CONCOMITANT PHOTOSYNTHESIS IMPLICATED IN THE LIGHT EFFECT ON TRANSLOCATION IN BEAN PLANTS

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Summary

Light supplied during a 3-hr translocation period stimulated [^{14}C]sucrose translocation in bean plants, both when the plants had spent the previous 30 hr in the light and when they had spent this period in the dark. Light during the translocation period was also very effective when the latter was shortened to 30 min and followed a 30-hr pretreatment period in the light. It is thus unlikely that the light effect is due to a change in overall leaf sugar level, since the latter will not have altered markedly during the final 30 min of a light period of more than 30 hr. Not only was more ^{14}C translocated out of the leaves in the light, but a far higher percentage of the ^{14}C so translocated reached the roots.

If illuminated plants were exposed to CO_2-free air during the translocation period (which was again 30 min, and followed a light pretreatment period of 30 hr) translocation was much depressed. In the dark, on the other hand, CO_2 deprivation during the translocation period had no effect. The amount of ^{14}C translocated in the dark was about equal to that translocated by illuminated plants deprived of CO_2.

DCMU, applied under conditions which were shown to depress $^{14}CO_2$ fixation by 60%, markedly reduced [^{14}C]sucrose translocation in the light. In the dark DCMU was without significant effect on translocation.

The results suggest that the ATP formed in non-cyclic photosynthetic phosphorylation plays a role in translocation, probably by promoting vein-loading in the leaves. Alternatively, the sucrose concentration in a compartment of the cell containing nascent sucrose may affect the rate of translocation.

I. INTRODUCTION

An effect of light on the translocation of sucrose has recently been reported from several laboratories (e.g. Bianchetti 1963; Hartt 1965; Geiger and Swanson 1965; Hartt and Kortschak 1967; Geiger and Batey 1967). During the course of an investigation into [^{14}C]sucrose transport in bean plants we ourselves noted that higher amounts of ^{14}C were translocated in the light than in the dark (Plaut 1966). Various possible mechanisms for such an effect might be envisaged. The influence of light might, for instance, be indirect, brought about via the level of sugar in the leaf. It might, alternatively, be related to ATP formation during photosynthesis. Or again it might be due to some light-activated process other than photosynthesis. In the present investigation we attempted to distinguish between these various possibilities. We here report experiments which clearly implicate *concomitant* photosynthesis in the effect of light on translocation.

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II. Materials and Methods

Bean plants (*Phaseolus vulgaris* cv. Brittle Wax) were grown, either in soil or in vermiculite, at 25°C under a light regime of 14 hr light, 10 hr dark. Light intensity was approximately 2500 f.c. at the level of the upper leaves.

Fourteen-day-old plants were used in the experiment. At this stage the primary leaves were expanded and the first trifoliate leaf was beginning to expand. Vermiculite-grown plants were transferred to half-strength Hoagland solution 4 hr before the experiments. When the latter involved treatment with 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), the inhibitor was added to the Hoagland solution at a concentration of 4×10^{-6}M.

A 0·01-ml drop containing 0·06 μmole of uniformly labelled [^{14}C]sucrose (specific activity 13 μCi/μmole) was applied to a primary leaf. The method of application and subsequent techniques for determining the extent of translocation, were as described by Plaut and Reinhold (1965). Sugar applied under these conditions has been shown to travel in the phloem (Plaut and Reinhold 1965, 1967). The length of the translocation period was in most cases 30 min. All experiments were carried out in triplicate or quadruplicate, each replicate being analysed individually.

In certain experiments where photosynthesis was estimated, the extent of CO_2 fixation in primary leaves of plants exposed to $^{14}CO_2$ in closed chambers was measured. The $^{14}CO_2$ was generated from 100 μmoles $Ba^{14}CO_3$, specific activity 2·5 μCi/μmole, and the volume of the chambers was such that total CO_2 concentration was 360 p.p.m.

III. Results

An example of an early experiment indicating that [^{14}C]sucrose translocation proceeded more rapidly in the light than in the dark is given in Table 1. In the experiment the translocation period was 3 hr. Light during this period stimulated

Table 1

Effect of light supplied during a 3-hr translocation period on the distribution of ^{14}C in bean plants treated with [^{14}C]sucrose

The plants were pretreated in darkness or in light for 30 hr before application of [^{14}C]sucrose to a primary leaf. The experiment was performed in quadruplicate

Pretreatment	Treatment	Total ^{14}C Translocated	
		counts/min	%*
Light	Light	12,990	81·4
	Dark	8,100	69·6
Dark	Light	6,710	69·0
	Dark	326	17·5
Standard error		1,950	5·1

* Total ^{14}C translocated out of leaf blades as a percentage of total ^{14}C in plant.

translocation, both when the plants had an immediate previous history of 30 hr light and when they had a history of 30 hr dark (Table 1). This table demonstrates further that more ^{14}C was translocated in the plants pretreated in the light, irrespective of the light conditions during the translocation period itself. The latter result suggested that the sugar level in the leaves at the start of the experimental period

was influential. The question thus arose whether the greater intensity of translocation in plants illuminated during the experiment period itself was also mainly the result of higher sugar levels in the leaves.

Experiments were accordingly carried out in which the translocation period was shortened to 30 min. The pretreatment was again 30 hr in the light. Thus at the start of the experiment the sugar content of the leaves was high, and it is unlikely that considerable changes in sugar level occurred during the subsequent 30 min. Geiger and Batey (1967) reported a 20% drop in sucrose concentration 60 min after the plants passed from a 4-hr light period to darkness. Table 2 shows that under these conditions also light during the translocation period was markedly stimulatory. More ^{14}C reached plant parts other than the leaf blade in the case of illuminated plants, and, further, a far higher percentage of the ^{14}C which was translocated out of the leaf reached the roots. The latter result is important because when the data are expressed in this form differences due to differing ^{14}C concentrations in the leaf are

TABLE 2

EFFECT OF LIGHT SUPPLIED DURING A 30-MIN TRANSLOCATION PERIOD ON THE DISTRIBUTION OF ^{14}C IN BEAN PLANTS TREATED WITH [^{14}C]SUCROSE

The plants were pretreated in the light for 30 hr before application of [^{14}C]sucrose to a primary leaf. The experiment was performed in quintuplicate. M, midvein; P, petiole; I_1, internode 1; I_2, internode 2; H, hypocotyl; R, root; G.P., growing point

Treatment	Total ^{14}C (counts/min) in:		^{14}C Translocated (%)*	^{14}C (%)† in:			
	Treated Leaf Blade	Rest of Plant		M+P	I_2+G.P.	I_1+H	R
			Experiment 1				
Light	176	261	61·6	28·4	23·1	13·7	36·9
Dark	65	125	61·0	50·5	22·9	15·0	11·6
Standard error	34	24	6·0	5·9	8·5	5·3	7·6
			Experiment 2				
Light	1916	3877	66·7				
Dark	1364	1568	40·9				

* ^{14}C translocated out of treated leaf blade as percentage of total ^{14}C in plant.

† As a percentage of total ^{14}C from midvein to root.

eliminated. In experiment 1, Table 2, the values for ^{14}C translocated out of the leaf expressed as a percentage of the total in the plant do not show a significant effect of light, though this effect can be seen in experiment 2. The leaf blade, after removal of the small ringed area where the [^{14}C]sucrose was applied, contained considerably more ^{14}C in the case of the illuminated plants (Table 2). A previous investigation (Plaut and Reinhold 1967) has indicated that movement out of the ringed area, even over short distances, involves phloem transport. Promotion of the latter by light would thus account for this effect. A possible influence of light on ^{14}C uptake by the leaf should also be considered (see Section IV).

Table 2 shows that light thus apparently stimulated phloem transport directly, and not only via a general effect on leaf sugar levels. The next experiment tested whether this stimulation was related to CO_2 fixation. Translocation was again measured over a 30-min period, following pretreatment for 30 hr in the light. Table 3 shows that if illuminated plants were exposed to CO_2-free air during the translocation period, the amount of ^{14}C subsequently detected in the leaf blade (after removal of the application site) was much reduced. As pointed out above, it has already been found (Plaut and Reinhold 1967) that movement in the leaf involved phloem transport. Further, in illuminated plants exposed to CO_2-free air, significantly less ^{14}C reached the roots. That this effect too, could not be accounted for on the basis of the differing levels of ^{14}C in the leaves is shown in Table 3, if the ^{14}C in the roots is expressed as a percentage of the total amount translocated out of the leaf, a markedly depressing effect of CO_2 deprivation is still apparent.

TABLE 3

EFFECT OF LIGHT SUPPLIED DURING A 30-MIN TRANSLOCATION PERIOD IN THE PRESENCE AND ABSENCE OF CO_2 ON THE DISTRIBUTION OF ^{14}C IN BEAN PLANTS TREATED WITH [^{14}C]SUCROSE

The plants were pretreated in the light for 30 hr before application of [^{14}C]sucrose to a primary leaf. The experiment was performed in quadruplicate

Treatment		Total ^{14}C (counts/min) in: Treated Leaf Blade	Rest of Plant*	Root	^{14}C in Root (%)†	^{14}C Translocated (%)‡
Light	Control	252,000	1,990	538	21·8	1·01
	CO_2-free air	104,700	1,270	197	12·1	1·40
Dark	Control	126,700	960	124	11·8	0·94
	CO_2-free air	143,000	1,110	140	11·1	0·93
Standard error		22,900	160	61	1·8	0·20

* Excluding root.

† As a percentage of total ^{14}C translocated out of treated leaf blade.

‡ ^{14}C translocated out of treated leaf blade as percentage of total ^{14}C in plant.

In the dark, on the other hand, a CO_2-free atmosphere was without significant effect (Table 3). The amount of ^{14}C translocated in both control and CO_2-free plants in the dark is approximately equal to that in illuminated plants deprived of CO_2. This is true for the ^{14}C moving from the application site into the rest of the leaf blade as well as for that transported to the rest of the plant. Percentage ^{14}C translocated out of the leaf was very low in this experiment—approximately 1%—and no significant effect of light was visible.

The effect of the photosynthetic inhibitor, 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), was next examined. Preliminary tests showed that under our conditions, in the light, $^{14}CO_2$ fixation was depressed by 60% by 4×10^{-6}M DCMU. No effect was visible on the negligible $^{14}CO_2$ fixation that took place in the dark.

Table 4 shows the effect of this DCMU treatment on translocation. In the light the inhibitor markedly reduced the amount of ^{14}C moving into the leaf blade from the treated spot. It also reduced the amount of ^{14}C detectable in all other plant parts. Even if the ^{14}C recovered from the roots is expressed as a percentage of the total amount translocated out of the leaves (in order to eliminate differences due to the differing ^{14}C concentrations in the leaf blades), the value for the DCMU-treated plants is lower than that for the controls. The percentage ^{14}C translocated out of the leaves was also reduced by DCMU. Thus it is clear that DCMU affected translocation apart from any possible effects there may have been on sugar uptake by the leaf from the applied solution. On the other hand, while values for the leaf blade indicate a small effect due to DCMU in the dark (Table 4), there is no significant difference between other parts of treated and control plants. This result seems to rule out the possibility that DCMU was affecting transport via a depressing effect on the translocation "sink".

TABLE 4

EFFECT OF LIGHT SUPPLIED DURING A 30-MIN TRANSLOCATION PERIOD, AND OF DCMU (4×10^{-6}M), ON THE DISTRIBUTION OF ^{14}C IN BEAN PLANTS TREATED WITH [^{14}C]SUCROSE

The plants were pretreated in the light for 30 hr before application of [^{14}C]sucrose to a primary leaf. Abbreviations as in Table 2. The experiment was performed in quadruplicate

Treatment		Total ^{14}C (counts/min) in:					^{14}C in Root (%)*	^{14}C Translocated (%)†
		Treated Leaf Blade	M+P	I_2+G.P.	I_1+H	R		
Light	Control	815,000	4,070	1,155	1,187	1,648	21·2	0·98
	DCMU	318,000	870	319	327	145	10·1	0·45
Dark	Control	480,000	940	325	393	193	10·9	0·42
	DCMU	280,000	1,040	447	510	260	11·7	0·65
Standard error		58,900	398	104	31	171	1·8	0·26

* As a percentage of total ^{14}C translocated out of treated leaf blade.

† ^{14}C translocated out of treated leaf blade as percentage of total ^{14}C in plant.

IV. DISCUSSION

The light effect shown in Table 1, where illumination during a 30-hr pretreatment period resulted in more rapid translocation irrespective of the light conditions during the translocation period itself, may well have been brought about through an effect on the sucrose level in the leaf. But higher sugar levels cannot account for the effects shown in Tables 2, 3, and 4. Here light was supplied during a translocation period which was very brief compared with the long light pretreatment, and a material change in sugar level during the translocation period itself is unlikely (cf. Geiger and Batey 1967).

The possibility should be considered that light may have affected the rate of uptake of [^{14}C]sucrose from the applied solution. That a light effect on stomatal opening was not a decisive factor is shown in Table 3. In CO_2-free air in the light far

less ^{14}C was detectable in the leaf blade whereas stomatal aperture would be expected to increase under these conditions. Light might conceivably affect sugar uptake by the mesophyll cells, however. The further possibility, that the amount of sugar entering may in turn have affected vein-loading, or some other step in translocation, may be discounted, since a negligibly small amount of sugar is applied (0·1 μmole) of which less than 10% is taken up. An effect of light on uptake might, however, affect the specific activity in the tissue. This would be corrected for when the results are expressed on a relative basis. When the results are analysed in a manner which eliminates differences due to differing ^{14}C concentrations in the leaf blade, however, clear-cut effects of light are apparent. This fact shows that, while promoted uptake might possibly contribute to some of our light effects, the latter cannot have been due to enhanced uptake alone. Furthermore, other workers (e.g. Bianchetti 1963; Hartt 1965) have reported effects of light on the movement of assimilates, i.e. in experiments where uptake of [^{14}C]sucrose from solution is not involved.

Hartt (1965) considered that the light effect on translocation in sugar-cane leaves was independent of CO_2 assimilation. This conclusion was based on experiments in which basipetal translocation was increased by light intensities which produced no net uptake of CO_2. The results of two experiments reported here, however, indicate the important role played by photosynthesis in the present investigation. Firstly, the light effect was abolished if the plants were exposed to CO_2-free air during the translocation period. Exposure to CO_2-free air had no effect, on the other hand, on translocation in the dark; and the level of the latter was approximately equal to that in the light under CO_2-free conditions. Secondly, DCMU had a markedly depressive effect on translocation in the light, but not in the dark. The latter result indicates that the inhibitor, though presumably present throughout the plant, was not affecting the principal translocation sink (in our case the root—see Plaut and Reinhold 1965). It is most likely that its effect was exerted via photosynthesis.

Some product of concomitant photosynthesis thus apparently influences the rate of translocation. It is conceivable that this product is nascent sucrose. The main sucrose pool may be spatially separated in the cell from the compartment containing nascent sucrose. While the overall sucrose concentration is unlikely to have been appreciably altered by light conditions during the translocation period, as pointed out above, the concentration in the compartment containing nascent sucrose may have been greatly affected. It is possible that the rate of translocation is influenced by the sucrose level in this compartment.

An alternative suggestion is that the ATP formed in photosynthetic phosphorylation may play a role in translocation. Bianchetti (1963) reached the same conclusion, but on the basis of very different results. He observed a stimulatory effect of light on the translocation of labelled assimilates in *Elodea* in the presence of *p*-chlorophenyl-1,1-dimethylurea (CMU) (and in the absence of CO_2) and concluded that the synthesis of ATP in cyclic phosphorylation (DCMU-resistant) was responsible. However, since both a CMU-free control and a control in the presence of CO_2 were omitted from the experiment, it is not known whether CMU or the lack of CO_2 did not in fact greatly diminish the light effect. Neither was information provided as to whether the applied CMU treatment affected photosynthesis under the conditions of the experiments. Our own experiments indicate that non-cyclic phosphorylation

is involved. Further, the fact that the light effect was abolished in CO_2-free air suggests that CO_2 fixation and the phosphorylation process important for translocation are closely linked.

As to the nature of the role played by photosynthetic ATP in translocation, two possibilities suggest themselves. Firstly, since the passage of sucrose from the mesophyll to the phloem is known to be against the concentration gradient (Mason and Maskel 1928), photosynthetic ATP may help to provide energy for this uphill process. Secondly, photosynthetic ATP may be involved in regulating the permeability of cellular membranes. It has recently been suggested that the permeability of *Nitella* membranes to cations is increased by light (see e.g. Raven 1968).

A stimulatory effect of light on the loading of sugar onto the veins in the leaves may thus be explained on the lines suggested above. But the question remains as to why sugar also moved more quickly along the plant axis in illuminated plants. (See the values for ^{14}C reaching the roots, expressed as a percentage of the ^{14}C translocated out of the leaves, in Tables 2, 3, and 4.) A possible explanation might be that the promotion of vein-loading produced a higher concentration of sugar in the leaf phloem sieve tubes, and consequently a larger head of hydrostatic pressure, which in turn brought about the faster flow of sugar to the roots.

If photosynthetic ATP does in fact have a role in the translocation of sugars we are faced with an interesting problem—how can ATP formed inside chloroplasts exert an influence on processes at a distance from the chloroplast-containing cells? The answer must await further investigation.

V. References

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