ACCUMULATION AND TRANSLOCATION OF SORBITOL IN APPLE PHLOEM

By R. L. Bieleski*

[*Manuscript received October* 21, 1968]

Summary

In intact apple plants, sorbitol, rather than sucrose, is the main carbohydrate involved in phloem transport. The behaviour of excised phloem, either freshly excised (fresh) or washed for 20 hr after excising (aged), towards sucrose, glucose, and sorbitol was studied. All three carbohydrates were accumulated rapidly, rates being higher when more concentrated solutions were supplied and when aged tissues were used. Both effects were more marked for sorbitol than for sucrose or glucose. When sucrose or glucose was accumulated by fresh or aged phloem, sucrose was the main product and no sorbitol was formed. When sorbitol was accumulated it was the main product, though in aged phloem sucrose was also formed. In comparison with sucrose sorbitol is readily accumulated from the more concentrated solutions, but is only slowly metabolized by phloem tissues. It is suggested that, in the intact plant, sorbitol, which is present in the leaf in high concentration, is preferentially accumulated into the phloem, but once there is metabolically rather inert and so is not altered until it reaches its destination. The tissue there, like aged phloem, can readily utilize sorbitol. Thus, sorbitol is well suited to translocation in apple.

I. Introduction

Sorbitol appears to be an early and major photosynthetic product in both apple (Hutchinson, Taper, and Towers 1959) and plum (Anderson, Andrews, and Hough 1961, 1962). Webb and Burley (1962) have suggested that sorbitol is also the major carbohydrate being translocated in apple phloem, though Whetter and Taper (1966) reported sucrose as the translocation product in apple seedlings. In addition, excised apple phloem readily accumulated sucrose from external solution, but failed to form any sorbitol (Bieleski 1966*a*). Possible causes for this apparent discrepancy in the role of sorbitol can be suggested. Webb and Burley (1962) studied the whole bark, not the phloem alone, and the sorbitol could have been a storage product in the cortex rather than in the phloem itself. The probability of this having happened is uncertain as the authors give no data on the duration of translocation, and any such redistribution would be much more pronounced after long translocation times. On the other hand the excised phloem used by Bieleski (1966*a*) had been removed from its normal source of carbohydrate, the leaf, and possibly the phloem was not able to synthesize sorbitol, though able to translocate it. Alternatively, the process of aging the phloem tissue (wherein the phloem pieces are aerated for 18 hr in several changes of 10^{-4}M $CaSO_4$) might have changed its metabolic behaviour. The experiments described in this paper were performed in order to learn more about the role of sorbitol in the translocation and accumulation of carbohydrate by apple phloem.

* Fruit Research Division, DSIR, Auckland, N.Z.

II. Materials and Methods

(a) *Materials*

Year-old, long shoots of *Malus sylvestris* (L.) Mill. cv. Granny Smith were either used on the tree (in translocation experiments), or were harvested and stripped to provide excised phloem tissues for accumulation experiments. Phloem was obtained free of both wood and stem cortex by the stripping procedure used earlier (Bieleski 1966*a*), and the pieces obtained were held at 0°C in 10^{-4}M $CaSO_4$ plus 10 mg/l ascorbic acid until the required amount had been collected. The pieces were then cut into 1·5-cm lengths and washed at 24°C on a rotary shaker in 10^{-4}M $CaSO_4$, either for 30 min in three changes of solution (fresh tissue) or for 20 hr in six to eight changes (aged tissue). [^{14}C]Glucose, [^{14}C]sucrose, and sodium [^{14}C]bicarbonate were obtained from the Radiochemical Centre, Amersham; [^{14}C]sorbitol was synthesized from [^{14}C]glucose by the method of Anderson, Andrews, and Hough (1962).

(b) *Nature of Carbohydrate in Translocating Phloem*

This experiment repeated that of Webb and Burley (1962) in more detail. A mature apple leaf was enclosed in a small plastic bag, which was then sealed around the petiole with adhesive tape and modelling clay. $^{14}CO_2$ was generated in a 500-μl gas-tight hypodermic syringe (Hamilton Co.) by sucking into the syringe first 50 μl (92 μCi) $NaH^{14}CO_3$ solution then 100 μl 10% perchloric acid. After the bubble of $^{14}CO_2$ had formed, excess solution was expelled from the syringe and the $^{14}CO_2$ remaining was injected into the plastic bag surrounding the leaf. The hole in the plastic was immediately sealed with a piece of adhesive tape. Photosynthesis was allowed to continue for 10 min, 20 min, 1 hr, or 3 hr, then the entire long shoot was harvested. The leaf blade and petiole were separated and killed in 10 and 3 ml respectively of methanol–chloroform–water (12 : 5 : 3 v/v/v) at −72°C, then phloem and cortex were stripped from the remaining stem. The phloem strip was cut into two pieces, corresponding to the regions 1 cm above to 2 cm below, and 2 cm below to 6 cm below the petiole junction; a sample of bark and of wood was taken from the region 1 cm above to 2 cm below the petiole junction; and each of the four samples was killed in 3 ml methanol–chloroform–water at −72°C. Sugars and amino acids were extracted by the procedure of Bieleski and Turner (1966).

(c) *Separation of Sugars and Amino Acids*

Sugars, amino acids, and sugar phosphates were separated into these three groups by thin-layer electrophoresis (TLE) on a cellulose layer at pH 2 for 5 min at 1000 V (Bieleski and Turner 1966). Sugars were individually separated by single-dimensional paper chromatography on Whatman No. 1 paper for 12 hr using methyl ethyl ketone–acetic acid–saturated boric acid solution, 9 : 1 : 1 v/v/v (Webb and Burley 1962). Amino acids were individually separated by two-dimensional TLE/thin-layer chromatography (TLC) (Bieleski and Turner 1966). In the final studies the separation of both amino acids and sugars was achieved on a single thin-layer plate as follows. Amino acids were first separated by TLE/TLC, as above. During this step, the sugars were left at the origin after TLE, and were then partly separated in the second dimension by the TLC step. Finally the plate was chromatographed once again, in the original TLE dimension, with methyl ethyl ketone–acetic acid–saturated boric acid solution (9 : 1 : 1, v/v/v). This separated the sugars and sorbitol, and did not modify the amino acid separation (Fig. 1).

(d) *Measurement of Radioactivity*

Chromatograms were autoradiographed to detect areas of radioactivity, then each of these areas was marked and the compound present identified. Radioactivity present in a compound was measured as follows. The appropriate region of a paper chromatogram was cut out, and one end was dipped in water so as to elute the compound up to the tip. The tip was cut off and put into a scintillation vial so that its radioactivity could be measured by scintillation spectrometry. A thin-layer plate was coated with cellulose acetate solution, and the appropriate regions cut out, lifted from the plate, and dropped into scintillation vials for counting (Bieleski and Turner 1966). Corrections for quenching, all less than 6%, were made by the two-channels ratio method.

(e) Rates of Accumulation in Excised Phloem

The procedure used for measuring rates of sugar accumulation has been described in detail by Bieleski (1966*a*). Fresh or aged phloem tissue samples weighing 0·25 g were aerated at 24°C in each of 10^{-4}, 10^{-3}, and 10^{-2}M [^{14}C]sucrose, [^{14}C]glucose, and [^{14}C]sorbitol upon a rotary shaker. Samples were taken after 1·25, 2·5, 3·75, and 5 hr; each sample was washed and then extracted in 2% formic acid. Aliquots of each extract were plated on planchets for radioactivity measurements with a thin end-window Geiger–Mueller counter. Standards were prepared and counted in the same way. Rates of accumulation were determined from the lines of best fit of accumulation *v.* time (Bieleski 1966*a*).

(f) Products of Accumulation in Excised Phloem

Fresh and aged excised phloem tissues were prepared. Samples were weighed out and incubated with sugar solutions as follows.

(i) *Fresh Samples.*—Samples of fresh tissue (1·0 g in 2 ml, 5 μCi, 10^{-3}M [^{14}C]sucrose; 1·0 g in 2 ml, 5 μCi, 10^{-3}M [^{14}C]glucose; and 0·5 g in 0·5 ml, 1·7 μCi, 10^{-3}M [^{14}C]sorbitol) were allowed to accumulate sugar for 5 hr at 24°C on a rotary shaker; they were then washed, blotted, and killed in 20 ml methanol–chloroform–water at −72°C.

(ii) *Delayed Samples.*—Samples of fresh tissue (1·0 g in 2 ml, 5 μCi, 10^{-3}M [^{14}C]sucrose; 1·0 g in 2 ml, 5 μCi, 10^{-3}M [^{14}C]glucose; and 0·5 g in 0·5 ml, 1·7 μCi, 10^{-3}M [^{14}C]sorbitol) were allowed to accumulate sugar for 5 hr at 24°C; they were then washed, and incubated a further 15 hr in 10^{-4}M $CaSO_4$ (i.e. aged) before being blotted and killed in 20 ml methanol–chloroform–water at −72°C.

(iii) *Aged Samples.*—Samples of aged tissue (1·0 g in 5 ml, 5 μCi, 10^{-3}M [^{14}C]sucrose; 1·0 g in 5 ml, 5 μCi, 10^{-3}M [^{14}C]glucose; and 0·5 g in 2·5 ml, 1·7 μCi, 10^{-3}M [^{14}C]sorbitol) were allowed to accumulate sugar for 5 hr at 24°C; they were then washed, blotted, and killed in 20 ml methanol–chloroform–water.

Sugars and amino acids were extracted from each sample, the components of each extract were separated by TLE and TLC, and the radioactivity of each component was measured.

III. Results

(a) Translocation Products in Intact Phloem

Though photosynthesis times ranging from 10 min to 3 hr were used, the amount of $^{14}CO_2$ fixed was the same in all leaves, 26–29 μCi. Presumably all the available $^{14}CO_2$ had been photosynthesized during the first 10 min. The remaining 70% was probably lost, during the same time, by leakage and diffusion through the permeable walls of the polythene bag.

After short periods of photosynthesis in $^{14}CO_2$, sugars, amino acids, and phosphate esters each contained a considerable portion of the radioactivity present in the leaf extracts, but after long photosynthesis times virtually all radioactivity was in the sugars alone (Table 1). Each petiole extract closely resembled the companion leaf extract in composition. On the other hand, the phloem extract always had over 97% of its radioactivity in sugars, even when the companion leaf had a large proportion of radioactivity in amino acids and phosphate esters (Table 1).

When the distribution of radioactivity between the various sugars was studied, it was found that sorbitol always contained the bulk of the radioactivity, whether after short or long photosynthesis times, short or long translocation distances, or whether in leaf, petiole, phloem, cortex, or wood (Table 1). Sucrose always contained about one-quarter as much radioactivity as sorbitol, while glucose and fructose contained still less radioactivity. There was no large or consistent pattern of change

TABLE 1

DISTRIBUTION OF ^{14}C IN COMPOUNDS EXTRACTED FROM VARIOUS TISSUES OF APPLE STEMS, FOLLOWING VARIOUS TIMES OF PHOTOSYNTHESIS

Amounts of extract equivalent to 5–20 mg tissue were analysed by one-dimensional TLE or one-dimensional paper chromatography. Value given is the percentage of the total radioactivity on the chromatogram which is present in the particular compound. Total radioactivity per chromatogram ranged from 200 counts/min (20-min and 1-hr phloem 1 and phloem 2 samples) to 6×10^5 counts/min (leaf samples). Phloem 1 was taken from the region of stem 1 cm above to 2 cm below the petiole junction, and phloem 2 from 2 to 6 cm below

Tissue	Thin-layer Electrophoresis				Paper Chromatography					
	Phosphate Esters	Sugars	Serine	Amino Acids	Origin	Sucrose	Glucose	Fructose	Sorbitol	Amino Acids
10-min sample										
Leaf	4·4	78·4	14·1	3·1	6·4	12·7	3·7	2·2	57·9	17·0
Petiole	10·8	66·3	16·9	6·0	13·6	6·8	4·8	4·7	48·7	21·3
Phloem 1	2·4	97·0	0·5	—	2·6	14·0	1·3	0·8	80·1	1·2
Phloem 2					8·8	15·9	13·3	5·9	56·5	—
Cortex					7·9	16·4	3·2	2·1	70·5	—
20-min sample										
Leaf	3·0	86·5	8·2	2·4	5·0	15·7	2·5	1·4	65·8	9·2
Petiole	9·8	75·5	11·0	3·5	12·0	9·2	3·1	3·2	60·0	12·5
Phloem 1	1·0	99·0	—	—	5·6	16·3	4·7	6·9	66·6	—
Phloem 2					4·5	17·1	5·3	3·8	69·4	—
Cortex					5·9	17·0	3·3	3·1	70·6	—
1-hr sample										
Leaf	0·9	97·5	0·7	1·1	1·7	13·1	1·0	0·5	81·3	2·3
Petiole	1·2	96·9	0·9	1·1	2·0	18·0	1·3	0·8	75·9	2·2
Phloem 1	1·5	98·5	—	—	3·3	21·1	3·4	3·9	68·4	—
Phloem 2					3·9	20·3	6·7	5·2	63·8	—
Cortex					6·3	17·8	4·8	2·5	68·7	—
3-hr sample										
Leaf	0·9	97·6	0·1	1·5	2·4	12·1	1·3	0·6	81·5	1·9
Petiole	0·9	98·0	0·1	0·8	1·6	12·4	1·0	0·5	83·5	1·0
Phloem 1	0·4	98·8	0·2	0·8	1·0	20·4	0·8	0·9	75·9	0·8
Phloem 2					1·3	22·8	1·7	1·7	71·0	1·4
Cortex					1·3	17·2	1·7	1·5	77·0	1·6
Wood					2·0	15·6	1·6	1·4	79·5	—

in the proportion of [^{14}C]sorbitol present in the various tissue extracts, and distribution of ^{14}C in the phloem sugars closely resembled that in the companion leaf. Only in the case of the 10-min and 3-hr phloem samples was sufficient radioactivity present to allow a more detailed study of extract composition by two-dimensional TLE/TLC. The general observations reported above were confirmed, and serine (*c.* 14% of total ^{14}C in extract) was identified as the amino acid which was disproportionately labelled during short photosynthesis times, and which was not translocated. Other important compounds were alanine (*c.* 2% of total ^{14}C) and succinate (*c.* 2%). The sugar raffinose (and possibly stachyose) was present in small amounts.

(*b*) *Accumulation of Sugars by Excised Phloem*

All three sugars, sucrose, glucose, and sorbitol, were actively accumulated by both fresh and aged phloem tissues. The rates of accumulation were much higher in aged tissues, and this was more notably so for sorbitol than for sucrose and glucose (Table 2). The sugars were most rapidly accumulated from the most concentrated

TABLE 2

RATE OF ACCUMULATION OF SUCROSE, GLUCOSE, AND SORBITOL INTO FRESH AND AGED APPLE PHLOEM TISSUE

Rates are expressed as n-moles per gram fresh weight per hour

Concn. of Sugar (M)	Rate of Accumulation in Fresh Tissue of:			Rate of Accumulation in Aged Tissue of:		
	Sucrose	Glucose	Sorbitol	Sucrose	Glucose	Sorbitol
10^{-4}	105	130	15	370	295	195
10^{-3}	370	420	120	1850	2000	1660
10^{-2}	775	810	445	4750	4220	5480

solutions, and this too was most notably so for sorbitol. Thus with aged phloem and 10^{-2}M solutions, sorbitol was more rapidly accumulated than either sucrose or glucose, but was accumulated at only one-seventh their rate into fresh phloem from 10^{-4}M solution.

(*c*) *Products of Sugar Accumulation in Excised Phloem*

The tissues were allowed to accumulate sugars, and were then extracted to give three main fractions (Table 3): aqueous phase (sugars, amino acids, etc.), chloroform phase (lipids, phospholipids, etc.), and residue (protein, polysaccharide, etc.). [^{14}C]Sucrose and [^{14}C]glucose behaved similarly throughout, the main feature being that the proportion converted into lipid was much smaller in the fresh tissue than in the delayed or aged tissues. [^{14}C]Sorbitol behaved rather differently, in that there was very little formation of either lipid or residue material in the fresh tissue, but a moderate formation of both in delayed and aged tissues.

When the components of the aqueous extracts were studied in greater detail (Table 4), it was found that when either sucrose or glucose was accumulated by fresh, delayed, or aged tissues, sucrose was the major accumulation product and virtually no sorbitol was formed (though endogenous sorbitol was present in the tissue).

TABLE 3

DISTRIBUTION OF ^{14}C IN FRACTIONS OF EXCISED PHLOEM TISSUES WHICH HAD BEEN SUPPLIED WITH ^{14}C-LABELLED SUGAR AT 10^{-3}M FOR 5 HR

Value given is the percentage of the total activity which is in the particular fraction

Fraction	Fresh Tissue Supplied with:			Delayed Tissue Supplied with:			Aged Tissue Supplied with:		
	Sucrose	Glucose	Sorbitol	Sucrose	Glucose	Sorbitol	Sucrose	Glucose	Sorbitol
Residue	27·4	26·2	2·5	39·6	37·8	22·0	16·2	16·7	13·6
Lipid	4·6	5·4	1·1	17·8	18·6	16·7	12·3	11·1	13·8
Aqueous	68·0	68·4	96·4	42·6	43·6	61·3	71·5	72·2	72·6

TABLE 4

DISTRIBUTION OF ^{14}C IN COMPOUNDS PRESENT IN AQUEOUS EXTRACTS OF EXCISED PHLOEM TISSUES THAT HAD PREVIOUSLY BEEN SUPPLIED WITH ^{14}C-LABELLED SUGARS

Amounts of extract equivalent to 10 mg tissue were analysed by two-dimensional TLE/TLC. Value given is the percentage of the total radioactivity on the chromatogram present in that compound. Total radioactivity per chromatogram ranged from 1,500 counts/min (delayed tissue supplied with sorbitol) to 20,000 counts/min (aged tissue supplied with sorbitol)

Compound	Fresh Tissue Supplied with:			Delayed Tissue Supplied with:			Aged Tissue Supplied with:		
	Sucrose	Glucose	Sorbitol	Sucrose	Glucose	Sorbitol	Sucrose	Glucose	Sorbitol
Sucrose	83·6	79·1	6·0	50·3	44·6	21·5	64·2	62·3	24·5
Glucose	4·5	6·1	3·2	23·2	26·6	5·6	15·0	13·1	5·1
Fructose	3·2	3·8	2·2	12·3	11·2	4·9	10·4	9·7	4·6
Sorbitol	0·6	1·3	78·9	0·9	3·7	43·6	0·4	1·7	48·3
Raffinose	0·8	1·1	—	1·5	1·8	0·7	1·1	1·0	0·5
Leucine	0·4	1·2	—	1·4	1·4	0·8	0·6	0·7	0·6
Other amino acids	1·4	1·3	—	2·3	1·2	1·2	1·3	1·3	1·5
Organic acids	5·5	6·2	9·6	8·2	9·3	21·5	6·7	10·3	15·2

When sorbitol was supplied to fresh tissue, sorbitol was the major accumulation product; in delayed and aged tissues there was almost as much sucrose as sorbitol in the accumulation product (Fig. 1). In all cases, the proportions of glucose and fructose found in the accumulation product were similar to each other, and were about one-quarter that of sucrose.

IV. Discussion

The first possibility to be examined in these experiments was that sorbitol, which in the experiments of Webb and Burley (1962) appeared to be contained in the phloem, might actually be present in the cortical tissues; and the second, that sorbitol might not be a primary translocation product, but be formed during subsequent metabolism of the primary form within the phloem. The results of the first part of this study indicated that neither was so. The phloem when separated from all other

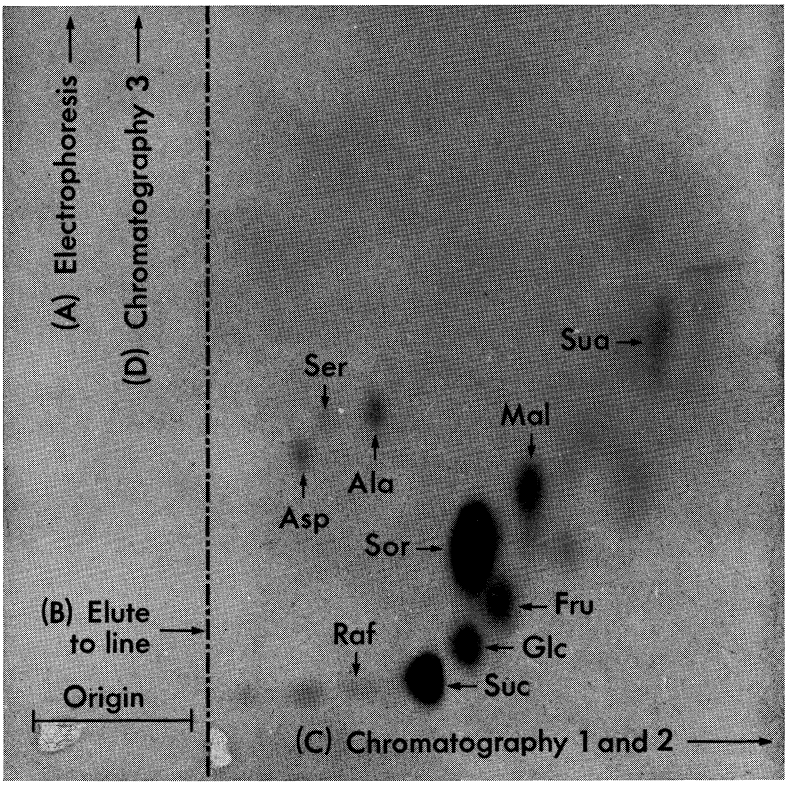

Fig. 1.—Autoradiograph of chromatogram of extract (equivalent to 10 mg tissue) from aged phloem previously fed [^{14}C]sorbitol for 5 hr. Extract was separated by thin-layer electrophoresis and thin-layer chromatography (*A–D*). Ala, alanine; Asp, aspartic acid; Fru, fructose; Glc, glucose; Mal, malic acid; Raf, raffinose; Ser, serine; Sor, sorbitol; Sua, succinic acid; Suc, sucrose.

tissues contained over 70% of the total soluble radioactivity in the form of sorbitol, just as reported by Webb and Burley (1962). Furthermore, phloem samples harvested within 10 min of the start of photosynthesis (when the radioactive front of translocation product should have moved only about 10 cm from the leaf; Canny 1960) still had sorbitol as the major translocation product. There were two other features of interest in these short-time samples. Serine and phosphate esters were major early photosynthetic products in the leaf, yet they did not appear in the phloem, and glucose appeared to be unusually prominent in translocation after the shortest

photosynthesis time and longest translocation distance. It seems that the phloem contents do not simply echo the leaf contents, though resembling them closely, and that some selection process operates when products of photosynthesis in the leaf are transferred to the phloem.

As the observations of Webb and Burley (1962) concerning the importance of sorbitol were clearly confirmed, it seemed that the discrepancy between experiments with intact plants and excised phloem must lie in the behaviour of the excised phloem. Possibilities to be considered here were that the aging process altered the behaviour of the excised phloem; or that excised phloem would not metabolize sucrose to sorbitol; or that sorbitol could not be accumulated by phloem (in which case the role of accumulation in phloem transport would come into question). Experiments to test these possibilities gave the following information. On the one hand, aging the phloem drastically altered its metabolic state (a similar aging effect has already been noted in vascular bundles; Bieleski 1966*a*). Accumulation rates were increased 4–10-fold, and metabolic rates probably increased as well, since the proportion of accumulated product being converted into lipid was doubled. On the other hand, aging did not change the distribution of ^{14}C in the various metabolites when [^{14}C]-sucrose or [^{14}C]glucose was supplied in the external solution. The results of Bieleski (1966*a*) were confirmed and extended: [^{14}C]sucrose did not form sorbitol when accumulated, either into fresh or aged phloem; and [^{14}C]glucose behaved exactly like [^{14}C]sucrose, forming sucrose but not sorbitol. This behaviour of phloem contrasts with that of the leaf, which rapidly converts absorbed glucose to sorbitol (Hutchinson, Taper, and Towers 1959).

The key to understanding the function of sorbitol in phloem translocation seems to lie in the behaviour of sorbitol during accumulation. The first point is that sorbitol is indeed accumulated, like other sugars, into phloem tissue. The rate of accumulation is much more influenced by the state of the tissue, fresh or aged, and the external concentration of the sugar, than are the accumulation rates of sucrose and glucose. Relative to the other sugars, sorbitol is most effectively accumulated when its concentration is high. The second point is that sorbitol, once accumulated, is metabolically more inert than sucrose or glucose. In fresh tissue, sorbitol stays as sorbitol; very little is converted to the other sugars or amino acids, or into lipids or residual proteins and polysaccharides. Only when, as in the aged tissue, there develops a system which can convert sorbitol to sucrose (probably via glucose) does [^{14}C]sorbitol enter the metabolic pathways. When this happens, the proportion of sorbitol falls and of sucrose rises, and other sugars, amino acids, lipids, etc. are formed (organic acids may lie on a separate pathway, as a higher proportion of organic acids was formed from [^{14}C]sorbitol than from [^{14}C]sucrose or [^{14}C]glucose in both fresh and aged tissues). It should be noted that this more rapid metabolism of sorbitol in aged tissue is not the sole cause of its more rapid accumulation, for when 10^{-3}M sorbitol was supplied accumulation increased 14-fold while the proportion of sorbitol in the accumulation product fell by half, so that the concentration of accumulated sorbitol was seven times higher in the aged tissue, even though its rate of conversion was also greater.

Is the role of sorbitol in phloem transport of apple then a passive or an active one? On one view, the unusual occurrence of sorbitol in both the leaf and phloem can

be used to support the mass-flow interpretation of movement in the phloem—sorbitol occurs as a passive component of the phloem because it is present as the main photosynthetic product in the leaf. Three points argue against this view. Firstly, evidence already presented indicates that sieve tubes in apple phloem have functional membranes (Bieleski 1966*b*). Secondly, if sorbitol is drawn passively into the phloem, so too should be serine, a major photosynthesis product after short photosynthesis times. Yet clearly serine is excluded in some way. Thirdly, using a high proportion of sorbitol in photosynthesis product and translocation product as evidence for mass flow begs the question, for even if the accumulation process observed in phloem is a secondary phenomenon, its occurrence should eventually result in the ^{14}C pattern of accumulation swamping the ^{14}C pattern of translocation materials. Yet sorbitol is as predominant after long translocation times as short ones, and in cortex and wood as in leaf and phloem. It is unlikely that this general distribution is solely due to passive processes.

An alternative view, which seems more consistent with the data, is that accumulation into the phloem is a primary process preceding translocation. Sorbitol, the main photosynthetic product, is present in the leaf in concentrations around $5–15 \times 10^{-2}$M (Anderson, Andrews, and Hough 1961; Whetter and Taper 1963, 1966). At such high concentrations, phloem accumulates sorbitol as readily as sucrose or glucose, and the ratio of sugars entering the phloem will resemble their ratio in the leaf. Further, once the sugars are accumulated there is very little tendency for sorbitol to be converted to sucrose, or sucrose to sorbitol. Thus the composition of the translocation stream, with sorbitol predominant, resembles the composition of the leaf photosynthate because sorbitol is readily accumulated by the phloem and sparingly metabolized. The selective nature of the accumulation system, interposed between leaf and phloem, would account for the failure of serine to pass from leaf to phloem. It could also account for a claim that sucrose, not sorbitol, is the transport sugar in germinating apple seedlings (Whetter and Taper 1966). If the sugar concentration in the cotyledon was to fall markedly, as during germination, sorbitol would be less effectively accumulated, sucrose or glucose would become the preferred sugar for accumulation, and the proportion of sorbitol in the translocation stream would drop. All that is needed for this state to continue is a control system within the leaf maintaining the relative proportion there of sucrose or of glucose to sorbitol.

These two features of sorbitol behaviour, namely ready movement into the phloem but restricted utilization there, make sorbitol a logical translocation substance. The only thing required to make it ideal is a ready utilization once it has reached its destination, the metabolic sink. The behaviour of the phloem itself, in metabolizing sorbitol after aging, shows that apple tissues can acquire or change their ability to use sorbitol. It would be expected that actively growing regions would use sorbitol readily and have low sorbitol concentrations, and that maturing tissues would tend to lose this ability and have higher sorbitol levels.

It is therefore felt that there is no conflict between the results of Webb and Burley (1962), Bieleski (1966*a*), and Whetter and Taper (1966), but rather that each represents a different view of a single situation. Sorbitol is a major translocation product because it is a major photosynthesis product. It can be accumulated as such by the phloem cells, and its conversion to or formation from other compounds

can depend on the state of the tissue. Its function is that of a somewhat unusual storage compound: there appear to be interesting parallels between the behaviour of sorbitol in the apple, and that of mannitol, ribitol, and other polyols in lichens (Lewis and Smith 1967; Richardson and Smith 1968).

V. Acknowledgments

The author thanks Miss Carol Hoelle and Mr. R. J. Redgwell for their excellent technical help.

VI. References

Anderson, J. D., Andrews, P., and Hough, L. (1961).—The biosynthesis and metabolism of polyols. Sorbitol (D-glucitol) of plum leaves. *Biochem. J.* **81**, 149–54.

Anderson, J. D., Andrews, P., and Hough, L. (1962).—The biosynthesis and metabolism of polyols. 2. The metabolism of ^{14}C-labelled D-glucose, D-glucuronic acid and D-glucitol (sorbitol) by plum leaves. *Biochem. J.* **84**, 140–6.

Bieleski, R. L. (1966*a*).—Accumulation of phosphate, sulfate and sucrose by excised phloem tissues. *Pl. Physiol., Lancaster* **41**, 447–54.

Bieleski, R. L. (1966*b*).—Sites of accumulation in excised phloem and vascular tissues. *Pl. Physiol., Lancaster* **41**, 455–66.

Bieleski, R. L., and Turner, N. A. (1966).—Separation and estimation of amino acids in crude plant extracts by thin-layer electrophoresis and chromatography. *Analyt. Biochem.* **17**, 278–93.

Canny, M. J. (1960).—The rate of translocation. *Biol. Rev.* **35**, 507–32.

Hutchinson, A., Taper, C. D., and Towers, G. H. N. (1959).—Studies of phloridzin in *Malus*. *Can. J. Biochem. Physiol.* **37**, 901–10.

Lewis, D. H., and Smith, D. C. (1967).—Sugar alcohols (polyols) in fungi and green plants. I. Distribution, physiology and metabolism. *New Phytol.* **66**, 143–84.

Richardson, D. H. S., and Smith, D. C. (1968).—Lichen physiology. X. The isolated algal and fungal symbionts of *Xanthoria aureola*. *New Phytol.* **67**, 69–77.

Webb, K. L., and Burley, J. W. A. (1962).—Sorbitol translocation in apple. *Science, N.Y.* **137**, 766.

Whetter, J. M., and Taper, C. D. (1963).—Note on seasonal occurrence of sorbitol (D-glucitol) in buds and leaves of *Malus*. *Can. J. Bot.* **41**, 175–7.

Whetter, J. M., and Taper, C. D. (1966).—Occurrence of sorbitol (D-glucitol) and certain related sugars in germinating seeds and developing seedlings of *Malus*. *Can. J. Bot.* **44**, 51–5.