THE UPTAKE OF SUCROSE BY BEAN LEAF TISSUE

II.* KINETIC EXPERIMENTS

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Summary

Leaf tissue of *Phaseolus vulgaris* takes up sucrose from external solutions by two processes. The first is an osmotic uptake into the free space. The second is a non-osmotic uptake into the osmotic volume. The kinetics of the non-osmotic uptake, from solutions less concentrated than 0·1M, fit the Michaelis–Menton equation. The tissue can accumulate sucrose against its concentration gradient from solutions less concentrated than 0·1M. There is no significant efflux of sucrose from the tissue. The correlation of the rate of carbon dioxide droduction with the internal sucrose concentration suggests that part, at least, of the cytoplasm is included in the osmotic volume for sucrose.

I. INTRODUCTION

A previous paper (Vickery and Mercer 1964) described a simple method for measuring the uptake of sucrose by leaf tissue of *Phaseolus vulgaris* L. cv. Brown Beauty. It was shown that the uptake from a 1% (w/v) solution of sucrose took place in three phases. During the first phase, which lasted 1 hr, the uptake was rapid and of the osmotic type; in the second phase, which lasted about 7 hr, the uptake was slower, constant in rate, and of the non-osmotic type; in the third phase the rate of uptake decreased with time.

The present paper is concerned with the relationship between the uptake and outside concentration of sucrose, the cause of the decrease in the rate of uptake during the third phase, and the site of accumulation within the cells of the tissue.

II. MATERIALS AND METHODS

In general, the materials and methods were similar to those described by Vickery and Mercer (1964); further details are given in the legend to Figure 1. Mature trifoliate leaves were cut into strips 1–2 mm wide and 2–3 cm long, injected with distilled water, and used within 2 hr of harvest. Uptake was determined by transferring known weights of leaf strips to sucrose solutions containing 0·2 μc/ml of randomly labelled [^{14}C]sucrose, and following the radioactivity of the external solution. Efflux was determined by measuring the radioactivity of initially non-radioactive solutions following transfer of tissue from radioactive solutions. The radioactivity of the efflux solutions and of solutions more concentrated than 0·1M was determined by internal sample liquid scintillation counting; the radioactivity of

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other solutions was determined with a Geiger–Müller counter. Rates of respiration were determined in the Warburg apparatus with the same ratio of tissue to solution as in the uptake experiments. All experiments were carried out in dim light at 25°C. It was found that respiration rates under these conditions were the same as those in the dark.

III. Results and Discussion

(a) *Effect of Concentration on Uptake*

(i) *Uptake against the Concentration Gradient*

In the previous paper (Vickery and Mercer 1964) it was shown that bean leaf tissue could accumulate sucrose against its concentration gradient from 1% solutions. One objective of the present experiments was to find whether this conclusion would be true for a wider range of sucrose concentrations. In order to demonstrate an active accumulation against the concentration gradient it is necessary to show that the concentration of sucrose inside the tissue rises until it exceeds *both* the initial concentration inside the tissue and the initial concentration outside the tissue.

Table 1

CONCENTRATIONS OF SUCROSE INSIDE AND OUTSIDE LEAF TISSUE AFTER TAKING UP SUCROSE FROM SOLUTIONS OF VARIOUS CONCENTRATIONS

Experiment I, single determinations; experiment II, means of two determinations

Initial External Sucrose Concentration		Experiment I (duration 26½ hr)		Experiment II (duration 12 hr)	
(mg/ml)	(M)	Sucrose Concn. inside Tissue (mg/g fresh wt.)	Sucrose Concn. outside Tissue (mg/ml)	Sucrose Concn. inside Tissue (mg/g fresh wt.)	Sucrose Concn. outside Tissue (mg/ml)
0·342	0·001	0·71	0·03		
1·37	0·004	0·49	0·10	1·19	0·0
2·74	0·008	0·74	0·23		
3·42	0·010			3·60	0·0
5·13	0·015	0·84	0·46		
10·28	0·030	3·4	1·23	18·4	0·05
34·23	0·100	9·5	22·9		
68·5	0·200	14·6	51·0		
102·73	0·300			74	82
Fresh tissue		0·81		0·31	

It is shown in Table 1 that only the uptake from 0·03M solutions met both the above criteria. However, it is believed that uptake from solutions more dilute than 0·03M did take place against the concentration gradient, and that this effect was obscured by the rapid utilization of sucrose. The concentrations of sucrose inside the tissues were calculated for hourly intervals on the assumption that the rate of loss of sucrose was equal to the rate of carbon dioxide production on a carbon basis (Vickery and Mercer 1964). In this way it was shown that, for initial external concentrations up to 0·03M, the internal concentrations rose to maximum values greater

than both initial internal and external concentrations before decreasing to the low values found at the end of the experiments.

For solutions more concentrated than 0·2M, the internal concentrations never exceeded the external concentrations. This observation was confirmed by the method of Weatherley (1953), using 1-cm disks of leaf tissue, and are consistent with the observations of Pennell and Weatherley (1958). The results for solutions of about 0·1M concentration were variable (see Tables 1 and 2). In bean leaf tissue in the present experiments incipient plasmolysis occurred at 0·15M sucrose concentration as determined by the apparent osmotic volume method. Uptake of sucrose into plasmolysed tissue would not necessarily result in the internal sucrose concentration becoming greater than the external sucrose concentration, since internal and external osmotic pressures should remain equal as the cells deplasmolyse. Calculations based on the linear rates of uptake for turgid tissue showed that tissue in 0·2M solutions should have regained turgor in 2 hr and tissue in 0·3M solutions should have regained turgor in 7 hr. Both periods were much shorter than the duration of the experiments (Table 1); therefore, other explanations must be sought for the failure to observe uptake against the gradient from the more concentrated solutions.

(ii) *Initial Rates of Sucrose Uptake*

The rates of uptake during the second hour of each experiment were taken as the initial rates of uptake for the second phase. Lineweaver–Burk plots of these initial rates, given in Figure 1, show that the results for solutions less concentrated than 0·1M fit the Michaelis equation. That is, under these conditions, the kinetics of the second phase of uptake are those characteristic of non-osmotic uptake. From these data the calculated concentrations (mM) at which half-maximum rates were achieved (K_m) were 5·2, 8·9, and 15·3 for experiments I, II, and III and the corresponding maximum rates of uptake (V_{max}), expressed as μmoles/mg nitrogen/hr, were 2·25, 4·18, and 4·80. The values varied considerably among experiments, but are of the same order of magnitude as those observed for the uptake of monosaccharides into carrot and corn root tissue (Grant and Beevers 1964) and are about 10 times higher than those for the uptake of sucrose by sugar-cane stem tissue (Bieleski 1960).

The initial rates of uptake from solutions with initial concentrations of 0·1M and higher fall below the straight lines drawn in Figures 1(*a*), 1(*b*), and 1(*c*), indicating that, initially, the rates of uptake during the second phase were greater than would be predicted by the Michaelis equation from the data for solutions less concentrated than 0·1M. These high rates of apparent uptake may be caused by dilution of the external solution by efflux of water during osmotic adjustment. The tissue was found to require 2–3 hr to come to weight equilibrium in the apparent osmotic volume method, in which the change in weight of the tissue was determined in mannitol solutions with concentrations ranging from 0·1 to 1M. This period overlaps the interval (from 1 to 2 hr from the start of each experiment) during which the initial rate of second-phase uptake was determined.

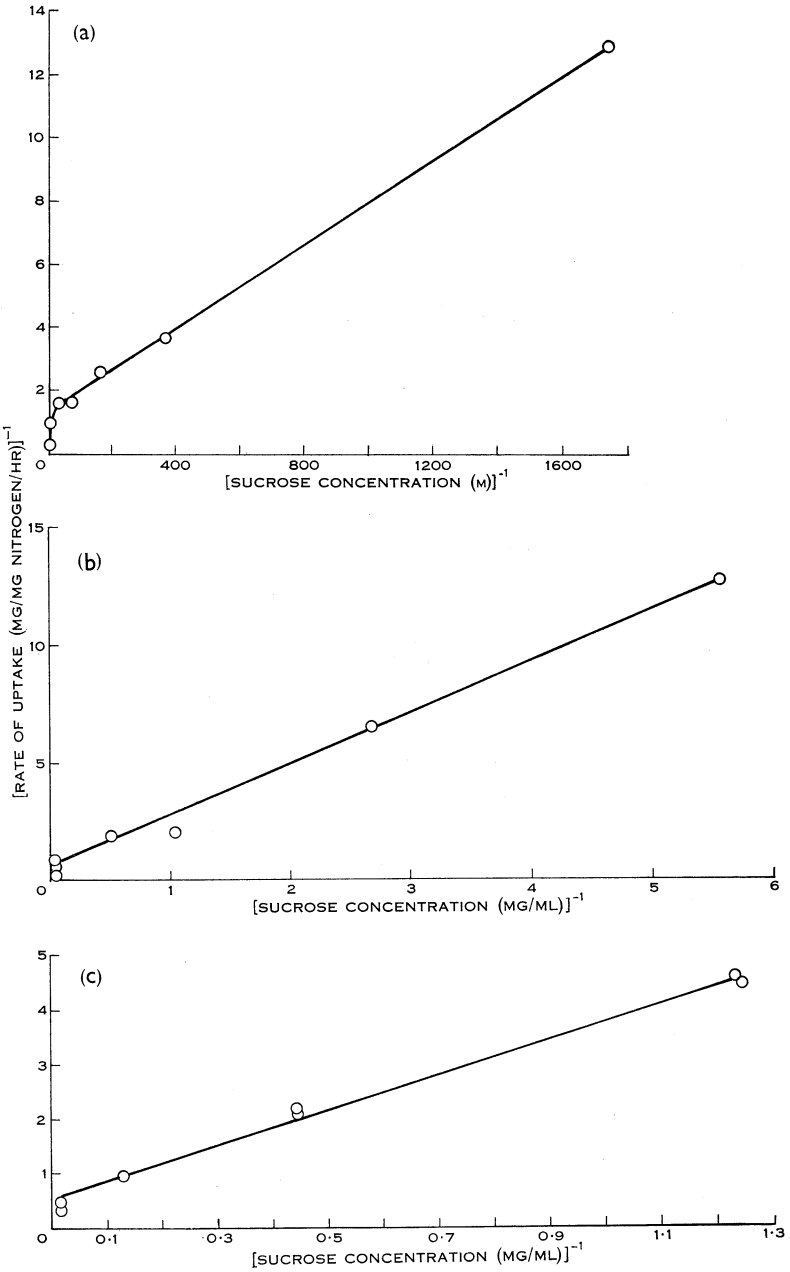

Fig. 1.—Effect of sucrose concentration on the rate of non-osmotic uptake. Aliquots of 3·5 g fresh weight of leaf strips were each placed in 15 ml of sucrose solution. The ranges of initial concentrations of sucrose were 0·001–0·20M [(*a*) and (*b*)] and 0·004–0·30M (*c*). The points on the curves show the rates of uptake and the outside concentrations of sucrose after the completion of osmotic uptake (usually 1 hr).

A similar divergence from the Michaelis relationship for sucrose uptake by tissue from immature internodes of sugar-cane was found by Glasziou (1961).

(iii) *Post-initial Rates of Sucrose Uptake*

The rates of uptake were calculated for hourly intervals throughout the second and third phases of the experiments and plotted against the external concentrations corresponding to the beginning of each hourly interval. The resulting graph for one experiment is shown in Figure 2. The solid line joins the "initial" rates. After a few hours the rates fell below this line, that is, they were less than would have been predicted from the initial rates. This divergence was more marked with the more concentrated solutions. It was postulated that this effect was caused by an efflux of sucrose from the tissue. The next section presents the results of experiments designed to detect such an efflux.

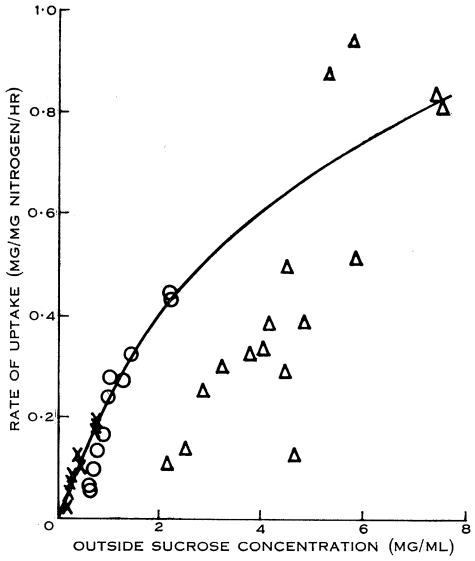

Fig. 2.—Data from one experiment showing the relation between successive short-term rates of uptake and the outside concentration of sucrose. ——— Initial rates of uptake. Initial outside concentrations as follows:

× 0·004M.
○ 0·010M.
△ 0·030M.

(*b*) *Efflux of Sucrose*

The results are given in Figure 3 and Table 2. The efflux was calculated as sucrose of the same specific activity as the sucrose taken up. This assumption was justified by the high ratio of sucrose taken up to endogenous sucrose (about 20 : 1), and by the absence of other significant carbohydrate reserves that could have equilibrated with the sucrose pool (Vickery and Mercer 1964).

The rapid efflux during the first hour in non-radioactive solutions [Fig. 3(*b*)] appears to be a reversal of the rapid, first phase of uptake. To confirm the similarity of these fluxes, the size of the apparent free space (AFS) was calculated (Table 2) from the uptake during the first phase [Fig. 3(*a*)]. From this was calculated the amount of sucrose in the free space at the time the tissue was transferred to the

non-radioactive solution ("sucrose in AFS at t_1", Table 2). The resulting quantities agreed quite well with the observed rapid efflux ("AFS efflux", Table 2) from the tissues in the 40 mg/ml sucrose solutions. The poorer agreement with the "AFS efflux" from the tissues in the 10 mg/ml solutions may be ascribed to non-osmotic uptake occurring while the tissues were being transferred from the radioactive to the non-radioactive solutions. It is concluded that the rapid uptake during the first phase is reversible and of the osmotic type.

The rates of efflux after 1 hr were very slow [Fig. 3(*b*); Table 2] and did not exceed one-tenth of the corresponding rates of uptake in the first part of the experiment. Efflux is not a significant factor in the kinetics of non-osmotic uptake of sucrose, a conclusion in accord with the results of Grant and Beevers (1964), who

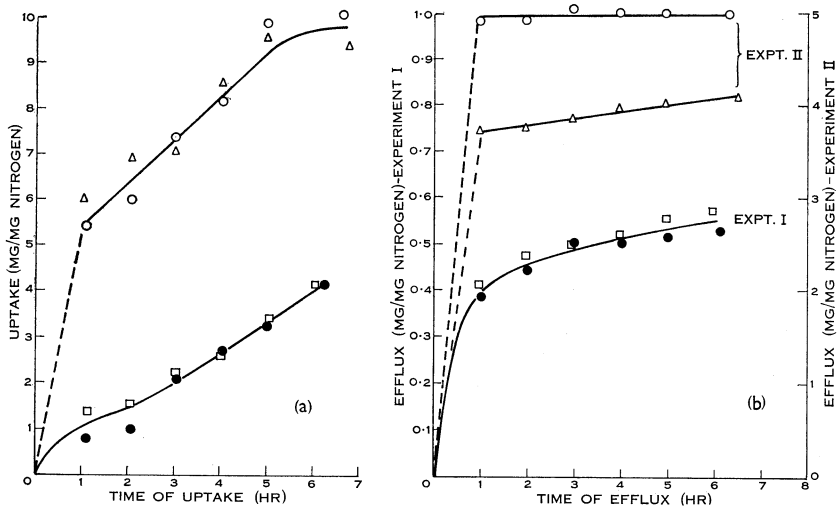

Fig. 3.—(*a*), (*b*) Uptake and efflux of sucrose. 3·5 g aliquots of tissue in 15 ml of solution. Initial concentrations of sucrose 10 mg/ml (□, ●) and 40 mg/ml (△, ○).

found no significant efflux of labelled sugars from carrot and corn root tissue immersed in water. Assuming that the efflux was in the form of sugar, the rates, when greater than zero, were of the same order as those in immature internodal tissue from sugarcane (Glasziou 1960).

The tissue continued to take up sucrose while the efflux was being measured. This is shown by the fall in concentration of the outside solutions (Table 2) from 10 mg/ml to about 5·9 mg/ml (average) and from 40 mg/ml to about 35·6 mg/ml (average). These results show that the mechanism of non-osmotic uptake of sucrose by bean leaf tissue is different from the "mobile carrier" mechanism demonstrated in yeast (Burger, Hejmova, and Kleinzeller 1959), erythrocytes (Rosenberg and Wilbrandt 1957), muscle cells (Morgan, Regen, and Park 1964), and *Escherichia coli* (Kepes 1960), in which the influx of sugar is coupled with an efflux.

(*c*) *Self-inhibition of Uptake*

The rate of efflux of sucrose was negligible. Therefore, it was concluded that neither the slowing down of the post-initial uptake, described in Section III(*a*)(iii), nor the failure to observe accumulation against the concentration gradient from concentrated solutions, Section III(*a*)(i), could be caused by efflux of sucrose from the tissue. It is postulated that both these effects are caused by sucrose inside the cell inhibiting further uptake.

TABLE 2

UPTAKE AND EFFLUX OF SUCROSE BY BEAN LEAF TISSUE

C_o = the concentration of sucrose in the solution, C_{AOV} = the concentration of sucrose in the apparent osmotic volume (AOV), t_0 = time at which tissue was placed in the radioactive solution, t_1 = time at which tissue was removed from the radioactive solution, t_3 = time at the end of the experiment. The values for the apparent free space (AFS) efflux were obtained by extrapolating the linear parts of the curves in Figure 3(*b*) to zero time. Values marked with asterisk calculated from radioactivity, other values determined by analysis

C_o at t_0 (mg/ml)	10	10	40	40
Fresh weight at t_0 (g)	3·50	3·50	3·50	3·50
Total nitrogen (mg)	14·3	14·3	14·2	13·5
AFS (averaged) (ml)	1·74	1·74	1·74	1·74
AOV (averaged) (ml)	1·49	1·49	1·49	1·49
Steady rate of uptake (mg/mg N/hr)*	0·68	0·68	0·94	0·94
C_o at t_1 (mg/ml)	5·4	5·3	31·7	32·8
Sucrose in AFS at t_1 (mg/mg N)*	0·86	0·77	4·6	3·4
AFS efflux (mg/mg N)*	0·43	0·43	5·0	3·8
Steady rate of efflux (mg/mg N/hr)*	0·026	0·016	0·000	0·071
C_o at t_3 (mg/ml)	6·1	5·6	35·8	35·4
C_{AOV} at t_3 (mg/ml)	46·0	46·7	59·6	58·3

Unsuccessful attempts were made to calculate a parameter for this inhibition by an iterative technique using a standard membrane–carrier model. These attempts failed because the parameters K_m and $V_{\max}$ varied with time. These variations are probably consequences of the effect of exogenous sucrose on the metabolism of the tissue—it has a marked effect on respiration [Section III(*d*)] and spares protein nitrogen during prolonged storage (Vickery, unpublished data). These parameters vary considerably among experiments, apparently in response to variations in the physiological state of the tissue.

Hatch (1964) has proposed that sucrose phosphate is an intermediate in sucrose accumulation in sugar-cane and Hawker and Hatch (1966) found this tissue to contain a sucrose phosphatase whose activity was inhibited 60% by 50 mM sucrose.

A similar system may account for the self-inhibition of sucrose uptake in bean leaf tissue.

(d) Location and Significance of the Sucrose "Pump"

A knowledge of where the non-osmotic mechanism of sucrose uptake, the sucrose "pump", is located in the leaf cells is important to studies of sucrose movement through the whole plant. Some information on this question was obtained by an analysis of the respiration data.

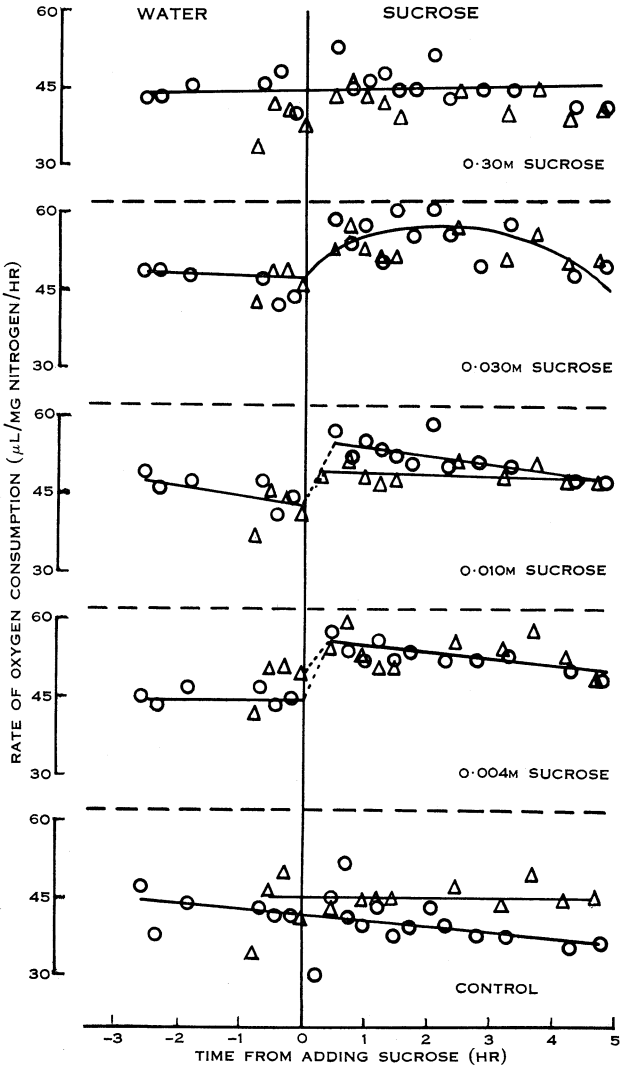

Fig. 4.—Rates of oxygen consumption by bean leaf strips in sucrose solutions of various initial concentrations. ○ Initial tissue harvest May 4, 1960. △ Tissue harvested May 9, 1960.

The increased rates of oxygen consumption caused by additions of sucrose were of short duration and independent of concentration (Fig. 4). The rate of carbon

dioxide production showed no correlation with the concentration of sucrose in the external solution (and, hence, in the free space) but was strongly correlated with the concentration of sucrose in the apparent osmotic volume (Fig. 5). This shows that the sites of carbohydrate metabolism are included in the osmotic volume for sucrose.

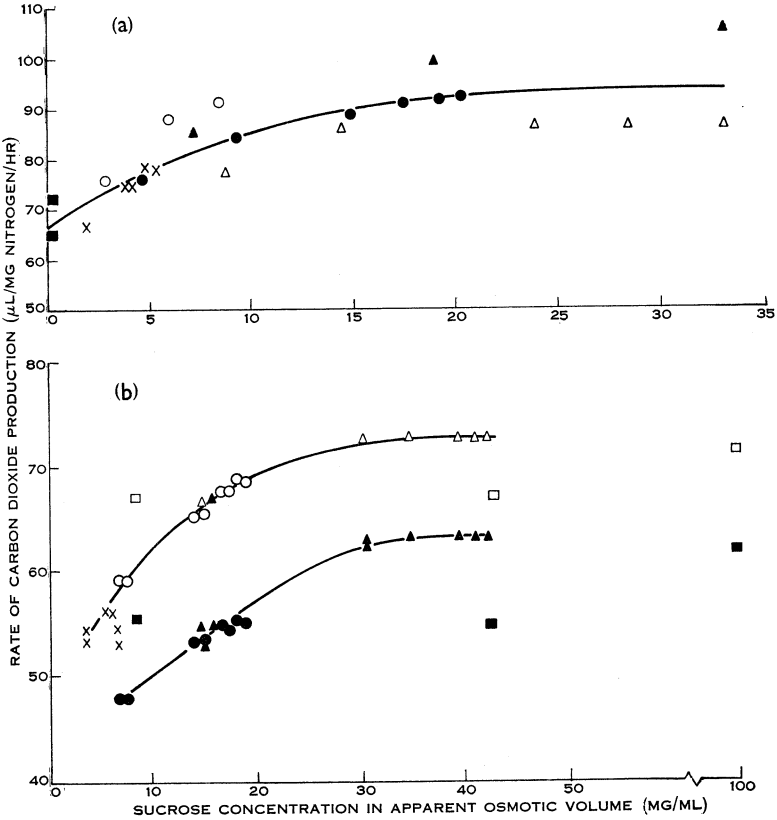

Fig. 5.—Relationship between the rate of carbon dioxide production and the concentration of sucrose in the apparent osmotic volume. (*a*) Respiration and uptake measured on the same day, initial outside concentration of sucrose 0·001M (■), 0·004M (×), 0·008M (○), 0·015M (●), 0·030M (△), and 0·100M (▲). (*b*) Respiration rates were measured with tissue harvested on May 4, 1960 (open symbols), and May 9, 1960 (solid symbols); uptake was measured with tissue harvested on May 5, 1960; initial outside concentrations of sucrose 0·004M (×), 0·010M (○, ●), 0·030M (△, ▲), and 0·300M (□, ■).

Therefore, part, at least, of the cytoplasm is included within the membranes carrying the sucrose pumps. Possible dispositions of the sucrose pumps within the cell are:

(1) On the plasmalemma only;

(2) On the tonoplast only;

(3) On other cell membrane systems only;

(4) On more than one membrane system.

The present results eliminate only disposition (2). Disposition (2) was proposed for immature storage tissue of sugar-cane by Sacher, Hatch, and Glasziou (1963), but they presented no evidence for this conclusion.

The overall concentration of sucrose in fresh bean leaf tissue is about 0·1 g/100 g fresh weight, and the concentration of sucrose in the phloem sap is, by analogy with other species, probably about 10 g/100 g fresh weight (Kursanov *et al.* 1958; Swanson and El-Shishiny 1958; Weatherley, Peel, and Hill 1959). The transport of sucrose from the chlorenchyma to the phloem is, therefore, against the concentration gradient and must require the intervention of a sucrose pump. The non-osmotic uptake mechanism investigated in these experiments has the location and the properties expected of such a pump.

IV. Acknowledgments

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V. References

Bieleski, R. L. (1960).—*Aust. J. biol. Sci.* **13**, 203.
Burger, M., Hejmova, L., and Kleinzeller, A. (1959).—*Biochem. J.* **71**, 233.
Glasziou, K. T. (1960).—*Pl. Physiol., Lancaster* **35**, 895.
Glasziou, K. T. (1961).—*Pl. Physiol., Lancaster* **36**, 175.
Grant, B. R., and Beevers, H. (1964).—*Pl. Physiol., Lancaster* **39**, 78.
Hatch, M. D. (1964).—*Biochem. J.* **93**, 521.
Hawker, J. S., and Hatch, M. D. (1966).—*Biochem. J.* **99**, 102.
Kepes, A. (1960).—*Biochim. Biophys. Acta* **40**, 70.
Kursanov, A. L., Chailakhian, M. K., Pavlinova, O. A., Turkina, M. V., and Brovchenko, M. I. (1958).—*Fiziologiya Rast.* **5**, 3.
Morgan, H. E., Regen, D. M., and Park, C. R. (1964).—*J. biol. Chem.* **239**, 369.
Pennell, G. A., and Weatherley, P. E. (1958).—*New Phytol.* **57**, 326.
Rosenberg, T., and Wilbrandt, W. (1957).—*J. gen. Physiol.* **41**, 289.
Sacher, J. A., Hatch, M. D., and Glasziou, K. T. (1963).—*Pl. Physiol., Lancaster* **38**, 348.
Swanson, C. A., and El-Shishiny, E. D. H. (1958).—*Pl. Physiol., Lancaster* **33**, 33.
Vickery, R. S., and Mercer, F. V. (1964).—*Aust. J. biol. Sci.* **17**, 338.
Weatherley, P. E. (1953).—*New Phytol.* **52**, 76.
Weatherley, P. E., Peel, A. J., and Hill, G. P. (1959).—*J. exp. Bot.* **10**, 1.