# Sucrose Concentration Gradients along the Post-Phloem Transport Pathway in the Maternal Tissues of Developing Wheat Grains[1]

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Sucrose concentrations were measured in serial frozen sections of the post-phloem transport pathway in developing wheat (*Triticum aestivum* L.) grains. In normally importing grains, there was an approximately linear concentration gradient along the pathway, with a difference between the ends of the pathway of about 180 mM. This indicates an unusually low resistance for cell-to-cell transport, due perhaps to the large size-exclusion limit for the pathway. However, the existence of concentration gradients raises presently unresolvable questions about the relative contributions of diffusion versus bulk flow to transport within the symplast. The concentration gradient disappeared when sucrose movement ceased (i.e. in excised grains or when endosperm cavities of attached grains were perfused with *p*-chloromercuribenzene sulfonate [PCMBS] or with 1660 mOsm sorbitol). PCMBS appeared to block solute release into the endosperm cavity, whereas the sorbitol treatment, previously shown to cause localized plasmolysis in the chalaza, appeared to block movement across the chalaza. Sieve element/companion cell unloading appears to be an important control point for assimilate import. The sucrose concentration gradient and, probably, turgor and osmotic gradients are extremely steep there. PCMBS blocked import without affecting the sucrose concentration in the vascular parenchyma around the phloem. Thus, blockage of unloading was more complex than a simple "backing up" of solutes in the vascular parenchyma.

Assimilate import by a sink is a multistep process involving distinct types of transport through several different tissues. Because this complexity occurs on a reduced physical scale, it is difficult to analyze the individual transport processes involved and the extent to which each might contribute to the control of import (Oparka, 1990). As noted more fully elsewhere (Wang and Fisher, 1994a), developing wheat (*Triticum aestivum* L.) grains provide some distinct advantages for this purpose. Transport steps are relatively separate anatomically, and solute concentrations and other transport-related parameters can be obtained readily from the sieve tubes and endosperm cavity. Based largely on the constancy of Suc import in the face of widely varying Suc and osmotic concentrations in the endosperm cavity, we have concluded that control of import is exerted by trans-

port processes within the maternal grain tissues, relatively independently of conditions in the endosperm cavity (Fisher and Gifford, 1986, 1987; Wang and Fisher, 1994a).

After Suc is unloaded from the SE/CC complex, it enters an extraphloem Suc pool from which it is released across the plasma membranes of the nucellar cells into the endosperm cavity (Fisher and Wang, 1993; Wang and Fisher, 1994a). In the context of $^{14}C$ tracer kinetics, we have shown that this extraphloem Suc pool behaves basically as a single, well-mixed compartment with a turnover time of about 1.3 h (Fisher and Wang, 1993). This property has been very useful in the interpretation of treatment effects, since changes in the size of the pool can be used as an indicator of which transport step was most affected by an experimental treatment. Thus, the pool decreases rapidly after grains are removed from the plant (Fisher and Wang, 1993; Wang and Fisher, 1994a) and increases when transport across the nucellar cell plasma membrane is blocked by PCMBS (Wang and Fisher, 1995), and when symplastic movement is blocked between the phloem and nucellus by sorbitol-induced plasmolysis of the chalazal cells (Wang and Fisher, 1994a). Each of these interpretations has distinct implications not only for the behavior of the total crease Suc pool but for the attendant alteration of the Suc distribution within the crease tissues.

The question of Suc gradients within the crease tissues also has important implications for the normal transport of Suc from the phloem to the endosperm cavity. A steep gradient at some site would suggest a high resistance to movement and the possibility that that step could exert significant control on import. Previously (Fisher and Wang, 1993), during pulse-labeling experiments with $^{14}CO_2$, we found a shallow gradient of [$^{14}C$]Suc across the nucellus and chalaza, with a steeper gradient in the vascular parenchyma between the chalaza and conducting tissues. Since, kinetically, the crease Suc pool is well mixed, we proposed that the [$^{14}C$]Suc distribution should provide an approximate quantitative indication of total Suc distribution. Thus, the shallow $^{14}C$ gradient through the nucellus and chalaza suggested a low resistance to Suc movement through that portion of the symplast and across the plasma membrane, whereas the steep gradient in part of the vascular parenchyma suggested a higher resistance there. However, this interpretation must be partly modified by our finding that

Abbreviations: PCMBS, *p*-chloromercuribenzene sulfonate; SE/CC, sieve element/companion cell.

Suc movement across the nucellar cell membrane is a reversible process (Wang and Fisher, 1994a, 1995), allowing at least some local exchange of crease Suc with endosperm cavity Suc, sometimes of greatly different specific activities.

To verify some of our interpretations of treatment effects and to investigate more explicitly the pattern of Suc concentration during normal transport, we assayed the Suc content of serial frozen sections from the crease tissues during normal import and after several experimental treatments.

## MATERIALS AND METHODS

### Plant Material

Wheat plants (*Triticum aestivum* L. cv SUN 9E) were grown in a growth chamber as described for previous work (Fisher, 1990). Briefly, plants were grown on a 16-h photoperiod at a PPFD of 450 $\mu$mol m$^{-2}$ s$^{-1}$ and a day/night regime of 22/16°C. Plants were irrigated with water at 16-h intervals and with a complete nutrient solution once a week. Plants used in these experiments were 17 d postanthesis.

### Freeze Sectioning, Sample Preparation, and Sugar Assays

To determine the crease Suc distribution, a grain was detached, surrounding glumes were removed, and the "cheek" regions were sliced from the ventral side of the grain (Fig. 1A). This provided a flat, moist surface for freezing the grain securely to the microtome chuck, a length of 5-mm-diameter water-soaked wood doweling. This surface was pressed into mounting medium (Electron Microscopy Sciences, Ft. Washington, PA) on the surface of the chuck and covered with powdered dry ice. About 1 min elapsed between detaching a grain and freezing it.

Freeze sectioning was carried out at −20°C in a microtome cryostat. The frozen grain was trimmed before sectioning by cutting away the basal and distal thirds of the grain and part of each side. The position of the green

pericarp, readily visible in the cross-section at each end of the trimmed grain, was used to orient the plane of sectioning parallel to the nucellar surface. Sections were cut at a thickness setting of 64 $\mu$m. When the endosperm cavity was reached, sectioning was momentarily stopped and the sides of the grain were retrimmed. The remaining sections were cut at as regular a rate as possible to minimize variations in section thickness. Sections were aligned in order along the surface of the microtome blade. They remained frozen during all subsequent manipulations until the samples were thawed for sugar analysis. This phase of sectioning took about 2 to 4 min and was terminated when sectioning passed through the colorless vascular parenchyma into the green outer pericarp (Fig. 1B). The sections were then transferred in order to a band of mineral oil on a precooled microscope slide. More cold mineral oil was added to completely immerse the sections. The slide was placed in a small box in an ice bucket containing dry ice and transferred to a −20°C cold room.

To provide as homogeneous a tissue sample as possible for analysis, the required tissue was cut from each section under a dissecting microscope, in the cold room, using a razor blade fragment. This was accomplished by two pairs of parallel cuts, one pair across the crease axis and the other pair parallel to the axis, just inside the readily visible boundary of the green pericarp (Fig. 1B). This produced a rectangular sample of the desired tissue, the length and width of which were measured with an ocular micrometer before transferring it to a small test tube.

Sample volumes were calculated from the sample areas, usually about 0.25 × 2 mm, and from section thicknesses (64 $\mu$m). Frozen sections of the endosperm cavity sap provided convenient material for verification of this calculation, since they could be handled identically with the tissue samples (above). When used for this purpose, care was taken during sectioning to immerse the sections in cold mineral oil immediately after they were cut to completely eliminate water loss. Actual sample volume was obtained by allowing the trimmed and measured sample to thaw under mineral oil and then measuring the diameter of the



**Figure 1.** Diagrams of the tissues in a cross-section of a wheat grain. A, Whole grain cross-section, showing the tissues trimmed away before freezing and the plane of sectioning. B, Arrangement of tissues in the crease and their approximate relationship to the sample positions shown in Figures 2 and 3. Samples consisted only of nonchlorenchymatous tissues (nucellus, chalaza, and vascular parenchyma with xylem and phloem). The green pericarp (shaded area) was removed by dissection while the sections remained frozen. nuc, Nucellus; ch, chalaza; par, vascular parenchyma; p, phloem; x, xylem.
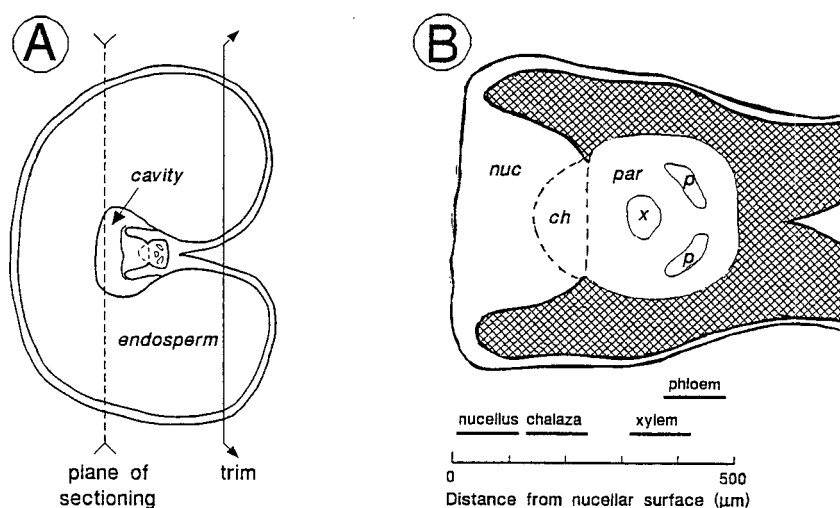
resulting droplet with an ocular micrometer in a compound microscope.

Suc plus Glc in the samples was assayed in the presence of invertase by the direct fluorimetric procedure of Jones et al. (1977). Almost all samples were between 1 and 10 nmol, well within the range of accuracy for the procedure. Because previous assays of the crease sugars in intact grains and in all experimental treatments except prolonged grain removal have shown the free Glc level to be about 10% or less of the Suc pool (Fisher and Wang, 1993; Wang and Fisher, 1994a; and additional measurements), the Suc content of the samples was taken to be the same as that of the total Glc after hydrolysis with invertase. However, the Suc distribution in several grains was also substantiated by measurements of soluble $^{14}C$, known to consist almost entirely of [$^{14}C$]Suc, in each sample (see "Results").

To identify the tissue type(s) represented in each sample, the tissue samples were recovered from the assay tubes and mounted, in order, on an adhesive-coated microscope slide. After staining with toluidine blue O, they were mounted and examined by bright-field microscopy.

## Endosperm Cavity Perfusion Treatments

For comparison with two treatments shown to block post-phloem transport, the endosperm cavities of attached grains were perfused with 10 mM PCMBS (Wang and Fisher, 1995) and with 1660 mOsm sorbitol (Wang and Fisher, 1994a). Two grains were perfused for each treatment; both treatments were carried out on the same ear. Experimental conditions were as described previously, including pulse labeling of the flag leaf with $^{14}CO_2$. Sorbitol perfusion was continuous, whereas the PCMBS treatment consisted of a 20-min preperfusion with 10 mM PCMBS followed by standard 300 mOsm perfusion medium for the remainder of the experiment. Five hours after labeling with $^{14}CO_2$, the four experimental grains were frozen, as were two control grains.

## Excised Grains

For comparison with earlier experiments using excised grains, several grains were removed from an ear and placed on moist filter paper to deplete their crease Suc pool (Fisher and Wang, 1993). After 4 h, two grains were frozen for determination of their crease Suc distribution. The remaining grains were sliced longitudinally through their endosperm cavities, parallel to the nucellar surface, and incubated in 400 mM Suc solutions as described earlier (Wang and Fisher, 1994b). After 4 h, two grains were frozen for the determination of their crease Suc distribution.

## RESULTS

The results of these experiments are given in Figures 2 and 3. Bars in the figures provide a guide to the tissue types present in each region of the profiles (Fig. 1B). They must be regarded as approximate, partly because more than one tissue zone was often present in the relatively thick sections used and partly because the plane of sectioning was often
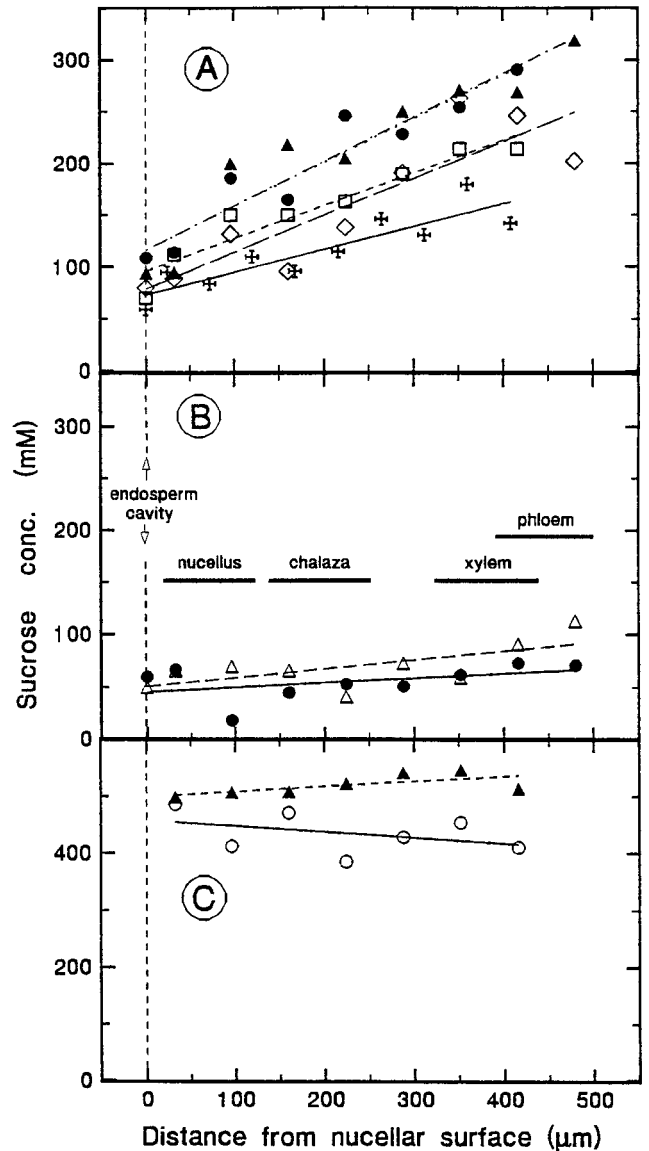


**Figure 2.** Suc distribution in the crease tissues of "attached" grains (i.e. frozen within about 1 min after removal from the ear) and in excised grains. Different grains are represented by different symbols. A linear regression is fitted to the data for each grain. The predominant positions of the major crease tissues are indicated. However, sections usually contained more than one tissue type (cf. Fig. 1B). "Xylem" and "phloem" simply indicate the presence of these tissues in a sample; vascular parenchyma accounted for almost all of the tissue in sections more than 250 $\mu$m from the nucellar surface. A, "Attached" grains. In one experiment (cross symbols), sections were cut at 48 $\mu$m instead of 64 $\mu$m. B, Excised grains incubated for 4 h on moist filter paper before freezing. C, Excised grains incubated for 4 h on moist filter paper, after which the grains were sliced longitudinally to expose the nucellar surface and incubated for 4 h in 400 mM Suc.
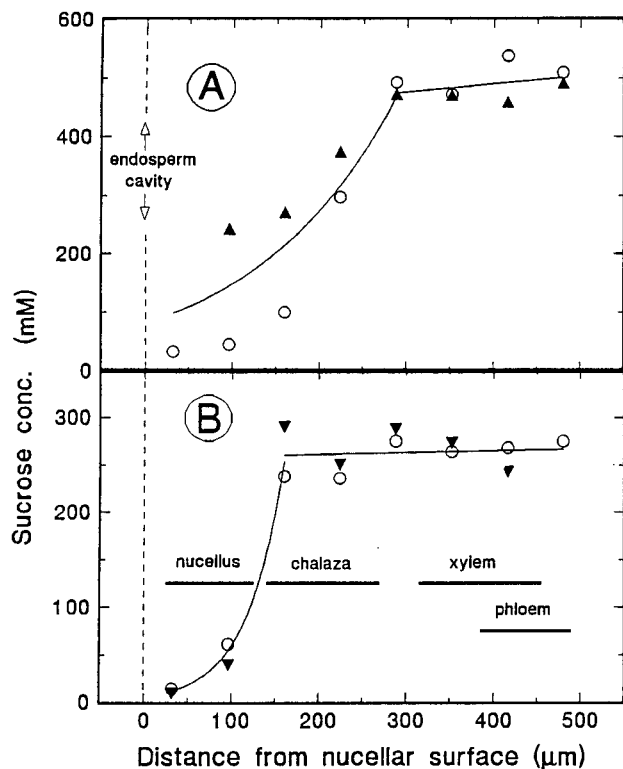
**Figure 3.** Suc distribution in the crease tissues of attached grains perfused with solutions known to strongly inhibit assimilate import into the grain. The Suc distributions for two nonperfused grains from the same ear are given by the filled symbols in Figure 2A. A, Grains perfused with 1660 mOsm sorbitol for 5 h. B, Grains preperfused for 20 min with 10 mM PCMBS, followed by standard perfusion medium for 5 h.

at a slight angle to the crease axis. Also, the extent of the tissues, especially of the nucellus, varied somewhat from to grain to grain. "Xylem" or "phloem" simply indicates the samples in which those tissues were present; most of their volume consisted of vascular parenchyma. As will be noted, however, the major features of Suc distribution did not appear to be significantly distorted by these deviations from tissue homogeneity.

The accuracy of the sample volume calculation appeared to be quite good. Seven comparisons were made of endosperm cavity sap volumes calculated from dimensions of the frozen samples and from subsequent droplet diameters. The former values were $102 \pm 5\%$ of the latter.

The distribution of crease Suc in attached grains (i.e. grains frozen about 1 min after excision) is shown in Figure 2A. In all cases, there was a regular, approximately linear, decrease in Suc concentration from the phloem region to the endosperm cavity. On average, the concentration difference along this pathway was about 150 to 200 mM.

Four hours after grains were excised from the ear, the Suc content of their crease tissues had declined markedly, and the concentration gradient had almost disappeared (Fig. 2B). At this point, the Suc concentration throughout

the profile was close to that in the endosperm cavity. When similarly starved grains were sliced through the endosperm cavity to expose the nucellus and then incubated in 400 mM Suc for 4 h, the crease Suc concentration increased fairly uniformly to somewhat more than 400 mM (Fig. 2C).

The effects on crease Suc distribution of perfusing the endosperm cavity with 1660 mOsm sorbitol or with PC-MBS, both of which have been shown to block post-phloem transport (Wang and Fisher, 1994a, 1995), are shown in Figure 3. In sorbitol-perfused grains (Fig. 3A), the Suc concentration throughout the vascular parenchyma was unusually high, at about 500 mM, with no evidence of a gradient. A steep concentration gradient occurred in the chalaza, presumably reflecting blockage of symplastic transport there. Although the Suc distribution was basically similar in both cases, the Suc level in the nucellus of one grain was appreciably higher than the other.

In grains that were preperfused for 20 min with PCMBS, the crease Suc concentration was a uniform 260 mM throughout the chalaza and vascular parenchyma, with a sharp decrease at the chalaza/nucellus boundary (Fig. 3B).

The crease Suc distributions in nonperfused grains on the same ear as the sorbitol- and PCMBS-perfused grains are shown by the filled symbols in Figure 2A. There, the high end of the Suc gradient around the phloem was about 300 mM. Thus, it appears that the effect of PCMBS was to cause Suc accumulation in the chalaza and adjacent vascular parenchyma but not in the vicinity of the phloem itself. In sorbitol-perfused grains, the Suc pool increased throughout the vascular parenchyma.

The ^{14}C-counting data for the PCMBS- and sorbitol-perfused grains and for the two control grains, all from the same ear, are not presented. However, the relative distribution of ^{14}C within the crease tissues of each grain followed almost precisely the relative distribution of Suc. Except where Suc was particularly low, count rates ranged from about 200 to 2000 cpm, providing good counting accuracy. Since about 90% of the ^{14}C is in the form of Suc, which is at uniform specific activity throughout the crease and endosperm cavity this long after pulse labeling (Fisher and Wang, 1993), the counting data fully confirmed the Suc assay data.

## DISCUSSION

The gradient in the volume-averaged Suc concentration along the post-phloem transport pathway was clearly associated with ongoing Suc transport. It was present only during transport and disappeared in all cases in which transport presumably ceased. The flatness of the distribution in the absence of transport agrees with autoradiographic and kinetics evidence against the occurrence of significant Suc compartmentation in these cells (Fisher and Wang, 1993). Thus, the gradient in the volume-averaged concentration appears to be an acceptable indicator of the gradient available for diffusive transport.

The uniformity of the Suc gradient found in these experiments differs in some features from previous inferences

based on the distribution of $^{14}C$ in plastic sections of freeze-substituted crease tissues after pulse labeling with $^{14}CO_2$ (Fisher and Wang, 1993). There we found a sharp $^{14}C$ gradient at the boundary of the chalaza and vascular parenchyma. At that time, however, we had regarded Suc release into the endosperm cavity as a unidirectional transport. Given subsequent evidence that Suc transport across the nucellar cell membrane is bidirectional (Wang and Fisher, 1994a, 1995; Fig. 2C), much of the inferred steepness probably resulted from a largely localized exchange of endosperm cavity Suc with Suc in the nucellus and chalaza. Our conclusion that the Suc gradient in the nucellus and chalaza is relatively uniform and shallow appears valid, however, with important implications for Suc movement within those tissues and across the nucellar cell membrane (see below).

Based on the gradients found in these experiments and on the Suc flux during grain filling (Fisher and Wang, 1993), a cell-to-cell diffusive permeability coefficient may be estimated for the post-phloem pathway. If we assume equal concentration steps along a 15-cell pathway between the phloem and nucellus, the gradient at each step would be about 10 to 13 mM. With a flux density of $6.5 \times 10^{-5}$ mol $m^{-2}$ $s^{-1}$ (Wang and Fisher, 1994b), the permeability coefficient would be about $6 \times 10^{-6}$ m $s^{-1}$. This is substantially greater than values measured for fluorescent dye movement in other plant tissues (about $1 \times 10^{-6}$ m $s^{-1}$; Tucker et al., 1989; Goodwin et al., 1990). The much larger size-exclusion limit for intercellular movement in the crease (Wang and Fisher, 1994b) quite likely accounts for the higher cell-to-cell conductance of this pathway.

These considerations suggest that diffusion alone may provide an adequate basis for post-phloem transport through the crease tissues. However, the tendency of concentration gradients to cause bulk flow must also be considered, especially because the hydraulic conductivity of a channel can be expected to be even more sensitive to changes in dimensions than its diffusive conductivity. Aside from Suc, concentration gradients must be expected for other mobile solutes, with no evident reason for solute gradients in the opposite direction. Thus, it seems reasonable to expect a total osmotic difference along the pathway of 200 to 300 mOsm (i.e. the Suc gradient plus 50–100 mOsm from other solutes), equivalent to an osmotic pressure difference of about 0.5 to 0.7 MPa. The turgor difference would evidently not be so large, however, since endosperm cavity water potential is higher by about 0.3 to 0.5 MPa than that of the outer pericarp (Fisher, 1985; and subsequent measurements). Thus, a turgor difference of about 0.2 MPa may be present between the nucellus and vascular parenchyma.

Admitting the possibility of bulk flow, however, poses difficult questions concerning the circulation of water in the grain. If we assume that bulk flow accounts for even half of the Suc movement through the chalaza, the volume of water moving into the endosperm cavity in a day's time would be approximately equivalent to the grain's entire water content. There is no evident basis for a comparable return flux to the outer pericarp, although a circulation of

water within the grain has been suggested (Jenner, 1985). These observations on solute gradients present basic questions about diffusive versus convective transport in the symplast that cannot yet be answered satisfactorily.

The transcrease Suc distributions resulting from sorbitol and PCMBS perfusion and from Suc uptake via the nucellus support our earlier interpretations of these treatment effects. The Suc gradient in sorbitol-perfused grains was sharpest within the chalaza, in agreement with our observation of localized plasmolysis there (Wang and Fisher, 1994a). Movement in PCMBS-perfused grains was blocked in the nucellus, consistent with the view that nucellar cell membranes are the site of PCMBS sensitivity (Wang and Fisher, 1995). However, the failure of the crease Suc concentration to increase above 260 mM in PCMBS-perfused grains is curious, since the Suc concentration in the crease sieve tubes is substantially higher (about 450–600 mM; Fisher and Gifford, 1986; Fisher, 1990). This suggests that movement out of the SE/CC complex was blocked by some other effect than a simple "backing up" of solutes in the surrounding parenchyma cells.

Four hours of Suc uptake via the nucellus resulted in a crease Suc concentration similar to that in the incubation medium. Because free Glc also increases somewhat during Suc uptake, especially when high Suc concentrations are used (data not shown), the apparent elevation of the Suc concentration above that in the medium may be due to the inclusion of Glc in our calculation of the Suc content.

Our observations point to movement out of the SE/CC complex as a likely control point for assimilate import. The largest Suc concentration difference along the entire source to sink pathway occurs here, over a distance of only 1 $\mu$m. Quite probably, the same is true of turgor and osmotic pressure gradients. The implications of the response to PCMBS have already been noted. Finally, our earlier observation that Suc efflux into the endosperm cavity declines immediately on detaching grains from the ear (Wang and Fisher, 1993) suggests that movement out of the SE/CC complex limits movement through the entire maternal pathway.

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