

VASCULAR TRANSFER CELLS IN THE WHEAT SPIKELET

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Summary

Xylem and phloem transfer cells are present at the nodal regions where the sterile glumes, lemma, palea, and caryopsis are attached to the rachis and rachilla. The course and distribution of the vascular tissues and the structure of the transfer cells are described. Experiments with colloidal lanthanum tracer fed via the transpiration stream have indicated that the walls of the transfer cells are relatively porous. The possibility that the transfer cells play an important role in the regulation of solute transfer to the developing grain is discussed.

I. INTRODUCTION

The transport of photosynthate from leaves and the movement of nutrients from the roots to the developing grain of wheat have been studied extensively (see e.g. Williams 1955; Carr and Wardlaw 1965; Thorne 1965; Kriedeman 1966; Lupton 1968; Evans and Rawson 1970), but the histology and cytology of the transporting tissues in the spike and spikelets have not been studied by modern methods. Further, although numerous studies have indicated that there is an interchange of solutes between the transpiration and translocation pathways at various levels within the plant, the site(s) of this exchange and the cells responsible for it have never been identified.

Recently, Gunning and Pate (1969) and Pate and Gunning (1969) have described the occurrence of "transfer cells" in a variety of situations in plants. These cells are characterized by the presence of wall ingrowths which increase the surface area of the cell membrane and the volume of free space associated with it. Gunning and Pate (1969) point out that these cells occur at many sites known to be involved in solute transfer and they predict that transfer cells should occur in any anatomical situation where "adverse surface area-volume relationships exist between donor and receptor compartments of the transport pathway and/or where the transported solutes are accompanied by a minimal flow of solvent". Pate and Gunning (1969) reported that vascular transfer cells were found only in one family (Zosteraceae) of the 42 families of monocotyledons they examined (only leaves were examined in their survey).

Since that time we have reported the occurrence of transfer cells at the coleoptilar node of wheat (O'Brien, Zee, and Swift 1970). In this paper, we describe the

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distribution of transfer cells at the regions where the sterile glumes, lemma, and palea are attached to the rachilla. The relationship between these zones of transfer cells and the modified tracheary elements that "block" the xylem at the base of the pericarp (Zee and O'Brien 1970) is also illustrated.

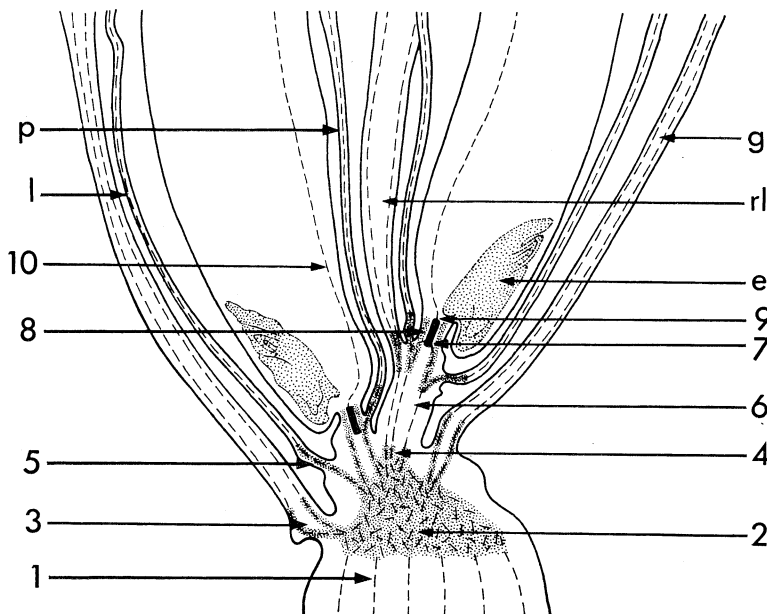


Fig. 1.—A sagittal section of a wheat spikelet showing the relation between the caryopses and the rachilla (*rl*) and rachis. The positions of the sterile glumes (*g*), lemmas (*l*), paleas (*p*), embryo (*e*), and the distribution of the vascular tissues (broken lines) and their associated transfer cells (dense fine stippling) are marked. [Numbers indicate the different levels illustrated in the figures that follow: level 1, Fig. 2; 2, Fig. 3; 3, Figs. 4 and 5; 4, Fig. 6; 5, Fig. 8; 6, not illustrated—identical to Fig. 2; 7, Fig. 7; 8, Fig. 9; 9, Fig. 10; 10, Fig. 11.]

II. MATERIALS AND METHODS

Spikelets of wheat (*Triticum aestivum* L. cv. Heron) at various stages of maturity after anthesis were obtained from plants growing in the glasshouse.

Whole spikelets with a small portion of the rachis attached were removed from the plant and fixed in a 0.025M phosphate-buffered solution of glutaraldehyde and acrolein (4%+3%) overnight at 0°C, dehydrated, and embedded in glycol methacrylate as described by Feder and O'Brien (1968). Serial longitudinal and transverse sections (0.5–2 μ m) were cut using glass knives and examined by optical microscopy after double staining with the periodic acid–Schiff's reaction and toluidine blue O.

For electron microscopy, additional material was fixed in 4% glutaraldehyde for 3 hr at room temperature, post-fixed in 2% osmium tetroxide, dehydrated in ethanol, and then embedded in the low-viscosity epoxy resin introduced by Spurr (1969). For tracing the path of lanthanum, the lanthanum tracer solution (prepared as in Revel and Karnovsky 1967) was administered through the rachis via the transpiration stream for a period of 6 hr. Following the uptake period, the region where the glumes, lemma, and palea are attached to the rachilla was severed from the plant and processed for electron microscopy as above, but omitting post-fixation with osmium tetroxide.

III. RESULTS AND DISCUSSION

(a) Distribution of Transfer Cells

Serial transverse and longitudinal sections through the spikelet of wheat reveal that the vascular tissues in the regions of attachment of the glumes, lemma, palea, and caryopsis are surrounded by numerous transfer cells. Figure 1 is a diagram of a sagittal section of a spikelet and shows part of the two lower florets and the rachilla that leads to the third floret of this cultivar. The courses of some of the main vascular bundles are shown as broken lines, and where transfer cells are present in either the xylem or the phloem, the broken lines are encased in a pattern of dots. In detail, the exact distribution of vascular tissue is more complex than Figure 1 suggests, especially in the plexus of vascular tissue that lies between levels 2 and 4. Nevertheless, Figure 1 provides a reliable guide to the courses of the main bundles supplying these floral organs and to the distribution of transfer cells in the vascular system.

For ease of presentation, the distribution of transfer cells within the parts of the spikelet is considered at the 10 levels shown on Figure 1:

- Level 1—The rachis of wheat is an internode, differing from the internodes of the culm only in its shape, being somewhat more flattened than those of the culm. In transverse view the rachis is shaped like a boat with 10 vascular bundles in the central tissue and two small bundles near the two pointed edges. The vascular bundles are collateral (Fig. 2) and each is enclosed by a layer of mestome sheath cells. There are no transfer cells in these bundles.
- Level 2—The separate collateral bundles of the rachis merge with each other and form a plexus of vascular tissues, from part of which (not shown in the diagram) arises a set of 10 collateral bundles which supply the next segment of the rachis immediately above the spikelet. The plexus of vascular tissue narrows, and from it branches diverge to supply the different organs of the spikelet, as well as the pair of empty glumes. The vascular tissues within the plexus contain numerous xylem (Fig. 3) and phloem (Fig. 4) transfer cells.
- Level 3—Each sterile glume possesses about nine vascular bundles which contain transfer cells only near the region where the glume is attached to the rachis. Figure 4 shows part of a vascular bundle of the lower sterile glume as it nears the vascular plexus in the rachis, and Figure 5 shows the region of fusion between two vascular bundles from the glume and the central plexus.
- Level 4—At this level the central vascular plexus of the rachilla divides into several branches that supply different parts of the florets. Figure 6 shows portion of one such branch in longitudinal view. Numerous transfer cells are present associated with the xylem and phloem, both of the central plexus and the branch trace.
- Level 5—The distribution of transfer cells in the vascular tissues of the lemma and palea is similar to that in the glumes. Transfer cells occur only in the basal portion of their vascular bundles, where these organs are

attached to the rachilla (Fig. 8) and are absent elsewhere along their length.

Level 6—The rachilla to which the second floret is attached varies somewhat in length (1–3 mm) from spikelet to spikelet. It is traversed by about six collateral vascular bundles each of which is identical to that illustrated in Figure 2. These bundles lack transfer cells except near their junctions with the traces of the palea, lemma, and caryopsis.

Levels 7 and 8—The continuity of the tracheary elements between the pericarp and rachilla is interrupted by a core of thick-walled cells (Zee and O'Brien 1970). Thus, the files of tracheary elements and xylem transfer cells in this region near the attachment of the palea come to a halt at the base of the core of thick-walled cells (see Fig. 1, level 7, and Fig. 7). However, the sieve elements and their associated transfer cells bypass the core of thick-walled cells forming a ring of phloem around the periphery of the core (Fig. 1, level 8, and Fig. 9) and finally meet the sieve elements of the pericarp bundle.

Level 9—At this level the core of thick-walled cells stops and the pericarp bundle, consisting of phloem (lying towards the groove) and xylem, begins (Fig. 10).

Level 10—Transfer cells are absent from all vascular tissues of the pericarp bundle, either near the region of the thick-walled cells or away from it (Fig. 1, level 10, and Fig. 11).

(b) *Types of Transfer Cell*

Pate and Gunning (1969) recognized four types of vascular transfer cell, two in the phloem (types A and B), one in the xylem (type C), and one in the endodermis or bundle sheath (type D). They also found examples of cells that were impossible to classify.

In the tissues of the wheat spikelet it is easy to recognize examples of types A (Figs. 13 and 15) and C (Figs. 3 and 12). Type A appears to be a companion cell, as suggested by Pate and Gunning (1969). They are distinguished by an acidophilic and electron-dense ground substance, numerous mitochondria (that commonly appear to have lost their stroma), plasmodesmata that lie within a swollen pit field, and wall protuberances that occur on all walls of the cell (Figs. 13 and 15). However, it is not always easy to prove that any particular parenchyma cell of the phloem is a companion cell in a primary vascular bundle, for the ontogenetic relationship

Fig. 4.—Transverse section (taken at level 3, Fig. 1) showing part of the vascular network of a rachis (bottom) and a portion of a vascular bundle coming from the glume (asterisk). *s*, sieve elements; *t*, tracheary elements; *tc*, transfer cells.

Fig. 5.—Transverse section taken at the attachment region between the glume and the rachis (level 3, Fig. 1, adjacent to that shown in Fig. 4) showing two vascular bundles of the glume fused with the vascular network in the rachis (bottom). Note the reduction in the wall protuberances (arrowheads) in the xylem parenchyma cells that lie further into the glume compared with the better-developed thickenings on cells proximal to the rachis (bottom). *s*, sieve elements; *t*, tracheary elements; *tc*, transfer cells.

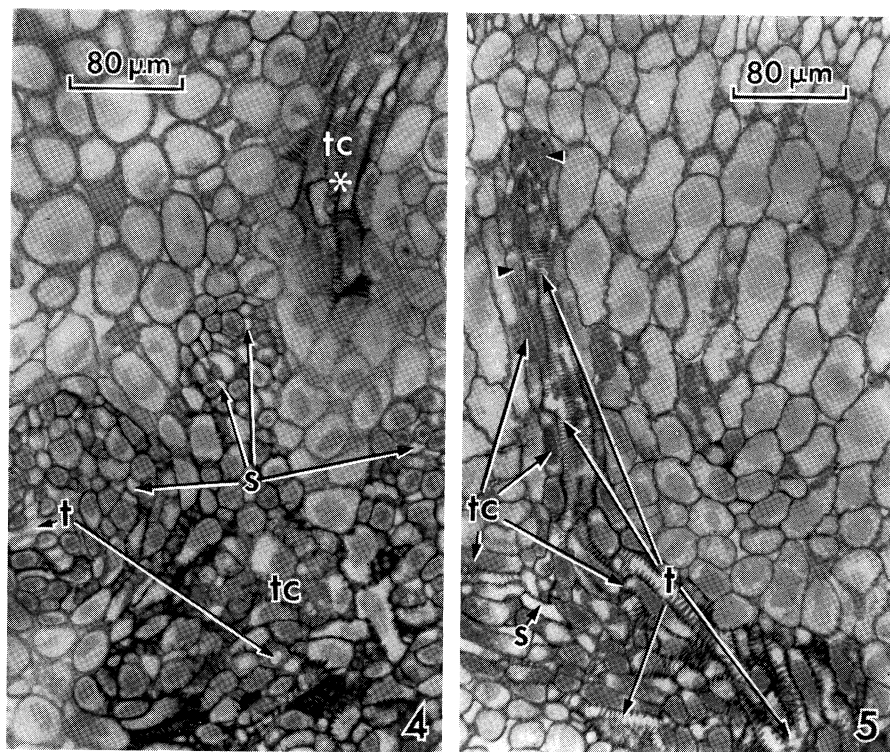
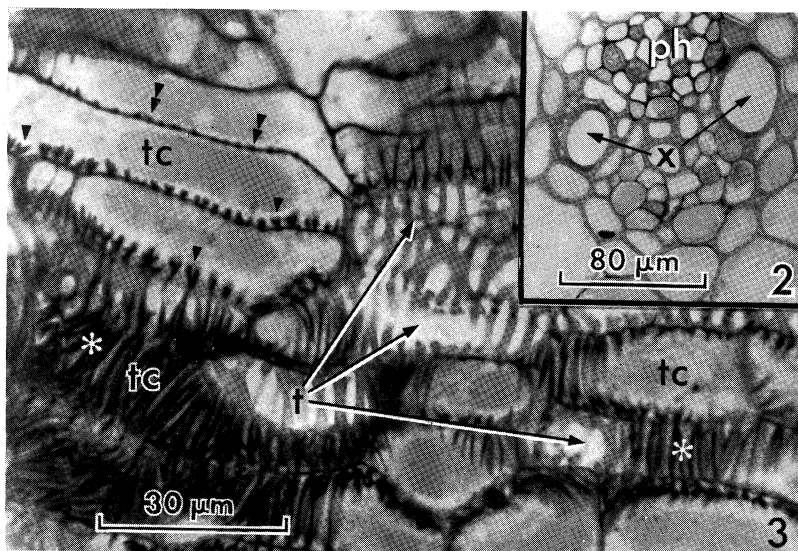


Fig. 2.—Transverse section of a vascular bundle of the rachis (cf. level 1, Fig. 1). *ph*, phloem; *x*, xylem.

Fig. 3.—Xylem transfer cells in longitudinal section (at about level 2 in Fig. 1). Note the development of the complex flanges (asterisks) in some transfer cells (*tc*) and the presence of Y-shaped flanges (arrowheads) and knobs (double arrowheads) in others. *t*, tracheary elements.

between the companion cell and sieve element may be hard to recognize. While it is difficult in a collateral bundle, it often becomes impossible in the case of the anastomosing networks of vascular tissue present at many of the levels of Figure 1.

This fact complicates the classification of many of the transfer cells present in these tissues. Clear-cut examples of type C (xylem parenchyma) cells are abundant in the lower portions of the vascular traces of the glumes, lemma, and palea (Figs. 3–8). Like those described in the coleoptilar node of wheat (O'Brien, Zee, and Swift

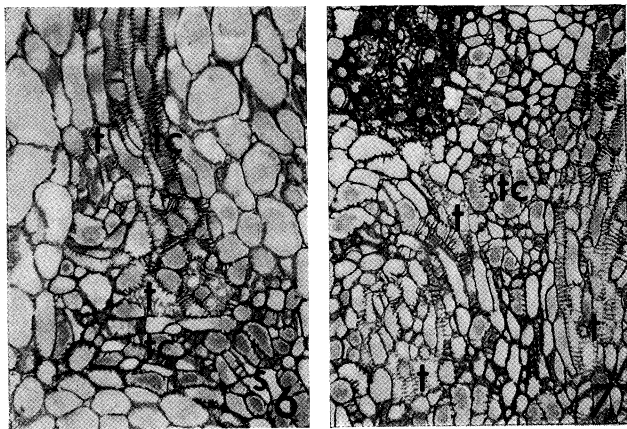


Fig. 6.—An oblique longitudinal section obtained from level 4, Figure 1. Section stained with periodic acid-Schiff's (PAS) reaction and the polychromatic stain, toluidine blue O. Note that the lignified walls of the tracheary elements (*t*) stain green, which contrasts sharply with the reddish purple of the unligified walls of the transfer cells (*tc*). *s*, sieve elements.

Fig. 7.—Longitudinal section of the rachilla at the junction between the thick-walled cells (*tw*) and the file of tracheary elements (*t*) from the rachis (see level 7, Fig. 1). Section stained with PAS and toluidine blue O. Note that the lignified walls (green) of the tracheary elements are sharply differentiated from the unligified walls (reddish purple) of the transfer cells (*tc*) and the orthochromatically stained (blue) thick-walled cells (*tw*).

1970), the thickenings consist of massive closely-spaced flanges that are developed preferentially on the side of the cell that abuts the tracheary element. The flanges are often grooved (appearing Y-shaped when seen in transverse section) and can be reticulate, mimicking the appearance of an incompletely differentiated tracheary element. However, they are readily distinguished from incompletely differentiated tracheary elements by the total absence of lignification (Figs. 6 and 7) and by the polarity of the distribution of the wall thickenings (Figs. 3 and 12). We are unaware of any reports of tracheary elements in which wall thickening is confined to one side

Fig. 9.—Transverse section of the core of thick-walled cells (*tw*) in the floral axis (level 8, Fig. 1) surrounded by a ring of sieve elements (*s*) and their associated transfer cells (asterisks).

Fig. 10.—Longitudinal section through the region between the pericarp bundle and the thick-walled cells (*tw*) (also see level 9, Fig. 1). *s*, sieve elements; *t*, tracheary elements.

Fig. 11.—Transverse section of the pericarp bundle taken at level 10, Figure 1. Note the absence of transfer cells. *c*, cuticle of seed coat; *s*, sieve elements; *t*, tracheary elements.

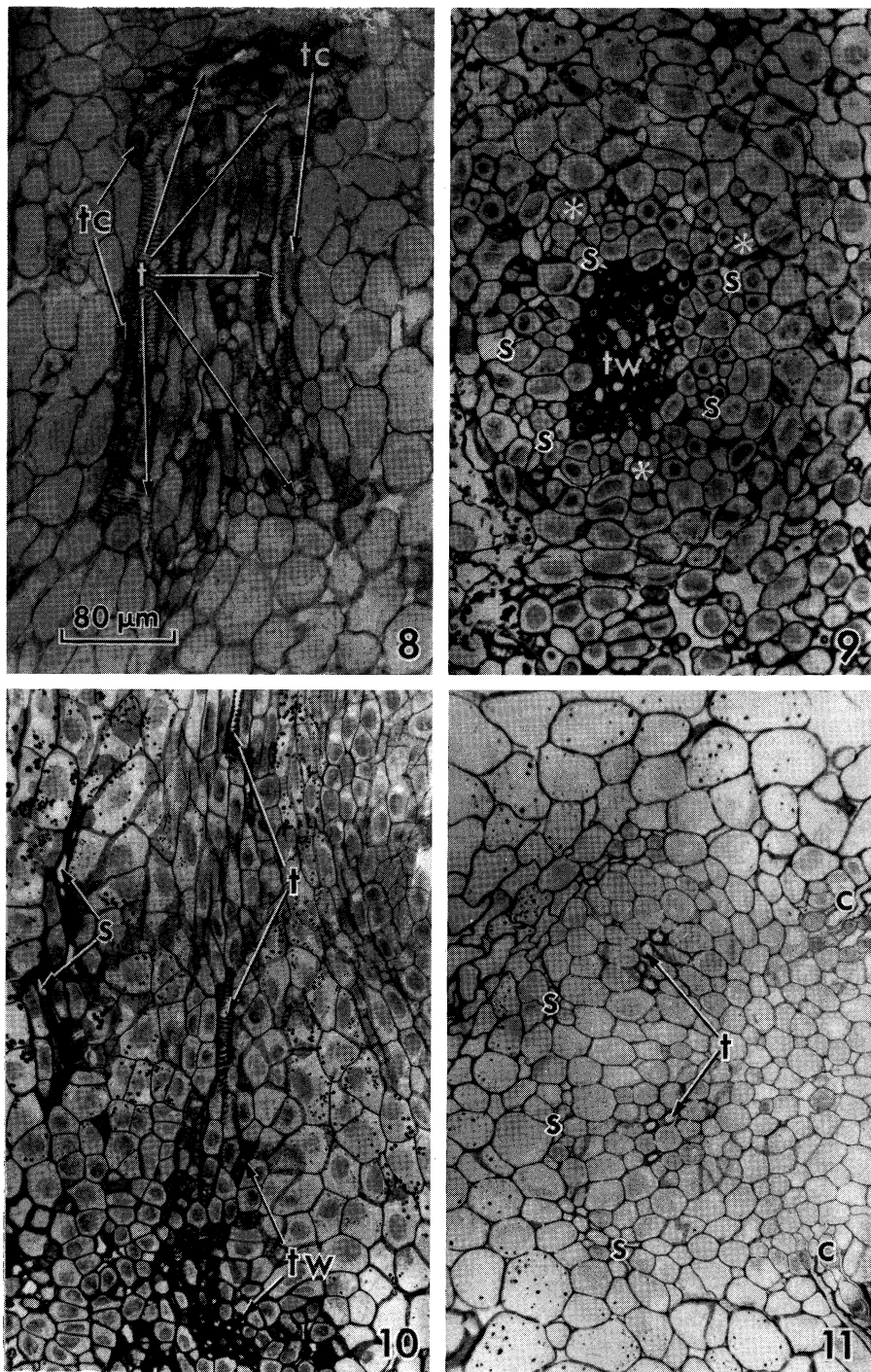


Fig. 8.—Longitudinal section of the basal portion of a vascular bundle of the lemma (see level 5, Fig. 1). *t*, tracheary elements; *tc*, transfer cells.

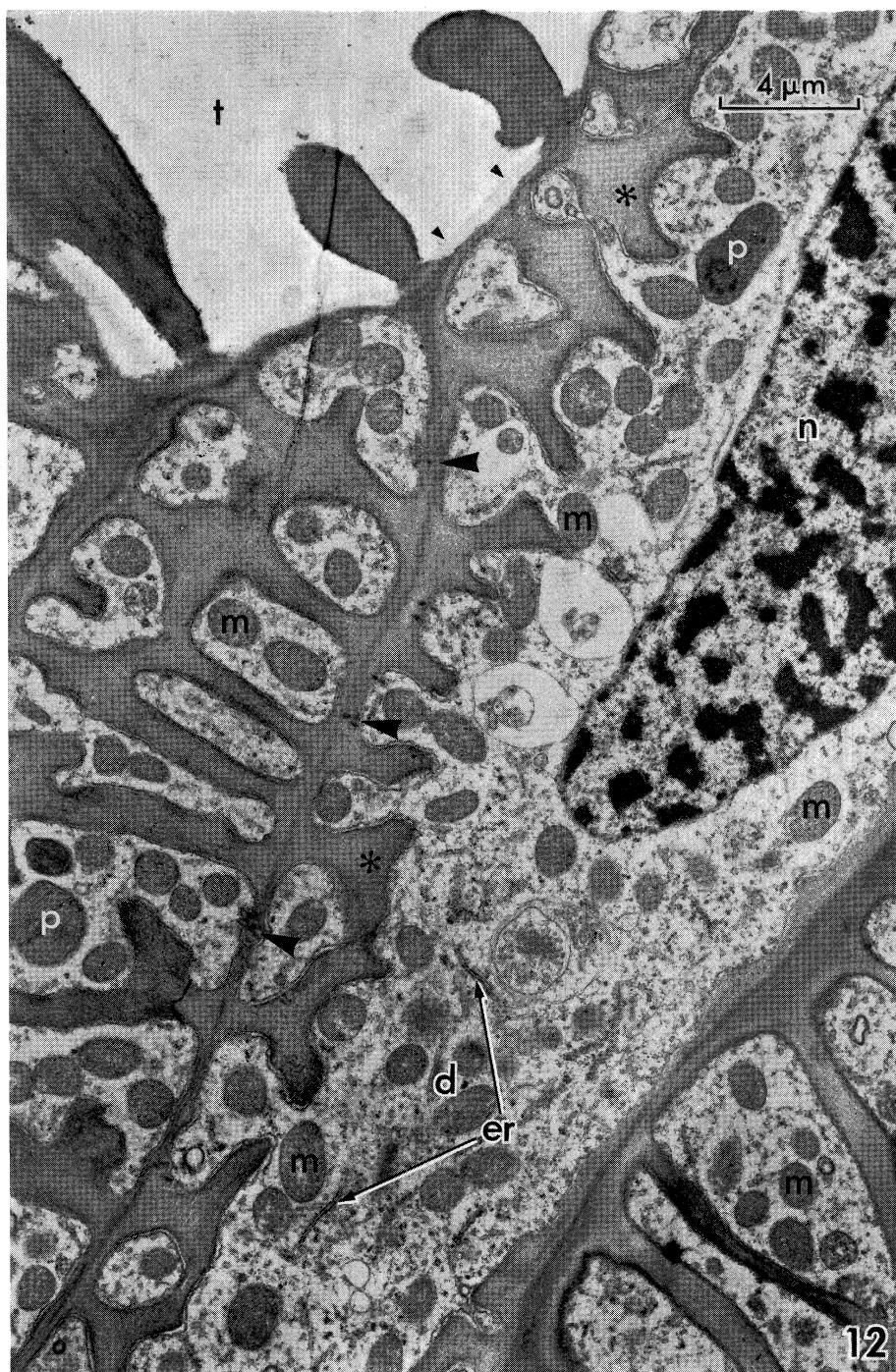


Fig. 12.—Longitudinal section of the C-type transfer cells. The cells on the left and right have flanges of wall thickening traversing the cells. The cell in the middle possesses Y-shaped flanges (asterisks) which are confined to only one side of the cell. Plasmodesmata (large arrowheads) pass through the wall between transfer cells. A portion of a tracheary element (*t*) with hydrolysed walls (small arrowheads) is also figured. *d*, dictyosomes; *er*, endoplasmic reticulum; *m*, mitochondria; *n*, nucleus; *p*, plastids.

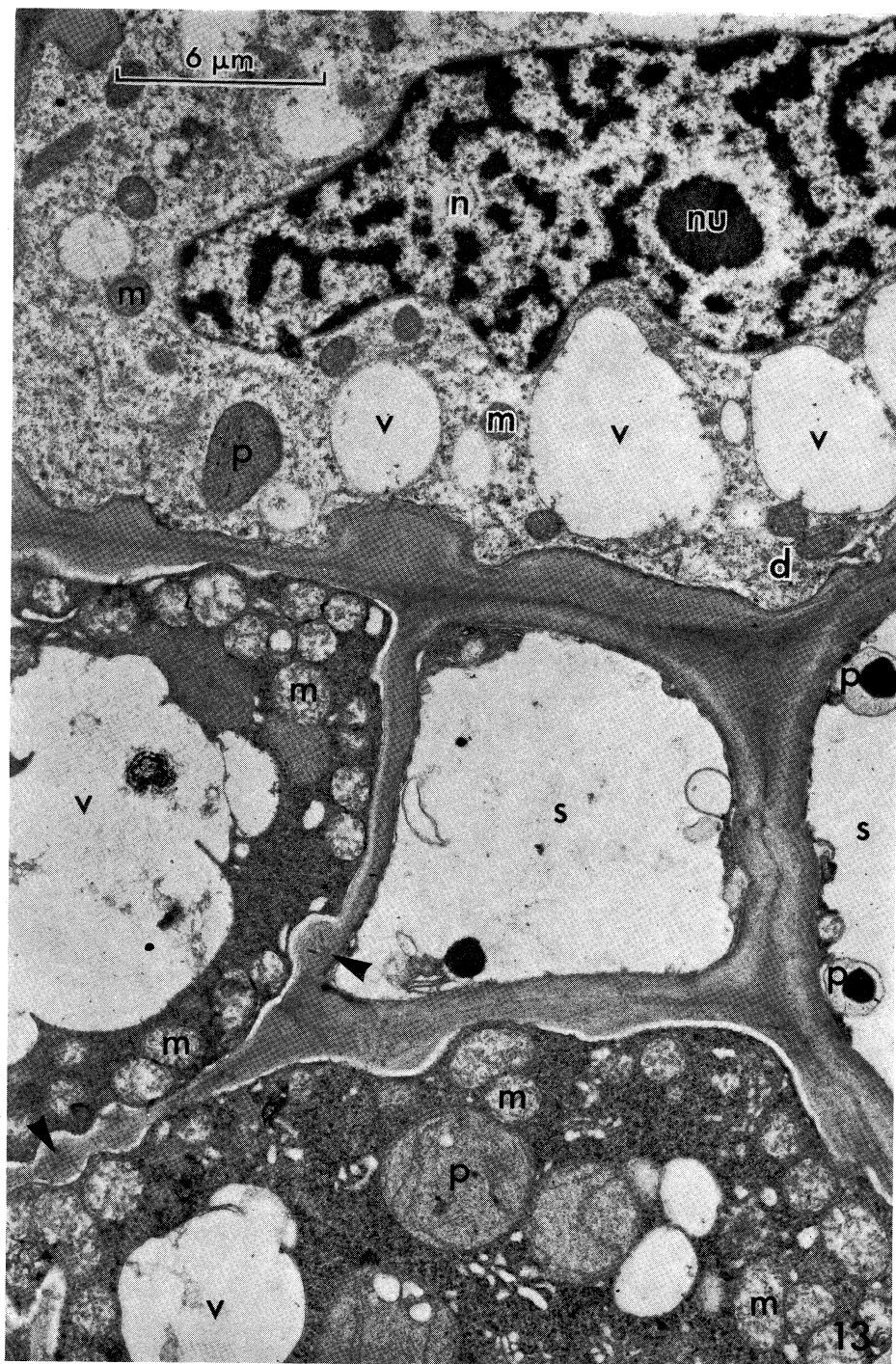


Fig. 13.—Electron micrograph of transverse section of sieve elements (*s*) and their associated A-type (left and bottom cell) and B-type (top) transfer cells. Note the contrast in the density of cytoplasm between the A- and B-type transfer cells and the presence of swollen plasmodesmatal pitfields in A-type cells (arrowheads). *m*, mitochondria; *n*, nucleus; *nu*, nucleolus; *p*, plastids; *v*, vacuoles; *d*, dictyosome.

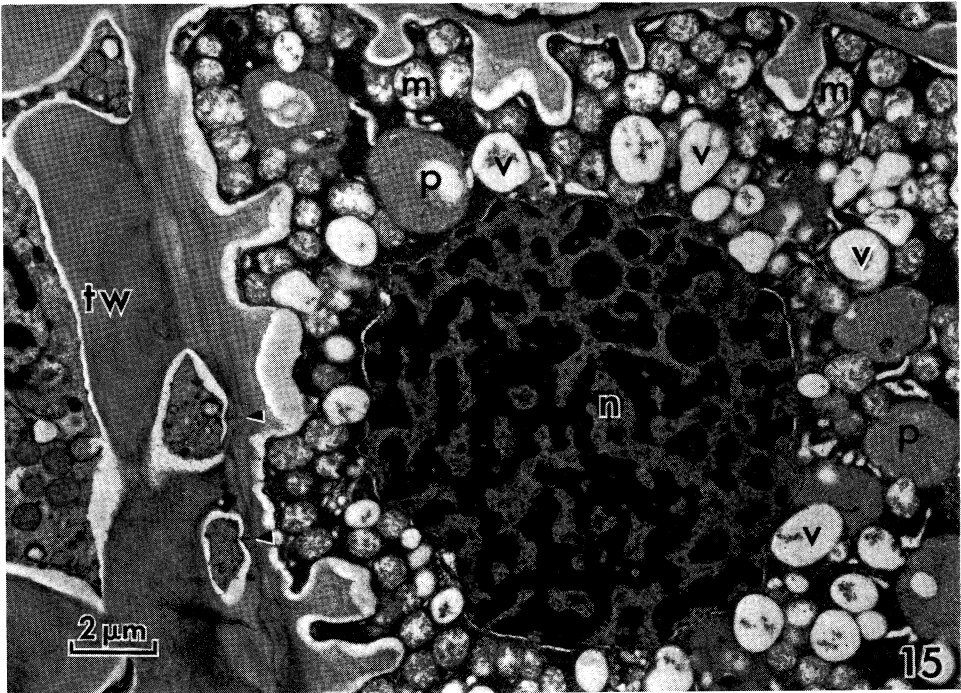


Fig. 14.—Electron micrograph showing the association of the thick-walled cells (*tw*) with associated transfer cells (*tc*). Note that the wall between the thick-walled cells and the transfer cells is traversed by numerous plasmodesmata (arrowhead).

of the cell. Also type C transfer cells differ in detail from those reported by Pate and Gunning (1969) in angiosperm leaves and by O'Brien (1970) in the pinnules of *Pteridium aquilinum*. In the leaf tissue, the wall protuberances of type C cells are always finger-like, never the massive flanges, reported in these nodal cells. Flanged thickenings were described in *Helianthemum* spp. (Cistaceae) on type A cells by Pate and Gunning (1969). Many of the type A cells in these wheat tissues have similar flanges of thickening.

Type B transfer cells differ from type A in leaf tissue by developing wall protuberances only where they abut a sieve element. However, in the complex anastomoses that occur between bundles in these nodes of the wheat spikelet, it is common to find parenchyma cells whose walls are in contact with two or more sieve elements, a sieve element and a type A cell, a sieve element and a type C cell, and in certain cases, with a sieve element and a tracheary element. Such parenchyma cells often cannot be classified easily even into xylem or phloem parenchyma, and it seems to us to be unwise to attempt to fit them into the classification developed by Pate and Gunning for the leaf. Unfortunately, a considerable number of the transfer cells present in the rachis nodes of wheat may be of this type.

To date we have not been able to reconstruct in detail the paths of the vascular traces from each of the organs of the spikelet, and until that is done, it is impossible to say whether bundles of particular organs develop only one type of transfer cell. However, it is our impression that the larger traces to the palea, lemma, and glumes are especially rich in type C cells, while regions of phloem continuity between the traces of the rachilla and those of the palea and lemma are especially rich in type A cells. The ring of phloem that encircles the core of modified tracheary elements at the base of the pericarp is rich in type A cells (Figs. 13 and 15) and in those that are impossible to classify (Figs. 14 and 18).

(c) *Lanthanum Tracer Experiments*

If the increased area of cell membrane created by wall protuberances is to be effective in promoting solute transfer, it is important that the free space that bounds the cell membrane be permeable to solutes. Revel and Karnovsky (1967) have shown that solutions of lanthanum nitrate adjusted to pH 7.2 give rise to colloidal species of lanthanum that can permeate spaces as small as 2 nm in the extracellular space of animal tissue. Gunning and Pate (1969) have shown that such lanthanum tracer will permeate to the cell membrane of transfer cells in *Trifolium repens* minor veins fed via the transpiration stream for 3 hr. They showed further that this tracer would not permeate into lignified thickenings of tracheary elements. Figures 16–19 confirm both of these observations for transfer cells in wheat spikelets fed via the transpiration stream for 6 hr. Lanthanum tracer may be detected in the free space of all types of transfer cell and in contact with the cell membrane. It seems reasonable to conclude that pores that may be as much as 2 nm in diameter exist in the free space in contact with the cell membrane of these cells. In animal tissue Revel and Karnovsky (1967) believed that the lanthanum was not readily adsorbed to the extracellular matrix

Fig. 15.—Shows the association of the thick-walled cell (*tw*) with an A-type transfer cell. Plasmodesmata (arrowheads) interconnect the two cells. *m*, mitochondria; *p*, plastids; *v*, vacuoles; *n*, nucleus.

for it could be leached from it very readily during specimen preparation. Our observations support this idea for we found that all the tracer could be lost from the walls unless the specimens were rapidly dehydrated after fixation.

In an earlier paper (Zee and O'Brien 1970), it was shown that dyes and ferric chloride do not cross from the xylem of the rachilla into the pericarp bundle. We established that the modified tracheary elements (Figs. 14 and 15) that comprise the core of thick walled cells offer no continuous pathway via their lumina, and movement of solution would have to be through their cell walls. When these cells were examined in the plants fed with lanthanum tracer, it was clear that the inner wall-layers of these cells contained no tracer (Fig. 17). Nor could lanthanum be detected in the upper parts of the core. It seems likely that lanthanum tracer arriving by the tracheary elements cannot spread readily into the free space of the neighbouring thick-walled cells.

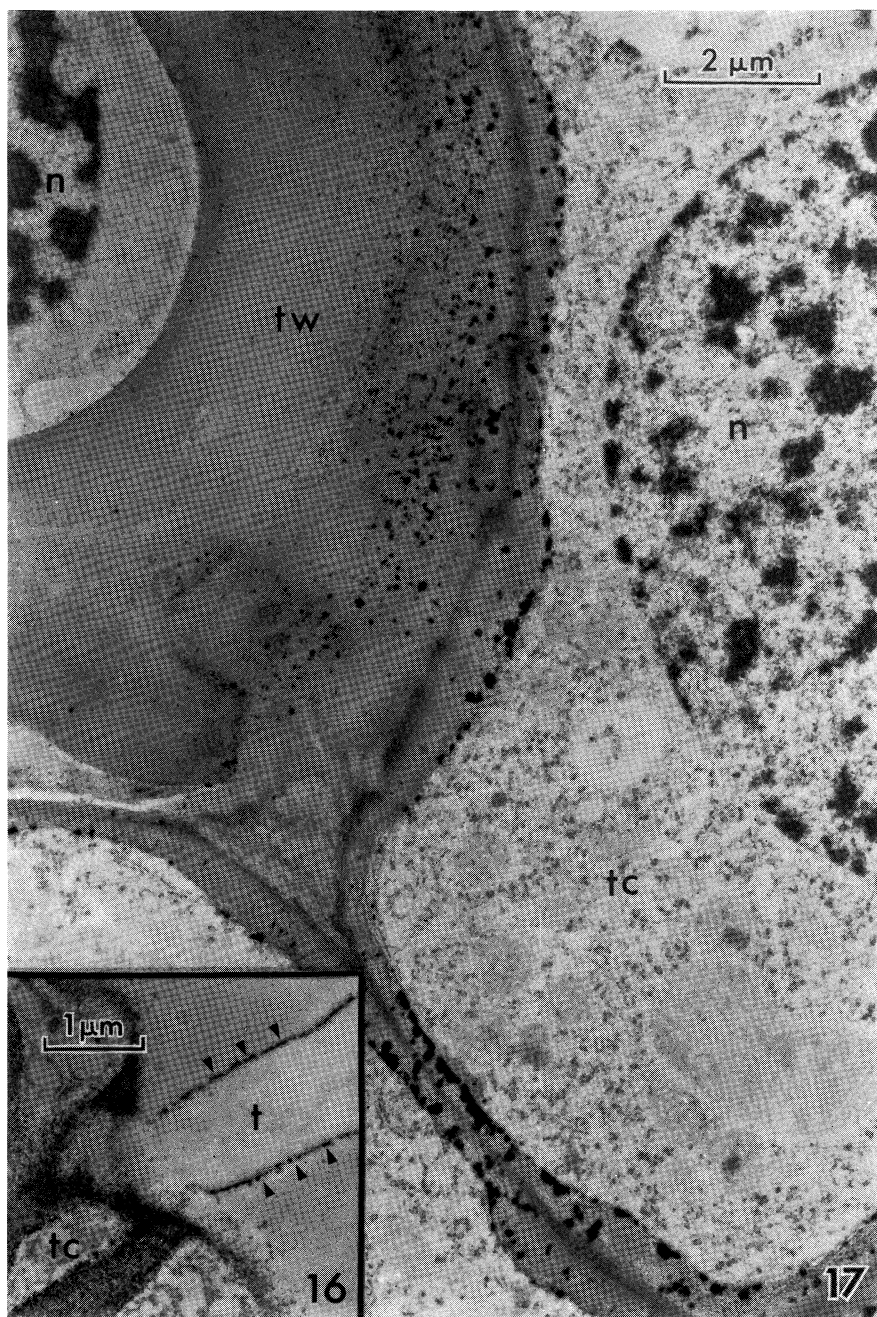
Figure 19 shows that the walls of sieve tubes exposed to lanthanum tracer contain very much less tracer than walls of their attendant transfer cells. The tracer appears to be confined chiefly to the innermost layer of the sieve-tube wall. Provided lanthanum has not been lost selectively from the other layers of the wall during specimen preparation this observation suggests that much of the free space bounding these sieve tubes may be rather impermeable to solutes. Could this function to aid solute retention by sieve tubes?

(d) General Comments

Pate and Gunning (1969) have shown that vascular transfer cells were absent from the leaves of members of 41 families of monocotyledons and we can confirm that they are absent from all of the normal collateral bundles of the leaf, leaf sheath, internode, rachis, rachilla, sterile glumes, palea, lemma, and pericarp. However, in the spikelet, vascular transfer cells occur in abundance at the "nodes" where sterile glumes, lemma, palea, and pericarp are attached to the rachis or rachilla. On these morphological grounds we propose that the type C cells extract solutes, and especially organic nitrogen, from the transpiration streams that supply the glumes, lemma, and palea and direct these solutes, via a symplastic route, to the sieve tubes which provide the only direct vascular continuity between rachilla and pericarp. It is more difficult to propose a function for the type A cells. However, it is well known that photosynthates synthesized in the glumes provide an important contribution to grain dry weight. Perhaps the type A cells help to ensure efficient transfer of sugar arriving from the various glumes to the ring of sieve tubes that supply the pericarp bundle, and through it, the developing embryo and endosperm.*

*Note added in proof.—Gunning, Pate, and Green [Transfer cells in the vascular tissues of stems: taxonomy, association with nodes, and structure (*Protoplasma*, 1971, in press)] have found that vascular transfer cells are present at nodes in more than half of the 190 species they investigated. The presence of vascular transfer cells in these floral nodes of wheat is clearly just one example of a phenomenon of widespread occurrence.

Fig. 17.—Gradient of lanthanum deposit within the wall of a thick-walled cell (*tw*). The tracer appears to be excluded from the inner layers of the wall in these cells. *n*, nucleus; *tc*, transfer cell.



Figs. 16–19.—These figures illustrate the way in which electron-opaque lanthanum tracer accumulates in the walls of the tracheary elements (Fig. 16), transfer cells (Figs. 16, 18, and 19), and the thick-walled cells (Fig. 17), following administration of the tracer solution through the stem via the transpiration stream. Figure 16 shows part of the lateral wall and wall ingrowths of a xylem transfer cell (*tc*) abutting on to a tracheary element (*t*). The tracer has penetrated into the walls of the transfer cells but not into the lignified thickenings (arrowheads).

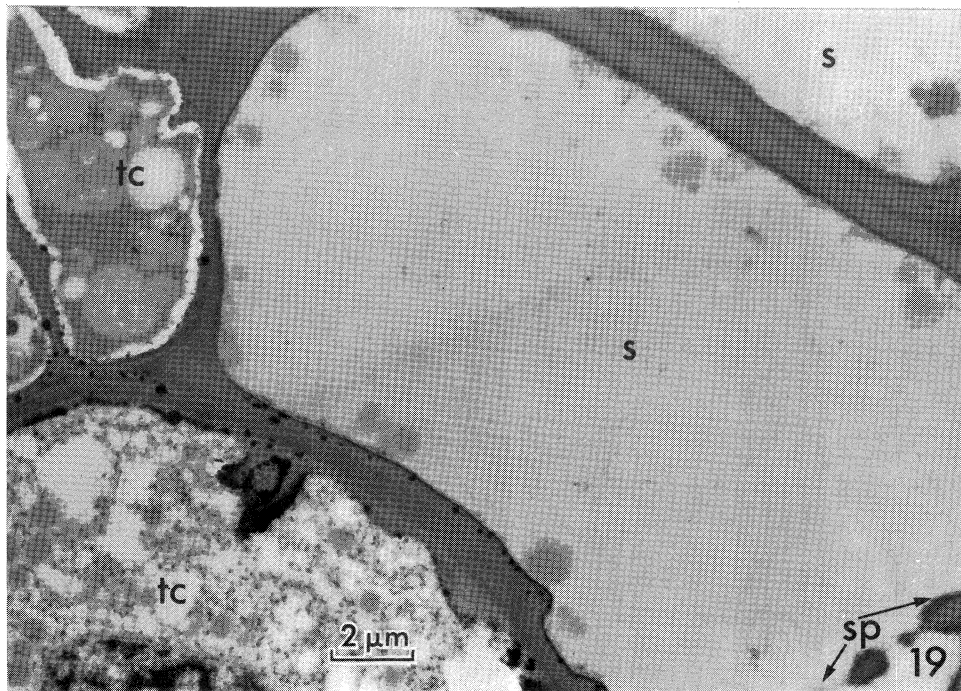
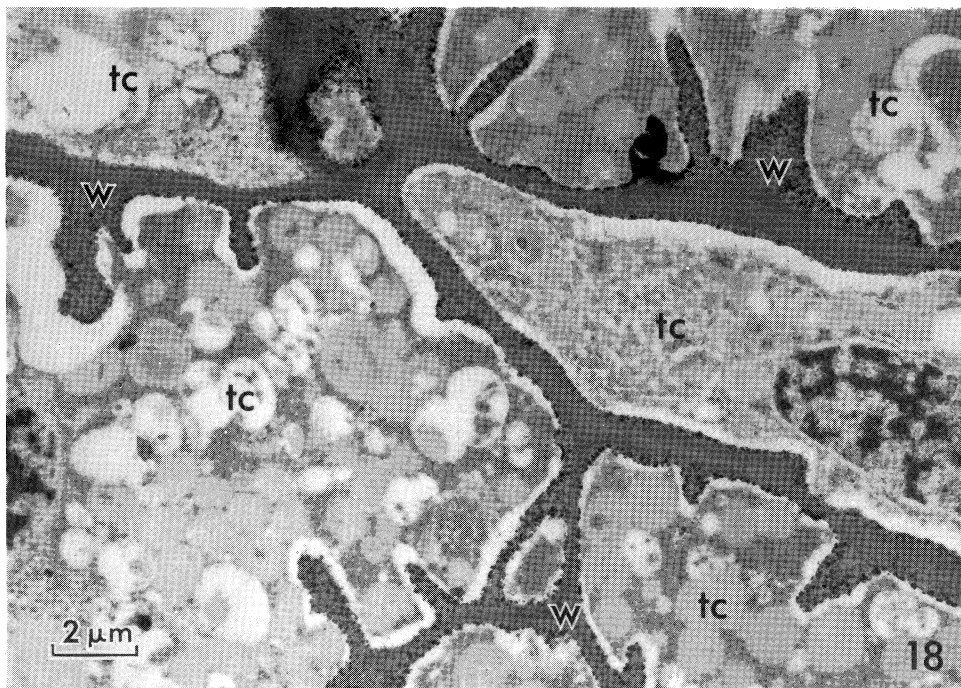


Fig. 18.—Phloem transfer cells (*tc*) showing the deposits of lanthanum tracer being confined to the cytoplasmic surface of the wall and to the wall ingrowths (*w*).

Fig. 19.—Portion of two sieve elements (*s*) and their associated transfer cells (*tc*). The tracer can be seen lining the inner layers of the walls of the sieve elements, but appears to be excluded from the primary wall. *sp*, sieve plate.

IV. ACKNOWLEDGMENTS

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V. REFERENCES

- CARR, D. J., and WARDLAW, I. F. (1965).—The supply of photosynthetic assimilates from the flag leaf and ear of wheat. *Aust. J. biol. Sci.* **18**, 711–19.
- EVANS, L. T., and RAWSON, H. M. (1970).—Photosynthesis and respiration by the flag leaf and components of the ear during grain development in wheat. *Aust. J. biol. Sci.* **23**, 245–54.
- FEDER, N., and O'BRIEN, T. P. (1968).—Plant microtechnique: some principles and new methods. *Am. J. Bot.* **55**, 123–44.
- GUNNING, B. E. S., and PATE, J. S. (1969).—"Transfer cells"—plant cells with wall ingrowths, specialized in relation to short distance transport of solutes—their occurrence, structure, and development. *Protoplasma* **68**, 107–33.
- KRIEDEMANN, P. (1966).—The photosynthetic activity of the wheat ear. *Ann. Bot.* **30**, 349–63.
- LUPTON, F. G. H. (1968).—The analysis of grain yield of wheat in terms of photosynthetic ability and efficiency of translocation. *Ann. appl. Biol.* **61**, 109–19.
- O'BRIEN, T. P. (1970).—Further observations on hydrolysis of the cell wall in the xylem. *Protoplasma* **69**, 1–14.
- O'BRIEN, T. P., ZEE, S., and SWIFT, J. G. (1970).—The occurrence of transfer cells in the vascular tissues of the coleoptilar node of wheat. *Aust. J. biol. Sci.* **23**, 709–12.
- PATE, J. S., and GUNNING, B. E. S. (1969).—Vascular transfer cells in angiosperm leaves—a taxonomic and morphological survey. *Protoplasma* **68**, 135–56.
- REVEL, J. P., and KARNOVSKY, M. J. (1967).—Hexagonal array of subunits in intercellular junctions of the mouse heart and liver. *J. Cell Biol. C* **33**, 7–12.
- SPURR, A. R. (1969).—A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* **26**, 31–43.
- THORNE, G. N. (1965).—Photosynthesis of ears and flag leaves of wheat and barley. *Ann. Bot.* **29**, 318–19.
- WILLIAMS, R. F. (1955).—Redistribution of mineral elements during development. *A. Rev. Pl. Physiol.* **6**, 25–42.
- ZEE, S. Y., and O'BRIEN, T. P. (1970).—A special type of tracheary element associated with "xylem discontinuity" in the floral axis of wheat. *Aust. J. biol. Sci.* **23**, 783–91.

