready and Hassid (1941) for barley shoots, 0·35; and by Porter and May (1955) for *Nicotiana*, 0·2. Permeability to sucrose at the rate of 2 mg/g fresh wt./hr is smaller than that measured by Weatherley (1953, 1954, 1955) for leaf disks of *Atropa* (5 of these units), but he states that this leaf has an abnormally high permeability. The more usual rate of penetration appears from his data to be around 1 mg/g fresh wt./hr. Porter and May (1955) record their permeability data in terms of mg/12 leaf disks/8 hr for *Nicotiana* leaves, but rough estimates of the area–density of tobacco leaves applied to their results yield a value of about 0·25 mg/g fresh wt./hr at 5 per cent. sugar in the solution. The phloem may thus be rated as highly permeable to sucrose.

Although nothing remarkable has been revealed about the physiology of phloem tissue, the results are nevertheless of some interest in the wider field of

translocation studies. The mere measurement of the sucrose content of phloem is interesting since all speculations about sucrose movement involve guesses at the concentration of the solution which is moving. Most workers have favoured a concentration of about 20 per cent. sucrose for the translocation solution, arguing from the measured rates of transfer of dry weight, and these estimates have received some support from the measured concentration of sucrose in phloem exudates which are commonly around 17–20 per cent. The question of whether the exudate represents either the contents of the sieve tubes or the translocated solution is still a vexed one, and has not been made any clearer by the fact that whenever measurements of sucrose content have been made on isolated phloem the sucrose is about 1 per cent. of the fresh weight. The present estimate of 2·5 mg/g fresh wt. in freshly isolated phloem is even lower. If sieve tube sap contains 25 per cent. sucrose this must occupy only one-hundredth of the phloem, and if this is so, it is difficult to see where the reserves of sap come from to supply large drops of exudate. From microscopic measurements, the proportion of the phloem occupied by sieve tube lumina has been generally estimated as about one-fifth. There is a paradox here that lies at the basis of the translocation problem.

The measurements of respiration rates of phloem presented in this paper constitute additional evidence that the very high rates reported by the Russians must be regarded with suspicion. Not only is the respiration rate of vine phloem remarkably similar to those of *Pelargonium* and *Heracleum* when supplied with sugar substrate, but when respiring only its endogenous substrates attains the modest figure of 90 μl CO_2/g fresh wt./hr. It is likely that this is closer to the rate in the intact vine cane, and is unlikely to represent a large part of the energy supply to translocation.

The high permeability of the phloem to sucrose is of some interest in relation to the question of plasmolysis of sieve tubes, for, as Weatherley (1955) points out, a sucrose permeability of this order will seriously alter the values obtained for suction pressure of the tissue by any plasmolytic method using sucrose as plasmolyticum. The values obtained by Currier, Esau, and Cheadle (1955) for suction pressures of sieve tubes may well be too high. They record that grapevine phloem often requires a 2M sugar solution to plasmolyse it, corresponding to a suction pressure of about 150 atm. The results presented above suggest a partial return to the older standpoint of permeable sieve tubes, and may explain some of the early difficulties in plasmolysing them.

Our failure to reveal any transport of labelled sucrose along phloem slices and the similar failure of Ziegler seem to indicate that although phloem isolated carefully from the plant can carry on many of its activities (including respiration and sucrose synthesis), and can be plasmolysed, its essential capacity for transport has been destroyed. It is becoming doubtful whether such measurements as those of Kursanov, Willenbrink, Ziegler, and those presented in this paper can ever tell us about those peculiar aspects of phloem physiology that are related to translocation. We have studied excised phloem and found it to be not very unlike other plant tissues. The remaining problem is more difficult, to study the physiology of the phloem while it is still on the plant and actively engaged in translocation.

V. Acknowledgment

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