

Sucrose Concentration at the Apoplastic Interface between Seed Coat and Cotyledons of Developing Soybean Seeds

Received for publication July 10, 1984 and in revised form November 15, 1984

ROGER M. GIFFORD*¹ AND JOHN H. THORNE²
Central Research and Development Department, Experimental Station, E. I. du Pont de Nemours, and Company, Wilmington, Delaware 19801

ABSTRACT

The apoplastic sucrose concentration at the interface between cotyledons and surrounding seed coats of developing soybeans (*Glycine max* L. Merr. cv Wye) was found by three indirect methods to be in the range of 150 to 200 millimolar. This is an order of magnitude higher than has been reported elsewhere for soybean. It was also higher than the overall sucrose concentrations in the cotyledons and seed coats, each of which was approximately 90 millimolar. By defoliating plants 24 hours before measurement, both the overall sucrose concentration in the cotyledons and the interfacial apoplastic sucrose concentration were reduced by three-fourths. However, there was no day/night difference in overall tissue sucrose concentration of cotyledons or seed coats from intact plants suggesting the existence of a homeostatic mechanism compensating for the diurnal photosynthetic cycle. About 7 hours were required for a tritiated polyethylene glycol-900 solution to fully permeate developing cotyledons (from ~220 milligram fresh weight embryos), implying high diffusion resistance through the tissue.

These results indicate that a high interfacial sucrose concentration may exist *in vivo*. They suggest that the saturable carrier-mediated component of sucrose uptake may be of little physiological significance in the outermost cell layers of the cotyledons.

After being unloaded from sieve tubes within the seed coat, solutes that are destined for delivery to a developing bean embryo must pass through an apoplastic (*i.e.* extracellular) region, since no symplastic connections have been observed between parent plants and embryos (2, 10). The solutes in this apoplastic region could be involved in the regulation of both efflux of solutes from the seed coat and uptake by the sink tissues. Rates of uptake of sucrose by isolated soybean embryos incubated in liquid media are concentration dependent up to at least 400 mM (6, 11). The rate of efflux of sucrose from seed coats appeared to be insensitive to a physiological range of osmotica placed in surgically accessed seed coat cups in soybeans (12).

At low sucrose concentrations (<20 mM), uptake kinetics by soybean cotyledons (6, 11), and by protoplasts isolated from cotyledons (9), are dominated by an active carrier-mediated mechanism. At higher concentrations, a diffusion-like, nonsatur-

able component becomes increasingly dominant. Thus, a prerequisite for developing an understanding of the possible role of apoplastic sucrose concentration in coordinating rates of sucrose efflux from the seed coat and uptake by the cotyledons is knowledge of the concentration of sucrose at the apoplastic interface between these two organs. By examining this question for soybean from three points of view, we concluded that the interfacial sucrose concentration was 150 to 200 mM.

MATERIALS AND METHODS

Plant Material. A determinate soybean cultivar (*Glycine max* L. Merr. cv Wye) was grown two plants per pot in Terra-lite Metro Mix 350 potting mix (Grace Horticultural Products, Cambridge, MA), without rhizobial inoculation, in a controlled environment growth room. Humidity was not controlled and watering was with half-strength Hoagland No. 2 nutrient solution and tap water. Temperatures day and night were 24 and 19°C, respectively. Illumination during the 12-h photoperiods was from a mixture of Sylvania cool-white fluorescent lamps (SHOF96T12/CW/SHO) and 100-w incandescent lamps giving PAR ranging from 400 $\mu\text{E m}^{-2} \text{s}^{-1}$ at the edge to 750 $\mu\text{E m}^{-2} \text{s}^{-1}$ in the center of the room at the plant height. Pot positions were rotated systematically such that growth started at 450 $\mu\text{E m}^{-2} \text{s}^{-1}$ and averaged about 700 $\mu\text{E m}^{-2} \text{s}^{-1}$ during the 25 d prior to using the plants. Planting density was 23 plant m^{-2} . Flowering occurred about 31 to 34 d after planting. Seeds were used 24 to 26 d after flowering. They contained embryos of 210 to 240 mg fresh weight, and 55 to 65 dry weight. Mature seeds contained embryos of approximately 150 mg dry weight.

Methods. Chemicals and enzymes used were PEG-400³ (Sigma) ³H-PEG-900 (New England Nuclear, 4.9 mCi g^{-1}), [¹⁴C]sucrose (New England Nuclear, 3.7 mCi mmol^{-1}). Cotyledons and seed coats were extracted at least 3 × 1 h with 80% ethanol at 70°C to recover sugars. The freeze-dried extracts were taken up in water, and assayed for glucose and sucrose. Glucose was assayed enzymically using Worthington's 'Flozyme glucose 340 nm' (based on hexokinase and glucose-6-P dehydrogenase). After glucose determination for a single sample, sucrose was assayed by stirring invertase (Sigma, 600 units mg^{-1}) into the solution in the cuvette and reading absorbance again after 4 min.

Seed coat cups, comprising the lower half of seeds attached at the funiculus to surgically opened, but otherwise intact pods, were prepared as described previously (12). Before removal of the half-embryo from the seed coat cup, silicon grease was brushed onto the cut edges of the seed coat. This prevented wicking over the edge of solutions placed in the cup. Flexible wire 'pipe cleaners' held pods at appropriate angles to prevent

¹ Visiting scientist from CSIRO, Australia with support from E. I. du Pont and Company. Present address: Commonwealth Scientific and Industrial Research Organization, Division of Plant Industry, GPO Box 1600, Canberra, A.C.T. 2601, Australia.

² Present address: E. I. du Pont de Nemours and Company, Agricultural Chemicals Department, Stine-Haskell, P.O. Box 30, Newark, DE 19714.

³ Abbreviations: PEG-400, polyethylene glycol (mol wt ~ 400); ³H-PEG-900, tritiated polyethylene glycol (mol wt ~ 900).

spillage of solutions pipetted into the seed coat cups.

The initial *in vivo* sucrose contents of seed coat cups were estimated indirectly by determining the ratio of sucrose content to alcohol extracted dry weight of the surgically removed top half of each seed coat, and by knowing the relationship between the top half and the bottom (cup) half in this regard. For a separate set of equivalent seeds, the top halves and cup halves of the seed coats were obtained as calibration samples and immediately extracted with 80% ethanol. The sucrose contents (s) and extracted dry weights (d) were determined for the calibration seed coats and the ratio $r = s/d$ computed. The ratio R , of r for the cup half (r_c) to that for the top half (r_t) (i.e. $R = r_c/r_t$) was determined for each calibration seed and an average taken for several replicates in each experiment. The average value for R of 44 such calibration seed coats in the size range used was 1.14 ± 0.02 . For any given experimental seed coat cup, i , having alcohol-extracted dry weight d_{ci} , the *in vivo* sucrose content (s_{ci}) was calculated as

$$s_{ci} = d_{ci} \cdot r_{ci} = d_{ci} \cdot R \cdot r_{ti}$$

Experiments involving the time-course of sucrose efflux from cotyledons were conducted in conditions that kept the fresh weight change of the tissue to less than 0.2%. This was achieved by running such experiments at melting-ice temperature and by including 140 mM PEG-400 as osmoticum in the external medium.

RESULTS AND DISCUSSION

Time-Course of Uptake of ^3H -PEG-900 by Cotyledons. In the design of exodiffusion experiments for sampling apoplastic sucrose, it is necessary to know the time required for the apoplastic space to reach diffusional equilibrium with a bathing solution. This was determined using tracer levels of ^3H -PEG-900 in PEG-400 osmoticum on the assumption that PEG-900 does not permeate the symplast. Knowing the dpm/ μl in the bathing medium containing ^3H -PEG allowed calculation of the μl of apoplastic volume equilibrated, based on the dpm recovered following incubation. It took about 7 h for the apoplast of cotyledon pairs to reach full equilibration with the bathing medium (Fig. 1); half-saturation was achieved at about 120 min. The final equilibrium volume (6.7 μl) represented 4% of the water in the tissue. The constancy of the level of PEG-900 in the cotyledons between 7 and 24 h (Fig. 1), at a level corresponding to only 4% of grain water, indicates that the molecule was not accumulated in the symplast of the tissue. Ho and Gifford (4) also found that PEG-900 was not accumulated into the symplast of developing wheat endosperm and that the same apoplastic volume was available to sucrose, mannitol, and the larger molecule of PEG-900.

To complete the calculation of apoplastic sucrose concentration, we at first considered determining the sucrose present in the apoplast using 'compartmental analysis', a common method to separate apoplastic efflux from symplastic efflux of endogenous or preloaded solutes. However, with such slow penetration of the apoplast by a solute (Fig. 1), compartmental analysis is invalid (15; and see "General Discussion"). Alternative approaches were necessary.

Concurrent ^3H -PEG-900 Influx and Endogenous Sucrose Efflux. As one alternative, experiments were designed to enable estimation of sucrose concentration in the apoplast of the surface layers of the cotyledons as follows. When cotyledons are first dropped into liquid medium containing ^3H -PEG-900, the label starts to diffuse into the surface layers of apoplast while sucrose diffuses out of the same space. However, within minutes symplastic efflux will start to augment the apoplastic efflux, and increasingly so with time. Dividing the total sucrose effluxed in time t by the volume penetrated by ^3H -PEG in the same time,

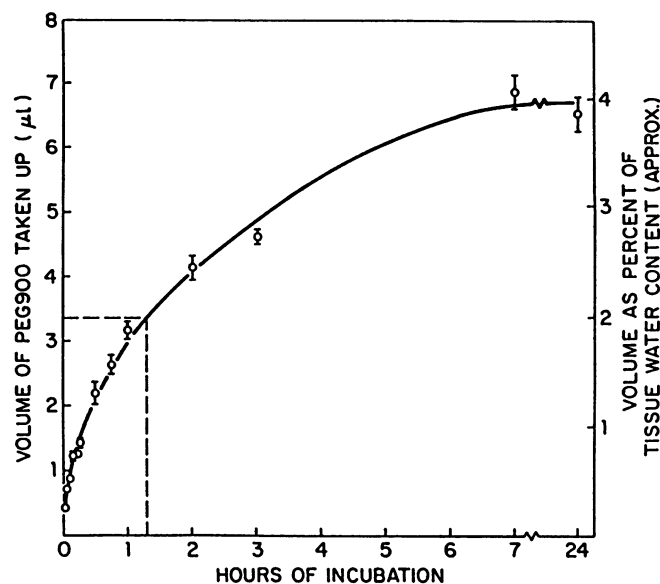


FIG. 1. The long-term time-course of equilibration of ^3H -PEG-900 with separated soybean cotyledons having the embryonic axis removed. Each point is the mean (\pm SE) of four pairs of cotyledons incubated on ice for various times in 2 ml of 140 mM PEG-400 containing 677 dpm/ μl ^3H -PEG for incubations longer than 30 min and 2500 dpm/ μl for shorter incubations. Following incubation, surface-contaminating ^3H -PEG was removed by a standard washing and drying procedure. (---), half-time for apoplastic equilibration with external solution.

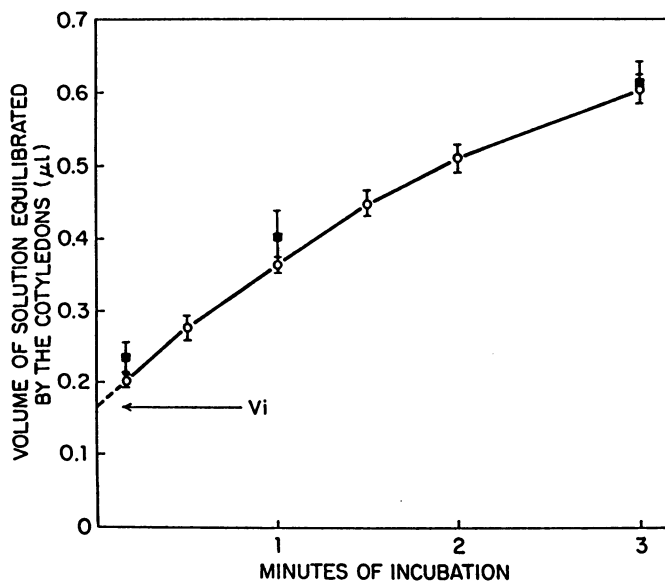


FIG. 2. The short-term time-course of equilibration of ^3H -PEG-900 with separated cotyledon pairs having the embryonic axis removed. In one experiment (\blacksquare), there were four replicate cotyledon pairs; in the other (\circ), there were ten replicates per data point. For details see Figure 1. The intercept at $t = 0$, V_i , is $0.16 = 0.03 \mu\text{l}$, this being taken as the volume of bathing medium left on the cotyledon surface by the standardized wash/dry procedure.

gives an apparent apoplastic sucrose concentration—an overestimate of true apoplastic concentration because of the symplastic contribution to efflux. By continuing the incubation for various periods, the apparent concentration can be extrapolated back to $t = 0$ to provide an estimate of apoplastic sucrose concentration close to the cotyledon surface.

Surface ^3H -PEG contamination was effectively removed by a 15-s water rinse and a 10-s blot on a pad of soft paper towels prior to extraction of ^3H -PEG. The surface contamination left by this procedure, V_i , was found to be $0.16 \pm 0.03 \mu\text{l}$ (Fig. 2).

Apparent apoplastic sucrose concentrations, S_{app} , were calculated for each time of incubation, t , as follows:

$$S_{app} = \frac{0.62m_t}{(V_t - V_i)}$$

where V_t is the volume of ^3H -PEG equilibrated in time t , m_t is mass of sucrose effluxed from the tissue in time t , and the factor 0.62 is the square root of the ratio of mol wt of sucrose to PEG-900 to correct for different coefficients of diffusion according to Graham's law of diffusion.

Figure 3 shows the results of three experiments, two using intact plants and one using plants that had been defoliated the day before. The apparent concentration showed a minimum after about 90 to 120 s. With prolonged incubation, S_{app} continued to increase steadily to over 0.5 M after 40 min followed by a decline thereafter (data not shown). Accurate extrapolation of the curve between 2 min and $t = 0$ is not possible because the pooled errors of the three terms in the above equation become large as t approaches zero, and because of the U-shaped character of the curve. However, it is clear that the curve could not be extrapolated to less than 100 mM for the intact plants even if one ignored the upturn between $t = 2 \text{ min}$ and $t = 0$. By contrast, for pods from plants which had had the leaves removed 24 h earlier (Fig. 3, \square), the value at the minimum was approximately one-quarter of the value for the nondefoliated plants. We tentatively interpret the U-shape of the curve reflecting a distinction between solely apoplastic efflux and apoplastic plus an increasing contribution by symplastic efflux.

Exogenous Sucrose Concentration Required To Prevent Decline of the Endogenous Sucrose Pool in Isolated Cotyledons. The observation that symplastic sucrose effluxed from cotyledons placed in a dilute sucrose solution, despite cotyledons being accumulating organs, suggested another method for estimating

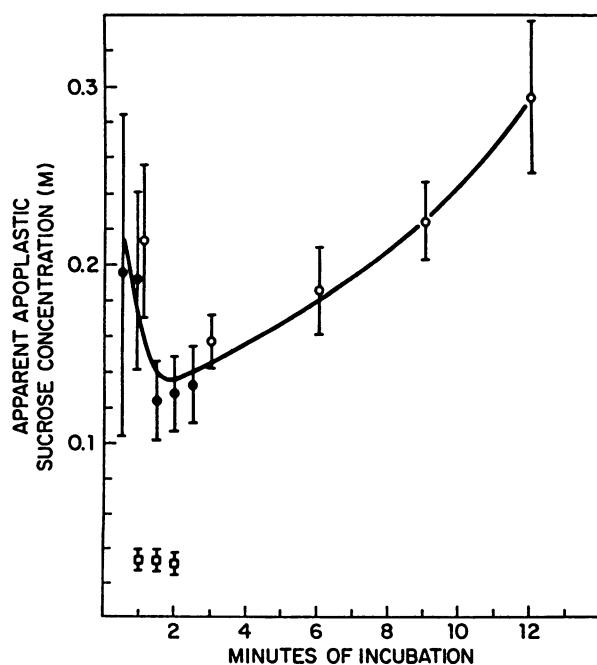


FIG. 3. The apparent apoplastic sucrose concentrations in the outermost layers of soybean cotyledons as a function of the time over which incubation at $\sim 0^\circ\text{C}$ occurred. The results are for two experiments using intact plants (\bullet , \circ) and one for defoliated plants (\square).

apoplastic sucrose concentration in the surface layers of the cotyledons. *In vivo*, there is a certain pool-size of sucrose in cotyledons (actually a composite of several pools) which may be drawn upon for starch and lipid biosynthesis and contributed to by uptake from the apoplastic solution at the surface of the cotyledons. At steady state, the input to the composite pool equals the output to respiration and biosynthesis. Cotyledons placed in *in vitro* solution culture continue to respire and to convert the sucrose pool to these insoluble storage products (6, 11).

If a freshly isolated cotyledon is placed into a sucrose solution having a concentration equal to the *in vivo* surface apoplastic concentration, then the size of the cotyledonary sucrose pool should remain constant. If the bathing sucrose concentration is higher than *in vivo*, then net uptake will at first exceed rate of biosynthesis and respiration and the sucrose pool will get larger. Conversely, if the external sucrose concentration is less than it was *in vivo*, then influx will be less than efflux and there will be net efflux into the bathing solution; hence the cotyledonary pool will shrink. Thus, the surface apoplastic sucrose concentration, *in vivo*, is equal to that sucrose concentration of an incubation solution (at *in vivo* temperature) causing no change in tissue sucrose content.

Figure 4 shows two experiments in which, for each cotyledon pair, one cotyledon was extracted to determine initial sucrose content and the other was incubated in a given sucrose solution for 2 h at 24°C . After 2 h, surface solution was washed off quickly so as to minimize removal of apoplastic solution (see below), and the cotyledon extracted to determine final sucrose content. The bathing sucrose concentration that resulted in zero change of internal sucrose content (null-balance point) was 163 mM for one experiment and 141 mM for the other, but the standard errors are such that the two estimates do not differ significantly. The method used to remove surface contamination of the cotyledon by excess solution differed between the two curves illustrated in Figure 4, but it appeared to be of little consequence to the result. For the curve with solid symbols, incubated cotyledons were water-washed for 5 s and then dab-dried between pads of paper towels for 10 s. For the curve with open symbols there was a 15-s wash, but also each paired cotyledon used for estimating

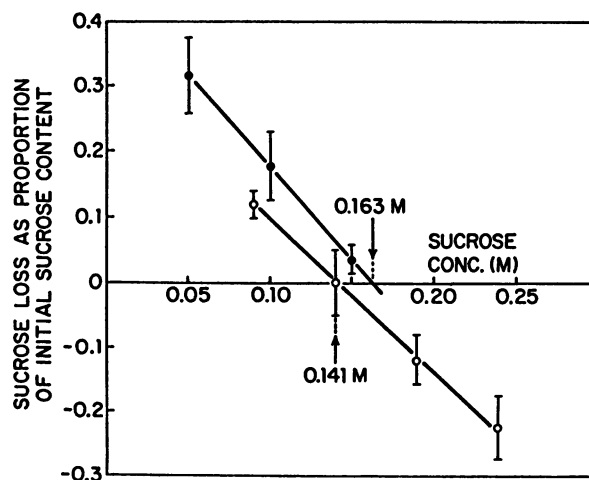


FIG. 4. The null-balance method for determining the surface apoplastic sucrose concentration of soybean cotyledons. Cotyledons were incubated at growth-room temperature for 2 h, washed in a standard quick procedure with cold water, and the net amount of sucrose taken up or lost by the tissue determined. The loss of sucrose is expressed as a proportion of the initial sucrose content of the cotyledon, negative values indicating net uptake. Two experiments are shown, each having five replicates per point ($\pm\text{SE}$).

initial sucrose content was first dropped into the appropriate incubation medium for 5 s before being given the same carefully timed 15-s wash plus 10-s drying.

External Sucrose Concentration Required To Prevent Decline of the Endogenous Sucrose Pool in Seed Coats. The same null-balance method used for cotyledons was used for surgically prepared seed coat cups having the embryo discarded. The seed coat, too, has pools of sucrose in various cell types, these being contributed to by unloading of the sieve-tube companion cell complex and depleted by efflux into the apoplast of the embryo. When the embryo is replaced by exogenous sucrose solutions, efflux from attached seed coat cups continues for soybean and also *Vicia faba*, but there may be concurrent influx too. That concentration which holds the seed coat pool constant at the *in vivo* value is proposed to represent the *in vivo* apoplastic concentration at the seed coat surface.

The first method used for cotyledons (involving ^3H -PEG influx) cannot be used for seed coats because there is an aerenchymatous parenchyma layer in seed coats (5, 10) which floods with several microliters when liquid is placed in the cup. This would lead to a substantial underestimate of apoplastic sucrose concentration if not corrected for. In applying the null-balance method, the flooded aerenchyma was finally purged of exogenous sucrose by a 4-min water wash which was found in a preliminary experiment, using [^{14}C]mannitol as tracer, to remove over 95% of it. As this wash must also remove some apoplastic, and possibly a little symplastic, sucrose, the method so applied to seed coats is presumably less precise than when applied to cotyledons.

Experiments were conducted on (a) completely isolated seed coat cups set in hollows in modeling clay, (b) on seed coat cups attached to detached pods, and (c) on seed coat cups attached to pods still attached to the plant. Since part of the logic of the method is that, at the null-balance point, the steady state sucrose pool should be maintained while a net sucrose flux through the pool continues, the fully attached seed coats should give the most reliable estimate. Detachment from the plant would partly or totally impair sieve-tube unloading and so the results for detached seed coats would be expected to be underestimates.

Figure 5 compares results for all three experimental arrangements. When sucrose concentrations placed in the cups were high enough, the sucrose pool increased in size. As expected, the null-balance concentration increased with the degree of integrity of the system.

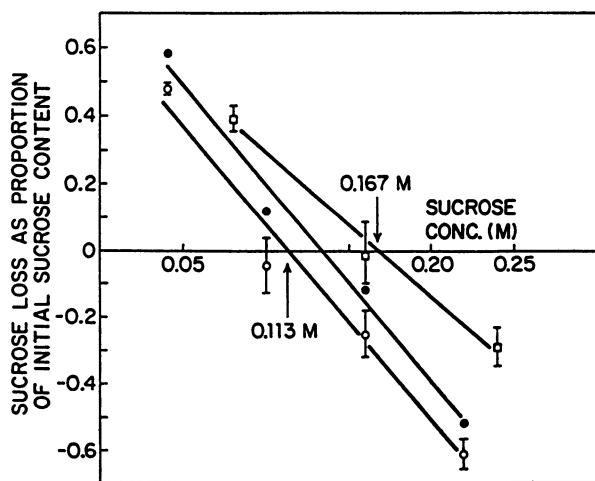


FIG. 5. The null-balance method for determining the surface apoplastic sucrose concentration of soybean seed coat cups. Three experiments are shown: (O), isolated seed coat cups; (●), seed coat cups attached to isolated pods; (□), seed coat cups attached to intact plants.

Within the limits of experimental error, the null-balance method applied both to cotyledons and to seed coat cups gave the same value for interfacial concentration, approximately 160 mM.

Overall Sugar Concentrations in Cotyledons and Seed Coats. The overall sucrose and glucose concentrations of cotyledons (less embryonic axes) or seed coats were obtained by obtaining fresh weight within seconds of dissection, freezing on dry ice, freeze-drying, obtaining freeze-dry weight, followed by 80% ethanol extraction to determine total glucose and sucrose content. Overall concentrations were calculated as (μg sugar extracted)/(fresh weight-dry weight). The results for three groups of seeds are shown in Table I. Experiment A was run in parallel with the null-balance experiment on cotyledons depicted by open circles in Figure 4, using pods randomly selected according to the same size criteria from the same plants. The estimated interfacial apoplastic sucrose concentration greatly exceeded the overall tissue sucrose concentration. In experiment C, some plants were defoliated 24 h earlier to remove the photosynthetic source. This caused a substantial decline in both overall sucrose concentration (Table I) and the surface apoplastic concentration (a parallel set of seeds on the same plants (Fig. 3)). However, a diurnal cycle in sucrose concentration did not occur in tissues of seeds on intact plants (Table I, experiment B). Glucose, while being present at much lower concentrations than sucrose, followed qualitatively similar patterns to sucrose.

GENERAL DISCUSSION

Three lines of evidence suggest that the sucrose concentration in the extracellular solution at the interface between the unloading seed coat and the developing embryo, 24 to 26 d after flowering, was 150 to 200 mM under the conditions of these experiments using intact plants. First, experiments involving ^3H -PEG-900 influx into cotyledons (to trace free-space penetration) and concurrent endogenous sucrose efflux, led to calculated apparent sucrose concentrations which extrapolated to about 200 mM at $t = 0$ (Fig. 3). Second, a novel 'null-balance' approach applied to cotyledons indicated that it took about a 150-mM sucrose bathing solution to hold the sucrose content of the live tissue at its *in vivo* size (Fig. 4). And third, a similar null-balance approach applied to half-seed coats, still attached to the plant by the funiculus, indicated that it required about 170 mM sucrose to maintain the tissue sucrose content at its *in vivo* level (Fig. 5).

While various considerations might raise questions concerning the precision of each of the methods, the convergence of all three of them on similar answers in the range 150 to 200 mM is mutually supportive. If a substantial gradient in apoplastic sucrose concentration in the cotyledon, between the interface with the seed coat and the center of the embryo, exists, then the methods described will underestimate the interfacial sucrose concentration. This is because the closely appressed flat faces of the cotyledon pair—at the center of the organ *in vivo*—were fully exposed to the bathing media. To that extent our estimates are minimal ones. But other uncertainties may act in the other direction: if, for example, Graham's Law of diffusion overestimates the ratio of diffusion coefficients for PEG to sucrose the first method might yield too high an estimate. PEG-900 being a larger and more linear molecule than sucrose, might conceivably not penetrate such a large apoplastic volume as sucrose. However, Ho and Gifford (4) found that both molecules led to the same estimate of apoplastic volume in wheat endosperm. If the balance between sucrose influx and efflux is affected by other solutes such as mineral ions, amino acids, or hormones, or by the osmotic concentration (7, 14), the null sucrose concentration might be shifted. These possibilities can be investigated for future refinements of the methods, but they are not expected to change the conclusion substantially, since the three distinct methods

Table I. Overall Sucrose and Glucose Concentrations in the Tissue Water of Developing Soybean Cotyledons and of Associated Seedcoats
Tissue water content was calculated as the difference between fresh wt and freeze-dried wt.

Experiment	Plant Status	Time of Day	No. of Replicates	Sucrose Concentration		Glucose Concentration	
				Cotyledons	Seedcoat	Cotyledons	Seedcoat
				<i>mM</i>			
A	Intact plants	Late morning (11–12 AM)	8	83.0 ± 3.7	103.5 ± 3.0	8.3 ± 0.3	15.0 ± 0.9
B	Intact plants	End of day (6 PM)	5	90.4 ± 2.8	89.6 ± 3.1	12.0 ± 0.5	13.6 ± 0.3
B	Intact plants	End of night (7 AM)	5	85.5 ± 4.6	97.3 ± 3.7	10.5 ± 1.0	14.8 ± 0.3
C	Intact plants	Late morning (11–12 AM)	5	93.2 ± 3.0		8.2 ± 1.1	
C	Plants defoliated morning before	Late morning (11–12 AM)	5	23.2 ± 1.7		4.1 ± 0.2	

yielded similar estimates.

The estimate obtained for the interfacial sucrose concentration is an order of magnitude higher than the 3.4 to 37 mM reported by others (1, 5, 6) as representing the apoplastic concentration of sucrose in soybean seed coats or developing embryos. The latter studies used 'compartmental analysis'. This method resolves a supposed multicomponent but smoothly declining wash-out curve into the sum of several exponential curves—one for each of three putative compartments arranged in series and corresponding to vacuole, cytoplasm, and apoplast—by a 'curve-stripping' technique which is replete with theoretical and practical difficulties (15). For a bulky tissue with such long apoplastic equilibration times as found here, symplastic and apoplastic efflux will inevitably occur concurrently within a few minutes. Compartmental analysis applied to diffusion out of a whole organ can only be validly applied when diffusion from the apoplastic compartment occurs at least an order of magnitude faster than transfer across the plasma membranes (15). Since the half-time for symplast to apoplast transfer of sucrose in cotyledons is certain to be substantially less than 12 h, compartmental analysis cannot be used reliably. Despite the mathematical formalization of curve-stripping, the assignment of a time that separates apoplastic from symplastic efflux is entirely arbitrary for a bulk tissue.

Using a variant of compartmental analysis for determining the apoplastic sucrose concentration in developing *Phaseolus vulgaris* embryos, Patrick and McDonald (8) give an estimate of 100 to 200 mM. This estimate agrees with the present one for soybean embryos. Patrick observed elsewhere (7) that sucrose efflux from detached seed coats of *Phaseolus* was balanced by influx when the bathing solution contained about 200 mM sucrose. Assuming that the flooding of seed coat aerenchyma was taken into account, that 200 mM null-balance concentration would, according to the concept developed here and the comparison of the left-hand and right-hand curves in Figure 5, be an approximate estimate (possibly an underestimate) of the apoplastic sucrose concentration in that tissue.

For both the cotyledons and the seed coats of intact soybean plants, the overall sucrose concentrations in the tissues (~90–100 mM; Table I) were considerably less than the estimated interfacial apoplastic sucrose concentrations of about 160 mM. This suggests that there is a downhill sucrose concentration gradient across the plasmalemma into cotyledon cells, and an uphill gradient for transport out of seed coat cells into apoplast. However, a large sucrose concentration gradient may occur in the apoplast from the outside to the inside of the cotyledon, as the long-time constant in Figure 1 suggests. If so, it cannot be precluded on present evidence that the symplastic and adjacent apoplastic sucrose concentrations are closely matched for any individual cell in the cotyledon. For the seed coats, on the other hand, being thin (~0.2 mm) and loose-celled (10), the apoplast may be essentially uniform in concentration. Thus, to maintain

the overall sucrose concentration lower than the interfacial concentration, either the seed coat cells pump sucrose out against a concentration gradient or only a portion of the overall tissue is involved in sucrose transport. A minute proportion of the seed coat volume comprises the sieve element-companion cell complex network, which is expected to have a symplastic sucrose concentration of up to 400 mM (3). To the extent that these cells unload symplastically down a concentration gradient into the parenchyma cells of the seed coat, it would appear that the latter may assist active unloading into the apoplast. However, the sieve element-companion cell complex might also unload passively down the gradient directly into the apoplast. Cooperation of both routes is not excluded, with the parenchyma acting as a buffer capable of taking up sucrose from the apoplast when the concentration there exceeds ~160 mM as in Figure 5. For each possible route, apoplastic sucrose concentration in the seed coat could potentially regulate net sucrose flux into the apoplast by (a) determining the rate of uptake by the storage parenchyma cells, or (b) by its contribution to the sucrose gradient from sieve tube symplast to apoplast, or (c) by being involved in a cell turgor-dependent mechanism of sieve tube unloading (7, 14).

The lack of diurnal variation in overall tissue sucrose concentration (Table I) suggests that a homeostatic mechanism may exist which maintains constancy of concentration in the tissues and, hence, constancy of growth by the embryo day and night. However, a limit to that mechanism was readily revealed by defoliation; within 24 h both the overall tissue sucrose concentration (Table I) and the minimum on the apparent apoplastic sucrose concentration curve (Fig. 3) had fallen by about three-quarters. Also, overall glucose concentration was halved. The parallelism between overall sucrose concentration and estimated interfacial sucrose concentration adds further support to the idea that the latter estimate has functional significance.

The uptake of sucrose by soybean cotyledons is concentration dependent even to as high as 400 to 500 mM sucrose (6, 11), so 160 mM in the apoplast is in a plausibly physiological range at which any variation would be reflected in embryo growth rate. Superimposed on a linear response dominant at these concentrations, sucrose uptake by an energy-dependent, carrier-mediated component occurs (6, 11). This saturable component has a half-saturating concentration of ~2 mM for cotyledon protoplasts (9) and ~8 mM for whole cotyledon tissue (6, 11). If apoplastic sucrose concentrations were high throughout the tissue, this high affinity saturable component would have virtually no role to play in sucrose uptake. However, with the likelihood of there being a gradient into the tissue, there may be a role for the saturable component in maintaining sucrose uptake at the center of the tissue despite a lower concentration there.

Acknowledgments—We are grateful to Shiela A. McKelvey for efficient and enthusiastic technical assistance, and to R. T. Giaquinta for inviting R.M.G. to take a study leave at the du Pont laboratories.

LITERATURE CITED

1. BENNETT AB, RM SPANSWICK 1983 Depression of amino acid-H⁺ cotransport in developing soybean embryos. *Plant Physiol* 72: 781-786
2. ESAU K 1965 *Plant Anatomy*, Ed 2. John Wiley, New York, p 624
3. FISHER DB 1978 The estimation of sugar concentration in individual sieve-tube elements by negative staining. *Planta* 139: 19-24
4. HO LC, RM GIFFORD 1984 Accumulation and conversion of sugars by developing wheat grains V. The endosperm apoplast and apoplastic transport. *J Exp Bot* 35: 58-73
5. HSU FC, AB BENNETT, RM SPANSWICK 1984 Concentration of sucrose and nitrogenous compounds in the apoplast of developing soybean seed coats and embryos. *Plant Physiol* 75: 181-186
6. LICHTNER FT, RM SPANSWICK 1981 Sucrose uptake by developing soybean cotyledons. *Plant Physiol* 68: 693-698
7. PATRICK JW 1983 Photosynthate unloading from seed coats of *Phaseolus vulgaris* L. General characteristics and facilitated transfer. *Z Pflanzenphysiol* 111: 9-18
8. PATRICK JW, R McDONALD 1980 Pathway of carbon transport within developing ovules of *Phaseolus vulgaris* L. *Aust J Plant Physiol* 7: 671-684
9. SCHMITT MR, WD HITZ, W LIN, RT GIAQUINTA 1984 Sugar transport into protoplasts isolated from developing soybean cotyledons II. Sucrose transport kinetics, selectivity, and modeling studies. *Plant Physiol* 75: 941-946
10. THORNE JH 1981 Morphology and ultrastructure of maternal seed tissues of soybean in relation to the import of photosynthate. *Plant Physiol* 67: 1016-1025
11. THORNE JH 1982 Characterization of the active sucrose transport system of immature soybean embryos. *Plant Physiol* 70: 953-958
12. THORNE JH, R RAINBIRD 1983 An *in vivo* technique for the study of phloem unloading in seed coats of developing soybean seeds. *Plant Physiol* 72: 268-271
13. Deleted in proof
14. WOLSWINKEL P, A AMMERLAAN 1984 Turgor-sensitive sucrose and amino acid transport into developing seeds of *Pisum sativum*. Effects of high sucrose or mannitol concentration in experiments with empty ovules. *Physiol Plant* 61: 172-182
15. ZIERLER K 1981 A critique of compartmental analysis. *Annu Rev Biophys Bioeng* 10: 531-562