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Accumulation and Conversion of Sugars by Developing Wheat Grains[1]

VI. GRADIENTS ALONG THE TRANSPORT PATHWAY FROM THE PEDUNCLE TO THE ENDOSPERM CAVITY DURING GRAIN FILLING

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ABSTRACT

Gradients along the transport pathway from the peduncle to the endosperm cavity were examined during grain filling in wheat. Sieve tube exudate was collected from severed aphid stylets established on the peduncle and rachis and on the vascular bundles in the creases of grains. Phloem exudate could also be collected from broken grain pedicels, and by puncturing the vascular bundle in the grain crease with a needle. Stylets on excised grains persisted exuding, indicating that grain sieve tubes are capable of loading solutes. There was little, if any, discernible gradient in osmolality or solute composition (sucrose, total amino acids) of sieve tube contents along the phloem pathway from the peduncle to the rachis or along the rachis itself. Neither was a gradient detected in osmolality along the sieve tube pathway from the rachis through the rachilla and grain stalk to the crease. Demonstrable solute gradients occurred only across those tissues of the grain crease between the crease sieve tubes and the endosperm cavity, a distance of just 1 millimeter. However, while the sucrose concentration in the sieve tubes was almost tenfold that in the endosperm cavity sap, total amino acids were only threefold higher, and the potassium concentrations of the two were equal. Our observations strongly implicate the movement of assimilates from the sieve tubes and across the crease tissues as important control points in grain filling.

In analyzing the transport of nutrients from source to sink tissues, particular importance must be assigned to those regions of the pathway where major gradients (concentration, composition, pressure, etc.) occur, as those sites must be regarded as major resistances and/or possible control points for transport into the sink. Almost all information on such gradients is indirect because of difficulties in sampling the transport pathway. For grain filling in wheat, restriction to assimilate movement to the grain in the sieve tubes is thought to be unimportant under most conditions (4, 18, 23). While a mild caveat to this view may apply to distal grains within a spikelet when assimilate availability is low (2), movement across the grain tissues between the grain sieve tubes and the endosperm cavity (the "crease" tissues) appears to offer a principal resistance in the pathway from the peduncle to the endosperm (11). Jenner (12, 13), however, while noting the resistance to movement across crease tissues, presents evidence from ear culture experiments that the limiting step(s) for entry into the grain must operate before the grain itself, and may be due to saturation of phloem loading or to restricted movement within the sieve tubes, or both. Termination of grain filling at maturity may possibly be due to increased resistance to transport across the crease tissues (25). That an appreciable gradient occurs there may be inferred from the low sucrose concentrations in endosperm cavity sap (about 10–100 mM) (10, 20, 21) in comparison to that typical of sieve tube sap (greater than 200 mM) (3). Jenner (11) has shown the average sucrose concentration within crease tissues to be higher than in the cavity, but the phloem constitutes only a minute proportion of crease tissue volume.

From several observations, it appears that the synthetic capacity of wheat grains may exceed their ability to import substrates for growth. In comparing the sucrose concentration in the endosperm cavity of wheat grains during linear grain filling with the sucrose response curves of starch synthesis in grains cultured *in vitro*, Ho and Gifford (10) concluded that *in vivo* apoplastic sucrose concentration is suboptimal for grain growth. Similarly, Jenner and Rathjen (14) found that, within a given wheat variety, the grain filling rate correlated with endosperm sucrose concentration.

The objectives of the present experiments were to identify as closely as possible the region(s) of the transport pathway where major gradients occur during the linear phase of grain filling in wheat and to quantify the gradients more precisely. Systematic sampling of the sieve tube contents from the peduncle to the grain, and of the contents of the endosperm cavity, confirmed that most, if not virtually all, of the concentration changes take place as solutes pass from the crease sieve tubes into the endosperm cavity. However, the gradients are substantially different for the major compounds translocated (sucrose, amino acids, K^+).

MATERIALS AND METHODS

Plant Material. Plants of *Triticum aestivum* L. (cv SUN 9E) were grown singly in pots of perlite-vermiculite in a glasshouse controlled at 21°C for 16 h of the day and 16°C at night. Tillers were removed from the plants as they appeared. The pots were supplied with modified Hoagland solution and water twice a day. Ears were tagged at anthesis and, unless indicated otherwise, were used for experiments when they were 20 to 22 d post-anthesis

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(during the linear phase of grain filling).

Some experiments were run with two other wheat cultivars (Cleveland and Gigas) grown under the same conditions as above.

Aphids. A virus-free colony of the oat-bird cherry aphid (*Rhopalosiphum padi* [L.]) was maintained on barley plants (*Hordeum vulgare* L.) grown in a growth cabinet. On the evening before an experiment, a plant was moved from the glasshouse to the laboratory and about 30 to 50 aphids were caged on the appropriate plant part. Aphids on the peduncle were located 10 to 12 cm below the ear. Except for placing aphids on the ear, no preliminary preparation of the plant was necessary, and a large proportion of the aphids would be feeding by the next morning. To encourage aphids to select grains as feeding sites, it was necessary to expose the crease regions of basal grains by breaking off the rachilla just above the lowermost grain and removing its palea. After thus preparing six to seven spikelets in the upper half of the ear, a few aphids caged there would usually feed on the crease areas, with several others feeding on the rachis. Because of the small crease area available to aphids feeding on the grain, all of these experiments employed the large-grained wheat cultivar, cv Gigas.

Aphids would also feed on grains that had been excised and placed overnight on moist filter paper in a Petri dish containing aphids.

The locations of several stylet tips of exuding stylets on grains were determined by quick-freezing the tissue piece containing the stylet, followed by freeze-substitution in methanol, embedding in L. R. White resin and serial sectioning at 3 μm. Sections were stained by the periodic acid-Schiff reaction for insoluble carbohydrates.

Sample Collection. *Stylet Exudate.* Aphid stylets were severed by radio-frequency microcautery (7). This operation and all others involved in exudate collection were carried out under a dissecting microscope. To allow easier handling of the plant and micromanipulator, ears were de-awned with scissors, removing about 80% of their length. For measurements of concentration and/or osmolality, exudate was collected under mineral oil by first constructing a small well(s) (usually segments of small tubing) sealed to the plant surface around one to four exuding stylets with Dow-Corning RTV silicone rubber. Before collecting a sample, exudate touching the plant surface was first sucked away with a fine-tipped pipet. Small amounts of exudate remaining on the plant surface were brushed away with a hair tip. The exudate droplet which followed was supported for some time by the stylet in the mineral oil without touching the plant surface, and was collected in a fine-tipped pipet silanized on its outer surface.

Grain Pedicel. Phloem exudate was collected from the broken stump of the grain pedicel. Before breaking off the grain, the lemma was removed and mineral oil was introduced between the grain and palea and placed on the base of the grain so that the broken surface of the pedicel was immediately covered with mineral oil upon breaking off the grain. After 20 to 30 s, accumulated exudate was collected with a fine-tipped pipet.

Crease. In addition to stylet exudate from the crease, phloem exudate could also be obtained from this site by making a shallow jab with a fine needle into the vascular tissue. The upper grains of a spikelet and the palea of the lower grain were first removed and the crease was flooded with mineral oil before making the needle puncture. For sequential exudate collections, an oil well was used. Only brief exudation occurred in the case of cv SUN 9E (and cv Gigas), but Cleveland grains continued exuding for up to 1 h. For this reason, quantitative data were obtained only for cv Cleveland.

Cavity Sap. The liquid contents of the endosperm cavity were sampled by slicing off the upper fourth or less of the grain and gently squeezing the basal portion to force out the cavity sap while collecting the sap by suction with a fine-tipped pipet. A small volume of mineral oil was sucked up before and after sap collection to prevent evaporation from the tip. In comparing cavity sap concentrations and compositions along the ear of the same plant, all of the basal grains in each spikelet ("a" position) were sampled first, then the "b" grains, etc.

Assays. Sample volumes were determined in one of two ways, the choice of which depended mostly on the volume range being dealt with in a particular experiment. For smaller volumes (mostly less than 0.2 μl, but ranging up to about 0.5 μl), the sample was ejected under mineral oil and its diameter measured at ×50 magnification. The droplet was then taken up in a fresh pipet for dilution to a standard volume. Larger sample volumes (mostly greater than 0.5 μl, but ranging down to about 0.2 μl) were determined by collecting the sample (usually, cavity sap) in 5 μl disposable pipets of uniform internal diameter drawn to a fine tip. Enough mineral oil was drawn in to pull the oil/sample interface past the taper, and the length of the sample was measured with a dissecting microscope.

Potassium concentrations in cavity sap samples were measured with a double-barreled ion exchange microelectrode. The measuring side of the double-barreled electrode (AM Systems, Everett, WA) was filled with 0.5 M KCl, with a column of K^+-sensitive liquid ion exchange resin (WP Instruments, New Haven, CT) occupying the electrode tip. On the reference side, a 10% acrylamide solution containing 0.5 M NaCl was polymerized in the tip and the remainder of the barrel was back-filled with 1 M KCl. (The use of polyacrylamide prevented flow from the relatively large [20–40 μm] electrode tips.) After inserting Ag/AgCl electrodes, their leads were connected to a Keithley model 610C electrometer and the electrode was calibrated with standard KCl solutions. After some initial drift, the slope of the calibration curve was quite constant, although some minor drift in the intercept sometimes occurred. The latter was easily accounted for by periodic checks with a standard solution. Samples were measured by expelling the sample onto Parafilm in a small moist chamber and inserting the combination electrode into the sample until a stable reading was obtained (usually 5–20 s).

Osmolality measurements were determined by freezing point depression using a nanoliter osmometer (Clifton Technical Physics).

Sucrose and total amino acids were determined from aliquots taken from diluted samples (usually the dilution was to 200 μl). Total amino acids were measured fluorometrically with fluorescamine (16), using glutamine as the standard. Sucrose was assayed by the methods of Jones *et al.* (15), with the exception that hexose isomerase was usually omitted. In assaying cavity sap samples, the value for sucrose was taken as the difference between replicates assayed with or without invertase. Assays with and without added hexose isomerase showed that equal amounts of glucose and fructose were released during the 10-min invertase hydrolysis period, showing that only sucrose was hydrolyzed during that interval. Longer times, however, resulted in a slow increase in the amount of glucose released, presumably from fructans (10).

Sample Stability. In several experiments, some of the samples remained at room temperature for various periods ranging up to almost 1 h for a few of the samples. To determine whether this might have an effect on the values obtained for their sugar and amino acid content, samples of sieve tube sap and cavity sap were divided into three roughly equal measured volumes, each of which received different pretreatments before analysis. One aliquot was frozen within 10 min of collection. A second was maintained at 23 to 25°C under mineral oil for several hours before freezing and a third was dried on a silanized microscope slide and stored for several days. All treatments of a given sample

were then assayed. Neither cavity sap nor phloem exudate showed analytically detectable differences among the treatments (data not shown).

RESULTS

Characteristics of Exudation from the Grain Pedicel and from Crease Punctures. When a grain was broken from a spikelet distal to the grain's subtending palea, the break usually occurred just above or, sometimes, just below the lodicules, in the region of the partially differentiated tracheary elements of the grain pedicel (24). The rate of exudation was quite variable, and its duration was usually brief (½–2 min), typically yielding 20 to 100 nl of exudate per stump. All samples from a given ear showed similar osmolalities (see below) in spite of wide variations in exudation rates. Both the reliability and volume of exudation were greater in plants exposed to strong illumination (*e.g.* full sun or continuous illumination in a growth chamber) during the immediately preceding two to three photoperiods. Consistent failure to obtain exudate was closely associated with the termination of grain filling. In one ear where upper grains had begun to yellow, exudate was obtained only from the lower grains. The composition and concentration of the exudate were similar to that collected from severed stylets on the same plant (see below).

Feeding Positions and Stylet Tip Location in Grains. While aphids would probe any exposed area of an attached grain, often inserting their stylets quite deeply, they produced honeydew only when positioned on the crease itself. Only severed stylets of the latter insects exuded when cut. Tips of three exuding crease stylets were located by serial sectioning; all were in sieve tubes.

Excised grains, placed dorsal surface down on moist filter paper, were likewise probed over their entire surface by exploring aphids. To our surprise, some were found feeding on the lateral surface of the grains (90° from the crease) about 3 to 4 mm from the base. (The lateral surfaces of attached grains remained covered by the lemma, so could not be probed by aphids.) Severed stylets of these aphids exuded for several hours. Tips of two exuding stylets were located in what appeared to be sieve tubes, most probably of the protophloem strands present in the pericarp of the ovary and young grains but previously thought to become nonfunctional soon after the start of grain growth (9, 17). While sieve plates were not identified, the small diameter of these cells (about 6 μm), their empty appearance, considerable length, and location in the grain indicated their probable identity as sieve tubes. In cross-section, two of these cells were surrounded by a rosette-like sheath of 8 to 10 elliptical parenchyhma cells, each about 15 × 20 μm. In contrast to the surrounding cellular debris from degenerating outer pericarp cells, the sheathing cells of the strand contained healthy appearing protoplasts.

Sieve Tube Sap Gradients. *Peduncle to Ear, and Along the Ear.* Comparisons of exudate osmolalities in samples taken from the peduncle (stylet exudate) and along the ear (grain pedicel exudate) showed little, if any, gradient in osmolality along this part of the pathway (Table I). In addition, there appeared to be no important gradients in concentrations of sucrose, or of total amino acids (Table II). Differences in light *versus* dark exudate compositions were similar at all points along the pathway.

Rachis to Crease. The final step in phloem transport, from the rachis into the sieve tubes of the grain itself, also occurred without any consistent change in osmolality (Table III). However, differences were more erratic than noted between peduncle and rachis, and were complicated by some peculiarities in exudation from the grain. In four of five instances where the osmolality of crease stylet exudate was followed with time, the osmolality showed a pronounced drop within 3 h, eventually increasing again (*e.g.* Fig. 1). Changes in rachis stylet exudate osmolality, while tending to change similarly, were not as marked.

A second peculiarity was that exudation could continue from grains even after their excision. As noted earlier, aphids were able to feed on excised grains, and their severed stylets continued exuding for many hours. One stylet, cut before the grain was removed from the ear, continued exuding for 7 d when the grain was placed under mineral oil. Exudate osmolality dropped from 800 to 460 mosmolal within 5 h after excision, but then showed a constant increase to its final value of 1185 mosmolal. The rise was interrupted only during a 9-h period (d 5) on an ice bath, during which exudation continued at a reduced rate but at the same osmolality.

Not only did stylets on excised grains exude, but exudate could also be collected from the grain base when an excised grain was placed under mineral oil or in a moist chamber. Several hundred nl would usually accumulate during 1 to 2 h. However, it was more dilute than that which exuded from the complementary broken surface of the grain pedicel on the intact plant. In one experiment where exudate osmolality was followed for three grains under mineral oil, the first samples collected (1 min after breaking) were almost 300 mosmolal less than the complementary grain pedicel exudates (about 860 mosmolal). While some dilution occurred during the 1 h sampling period, the osmolality dropped only by about 150 mosmolal during that time, and clearly did not extrapolate back to a zero-time value much higher than the original measurement.

Finally, in comparison to most stylets on the peduncle or rachis, exudation rates from crease stylets were consistently low (about 10 nl/h, *cf.* 50–100 nl/h for most peduncle and rachis stylets).

Gradients in Endosperm Cavity Sap Contents Along the Ear and Along Spikelets. Samples of endosperm cavity sap showed no evidence of systematic variation in potassium, total amino acids or osmolality, either along the length of the ear or along a spikelet (Fig. 2, A and B). Sucrose and glucose concentrations were more variable (Fig. 2B), possibly indicating, at least for sucrose in this experiment, an increase in concentration from basal to distal grains of an individual spikelet. However, in another experiment comparing only "a" and "b" grains, sucrose was generally somewhat lower in the "b" grains.

Table I. *Sieve Tube Sap Osmolalities Along the Pathway: Peduncle—Lower Ear—Upper Ear*

Peduncle samples were taken from exuding stylets. Samples along the ear came from exuding grain pedicels. The mean number of measurements per sample was 3.1. Each sequence of measurements is for a separate plant.

Peduncle	Ear	
	Lower fourth	Upper fourth
	mOsm ± SD	
ND[a]	655 ± 43	695 ± 35
680 ± 28	635 ± 26	690 ± 20
775 ± 7	723 ± 4	807 ± 46
850 ± 26	820 ± 3	845 ± 96
568 ± 39	539 ± 19	565 ± 23
846 ± 11	879 ± 17	838 ± 38
ND	808 ± 68	868 ± 48
Mean: 744	723	758

[a] Not determined.

DISCUSSION

The collection of phloem exudate by various means from a variety of positions on the peduncle and ear, in addition to endosperm cavity sap collection from individual grains, allows a detailed examination of transport gradients associated with grain filling. The discovery that phloem exudate could be collected

Table II. *Sieve Tube Sap Compositions Along the Pathway: Peduncle—Lower Ear—Upper Ear*

Peduncle samples were taken from exuding stylets. Samples along the ear came from exuding grain pedicels. Samples were obtained from one plant just after a normal night period ('Dark'), and from another in the afternoon ('Light') on the same day.

Measurement	Peduncle	Ear	
		Lower third	Upper third
Dark			
mOsm ± SD	556 ± 41 (n = 6)	539 ± 19 (n = 4)	565 ± 23 (n = 4)
Sucrose (mM)	287 (57)[a]	270 (55)	274 (53)
Amino acids (mM)	140 (25)	156 (29)	141 (25)
Light			
mOsm ± SD	836 ± 19 (n = 6)	879 ± 17 (n = 4)	838 ± 38 (n = 4)
Sucrose (mM)	512 (73)	552 (77)	564 (82)
Amino acids (mM)	142 (17)	133 (15)	143 (17)

[a] Numbers in parentheses indicate the percent contribution of sucrose or amino acids to total exudate osmolality.

Table III. *Sieve Tube Sap Osmolalities Along the Pathway: Rachis—Ovule—Crease*

Rachis samples were taken from exuding stylets. Crease samples were also taken from exuding stylets (cv Gigas) or from a shallow puncture (p) into the crease (cv Cleveland). Pedicel samples were taken from the bleeding sap obtained by breaking off a grain.

Rachis	Pedicel	Crease
	mOsm	
505		690
430		685
1060		1055
	620	620
	1010	1025
1070	1135	1125
1080	1000	875
	835	820 p
	875	630 p
	835	780 p
660		580 p

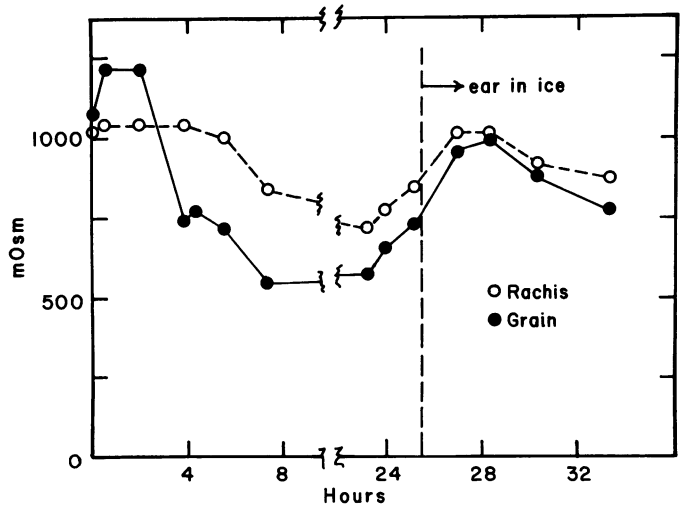

FIG. 1. Time course of stylet exudate osmolality for samples collected concurrently from stylets in the grain and rachis of a Gigas wheat ear. At about 25 h, the ear was slipped into a test tube which projected above the surface of an ice bath. The ear was removed for about 3 min at each sampling time.

from broken grain pedicels provided an important supplement to data obtained from exuding stylets. The phloem origin of grain pedicel exudate is indicated by its high osmolality (>600 mOsm), its similarity in composition and concentration to stylet exudate, and by the duration of exudation. While usually exuding for less than 3 min, grain pedicels continued to exude for up to 15 min on plants previously exposed to long periods of high light intensity. Although less well characterized with regard to composition, similar observations on composition and concentration apply to exudate collected from the grain crease by making shallow jabs with a sharp needle. Inability to collect grain pedicel exudate at the end of the grain filling period indicates that structural changes in the pedicel, as well as in the crease, need to be examined in relation to cessation of transport into the grain towards maturity.

The ability of aphids to feed on grain tissues other than the crease vascular bundle raises additional questions regarding grain vasculature. Structural investigations of the wheat grain (9, 17) have established the presence of one dorsal and two lateral protophloem strands in the ovary at anthesis, but they appeared to be obliterated during subsequent grain elongation. Our results almost certainly demonstrate that the lateral phloem strands remain functional at least past the midway point of grain filling. (The same may be true of the dorsal vein, but it was not accessible to probing by the aphids in our experiments.) Since all aphids feeding on noncrease tissues of the grain were within a few mm of the base, it is possible that the veins are functional for only a fraction of the grain's length. This question needs to be resolved, since continued functioning of these noncrease veins would allow more ready transport of solutes from pericarp degeneration and photosynthesis to the endosperm.

Measured exudate concentrations appeared to reflect accurately the concentration of sieve tube contents. This is suggested by the similarity of exudate concentrations and osmolality at various positions in the same ear, but may also be inferred from the general lack of dependence of osmolality on exudation rate. Grain pedicel exudation occurred at substantially different rates in the same ear but showed similar osmolalities.

The absence of any demonstrable concentration gradient along the phloem transport pathway indicates that movement through sieve tubes does not, at any part of the pathway we examined, limit or control the rate of grain filling. (Note that the variability in measurements from any particular site would likely make undetectable any gradients associated with osmotically generated pressure flow over the short distances of phloem transport examined.) Bremner and Rawson's (2) caveat regarding a possible significant resistance in phloem transport to the distal grains of

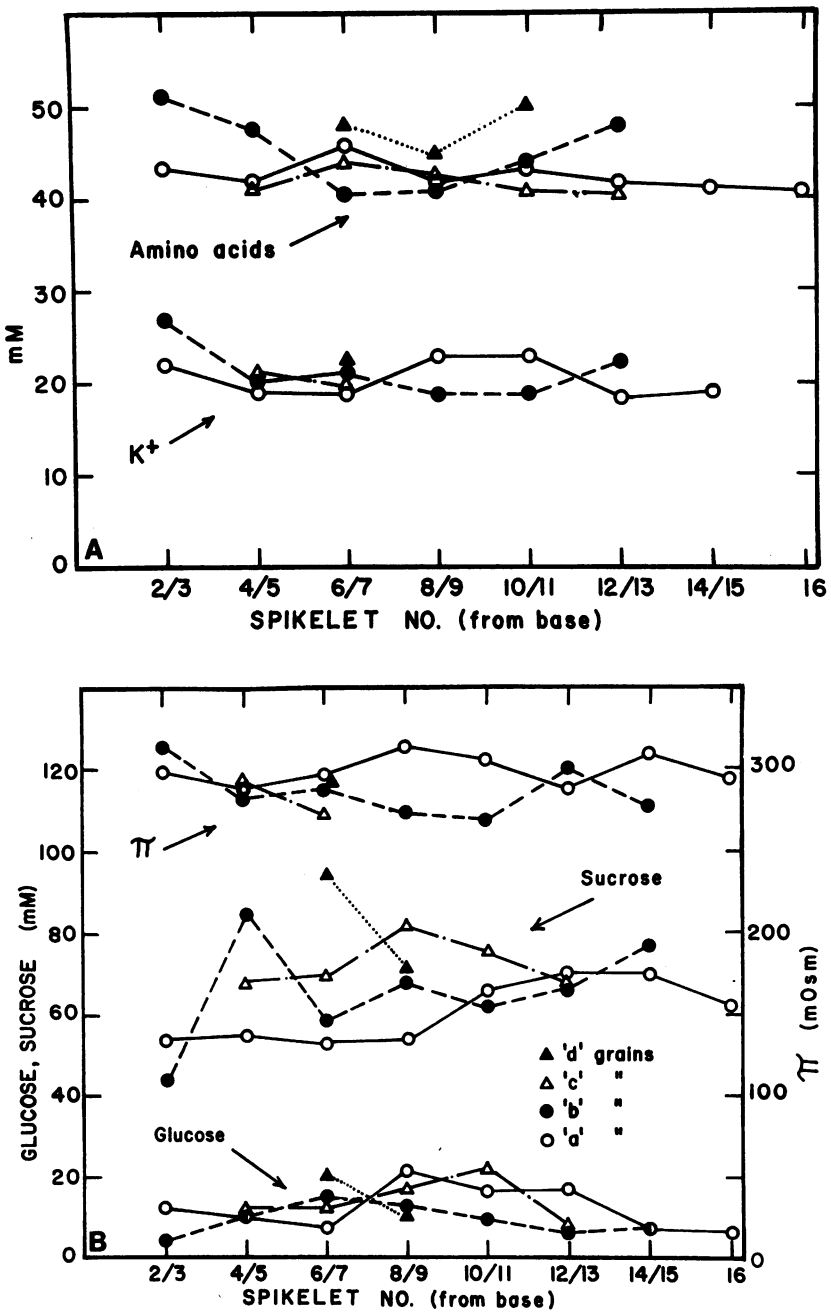

FIG. 2. Cavity sap osmolality and composition as a function of grain position in a wheat ear. A, Potassium and total amino acids; B, sucrose, glucose and osmolality. Grain positions within a spikelet are indicated as "a" (basal) to "d" (terminal).

spikelets under conditions of low assimilate availability may nevertheless be applicable, as our experiments did not adequately address that question. If the factors limiting grain filling at high levels of assimilate supply operate before the grain itself, as proposed by Jenner (12, 13), this would leave phloem loading as the deciding factor for the control of grain filling. However, in view of the variability in wheat sieve tube sap concentrations during grain filling (6) or under different conditions (8), including particularly high concentrations in water- or salt-stressed plants (5; DB Fisher, unpublished data), we feel that movement of assimilates from the sieve tubes and across the crease tissues in the grain must be considered as the most likely point(s) of control for grain filling.

Since no gradient occurred along the ear sieve tubes and the endosperm cavity contents were similar for all grains, the gradients across the crease tissues must likewise have been very similar in each of the grains. In terms of osmolality and concentrations of sucrose and total amino acids, the gradient was substantial, and occurred over a distance of only about 1 mm. While our values for sucrose concentration in the endosperm cavity sap are somewhat higher than the 10 to 50 mM reported by Ho and Gifford (10) for this cultivar, the potassium concentrations are quite similar. They, too, found no detectable gradients in cavity sap concentrations in comparing pooled samples of the a, b, and c grains of the ear as a whole. The ear appears to function, then, as a manifold distribution system having a central low resistance pathway (the sieve tubes) whose contents are "bled off" across lateral pathways of high resistance (unloading in the crease tissues), thus allowing similar rates of unloading and hence grain growth along the entire length of the ear.

While the major transport gradient was associated with movement across the crease tissues, a number of ambiguities arise concerning phloem transport within the sieve tubes of the grain itself, especially along its pedicel. First, owing to the xylem

Table IV. *Comparison of Representative Concentrations in the Sieve Tube Sap and Endosperm Cavity Sap in the Light*

	Sieve Tube Sap	Cavity Sap	Ratio (Sieve Tube/Cavity)
Constituents (mM)			
Sucrose	540	65	8.3
Glucose	0	15	0
Amino acids	145	45	3.2
Potassium	21[a]	22	1.0
Total solutes (mOsm)	860	290	3.0
"Missing" π[b]	40 (5%)	140 (48%)	

[a] Ref. 6.

[b] Total osmolality minus the osmotic contributions of sugars, amino acids, and potassium.

discontinuity in the grain pedicel (24), it is likely that the water potential of the grains was several bars lower than that of the remainder of the plant (1). In this context, it should be noted that, since the water potential of developing wheat grains tends to remain fairly constant (1), the opposite condition of grain *versus* plant water potential may occur in plants with low water potential (1). Second, the unloading of solutes and water from sieve tubes in the grain may be expected to affect substantially their water relations. Possibly the presence of an exuding stylet disturbed normal patterns of water and solute movement, but exudation accounted for only 2 to 3% of the average grain growth rate, suggesting only a minor effect from the stylet's presence. Both the lower grain water potential and, presumably, competition with unloading into the grain may have accounted for the generally lower exudation rates from crease stylets, but would imply that a sharp pressure drop accompanied movement through sieve tubes of the grain pedicel. Since the method for obtaining crease exudate necessitated removal of the palea, it should be noted that Radley (20) found that assimilate movement into grains was reduced within hours of removing their surrounding glumes and paleas. If this effect were variable, it might account for the somewhat more erratic comparisons, both instantaneous (Table III) and with time (Fig. 1), between crease samples and grain pedicel or rachis samples than were noted for other parts of the pathway. Finally, grain sieve tubes had the capacity to actively load solutes, or at least could develop that capacity. This is demonstrated by the prolonged exudation from stylets on excised grains and may possibly account, in part, for exudation from the broken grain pedicel on the grain itself. The lower osmolality of the latter exudate may reflect a limited capacity for sieve tube loading in the grain and/or competition with unloading. Since even stylets on excised grains could exude, exudate properties from stylets on attached grains cannot necessarily be regarded as derived exclusively from the incoming assimilate stream. However, the increase in crease sieve tube exudate osmolality in a chilled ear (Fig. 1; also observed in another experiment) and the parallel behavior of rachis and crease exudate osmolality in the chilled ear (Fig. 1) indicate that the movement of solutes into crease sieve tubes was not dependent on metabolism in the ear. Since low temperature does not affect sieve tube transport in wheat (19), these observations indicate continuity in the sieve tube connections along the grain pedicel. This contrasts with the discontinuity of the xylem vessels (24), which may result in grain water potentials lower (in well-watered plants, as in our case) or higher (in water-stressed plants) than in the remainder of the plant (1). This, in conjunction with solute and water movement from the sieve tubes immediately distal to the pedicel, substantially complicates interpretations of the possible behavior of water potential and its components (osmolality and pressure) in the pedicel and crease sieve tubes.

Gradients across the crease were not similar for all transported compounds. Table IV provides a summary of representative values found in the present experiments for solute levels in the sieve tube and endosperm cavity. The case of potassium is particularly interesting, as there was no demonstrable difference in its concentration in sieve tube *versus* endosperm cavity saps. In contrast to their relative proportion in the sieve tube, sucrose and amino acid concentrations in the cavity sap were more closely comparable, and are similar to values reported by Radley and Thorne (22) for wheat endosperm cavity sap. However, their relative proportion is not constant, as similar analyses performed for experiments described in a subsequent paper (8) gave cavity sap amino acid concentrations that were consistently higher than those of sucrose. In contrast, Radley (20) found a higher proportion of sucrose to amino acids than those reported here.

Metabolism by surrounding tissues apparently makes an important contribution to the endosperm cavity contents. The absence of analytically detectable changes when cavity sap was incubated for long periods at room temperature indicates that it serves as an essentially nonmetabolic compartment interposed between maternal tissues and the endosperm. However, a substantial proportion of cavity sap constituents are not present in the sieve tube; glucose is one, but others are not yet clearly accounted for. Fructans (10) probably make up much of the "missing" osmoticum (Table IV). In addition, high mol wt constituents caused the viscosity of the cavity sap to range from watery to jelly-like even in grains from the same ear, although they appeared to make essentially no contribution to total osmolality.

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