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SPECIALIZED "TRANSFER CELLS" IN MINOR VEINS OF LEAVES AND THEIR POSSIBLE SIGNIFICANCE IN PHLOEM TRANSLOCATION

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Translocation of organic substances in the phloem of higher plants has been investigated for almost a century, but remarkably little is known of the mechanisms whereby assimilates are drained from the mesophyll of photosynthesizing leaves and transferred to sieve tubes in the leaf veins. Certain modified companion cells (3, 5–7) associated with the sieve tubes of minor veins have long been suspected of having a special role in this respect (11). Indeed, the term "Uebergangszellen," introduced by Fischer in 1884 (9) to denote these densely staining cells, implies such a function. We shall employ "transfer cells" as a literal translation to preserve the physiological connotation. The present communication is concerned with a specialized type of transfer cell found in certain higher plants, and we wish to present and interpret cytological information relevant to its translocatory function.

Fig. 1 shows a cross-section of a vein from a mature leaf of *Pisum arvense*. Two tracheary elements (*x*), three narrow sieve elements (*s*), and two types of phloem parenchyma cell are visible. One type (*p*) has sparse contents; the other (*t*) has dense contents. The latter is the transfer cell. Its nucleus is large and usually central (Fig. 1). In its cytoplasm are vacuoles, chloroplasts with grana and starch grains, and numerous mitochondria with well-developed cristae (Figs. 7, 8). Dictyosomes and rough endoplasmic reticulum occur, and there are many polyribosomes; the ground substance is denser than in most plant cells (Fig. 11). A somewhat similar picture of the protoplast of companion cells has emerged from numerous other studies, including those few concerned with minor veins (7, 8).

The specialization characteristic of the transfer cells described here is their protuberant wall structure (Figs. 1–8, 11) To our knowledge there is only one previous micrograph of this facet of minor vein structure; this was included by Ziegler (25) in a paper on plant glands. However, wall protuberances occur commonly in other situations, e.g. salt glands (18, 22), other leaf glands and nectaries (21, and see bibliography in reference 18), embryo sacs (13), and the haustorial foot of a moss sporophyte (19) and of a parasitic angiosperm (4).

The transfer cell protuberances are intensely metachromatic (Fig. 1), stain with Schiff-type reagents after periodic acid oxidation (Figs. 2–5), and are positively birefringent with respect to their long axis. They form part of an irregular layer of wall material deposited secondarily on the primary wall (Fig. 8), and their loose microfibrillar texture can be seen at high magnification (Fig. 11). Wall microfibrils may be seen in a space, possibly created by fixation damage, which lies between

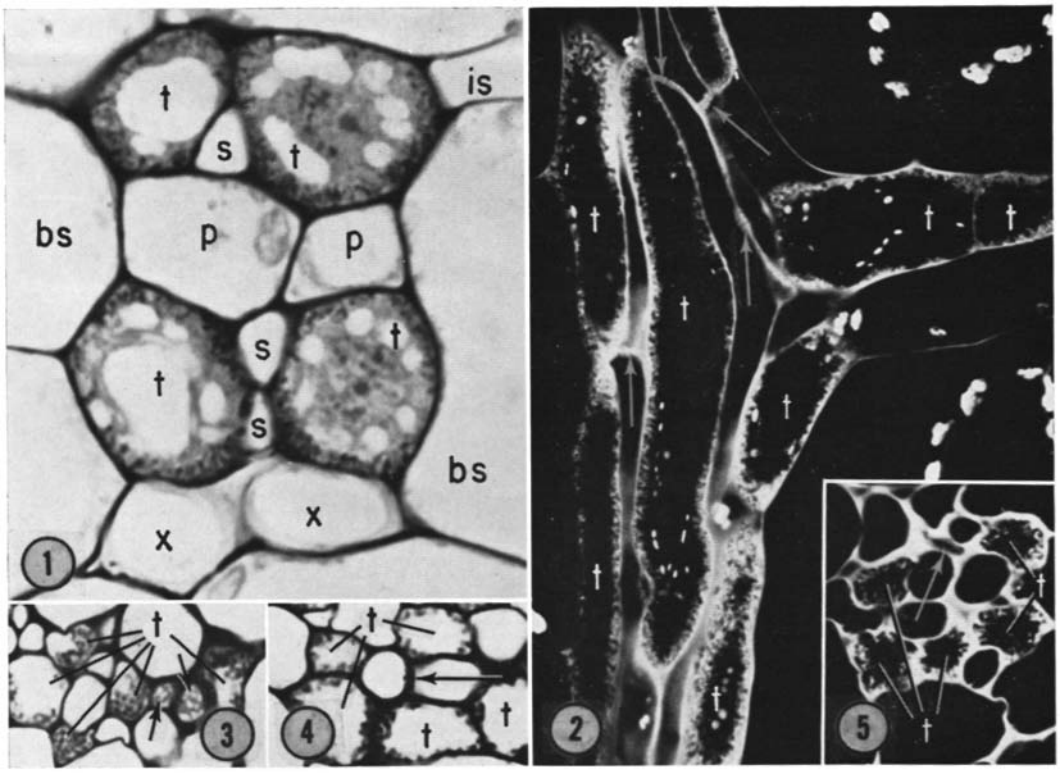

FIGURE 1 Transverse section of a *Pisum arvense* minor vein. *bs*, bundle sheath; *is*, intercellular space; see text for other symbols. Glycol methacrylate embedding. 1–2 μ section, acrolein fixation (as in Figs. 2–5). Toluidine blue. × 2000.

FIGURE 2 Longitudinal section through a vein branch in *Pisum arvense*. Wall protuberances identify the transfer cells (*t*) and sieve plates (arrows), the sieve elements. Periodic acid, acriflavine (14); dark-ground fluorescence. × 420.

FIGURES 3–5 Transverse sections of minor vein phloem in *Odontites verna*, *Galega officinalis*, and *Sonchus oleraceus*, respectively, each showing five transfer cells (*t*), to illustrate the range in form of protuberances. Arrows, sieve plates. Figs. 3 and 4, periodic acid-Schiff's reagent; Fig. 5, as Fig. 2. All × 1400.

the plasma membrane and the wall (Figs. 6–8, 11). The plasma membrane appears to have granular or fibrillar material deposited on it (Fig. 11).

Two contrasting specializations might be envisaged as favoring efficient absorption of materials into a cell: one in which symplastic transfer is enhanced by abundant plasmodesmata, and the other in which the capacity for uptake from the extracellular environment is promoted by increasing the cell's surface:volume ratio. The transfer cells described here are clearly specialized in the latter direction, as must be the glands and other structures listed above and known to be intensively engaged in solute uptake. We have estimated that if wall protuberances are well developed in a *Pisum arvense* transfer cell, the area of its plasma membrane may be increased by more than 10 times that of a smooth-walled cell of similar overall dimensions. This factor may be much larger in plants whose transfer cells possess numerous filiform protuberances, e.g. *Odontites* (Fig. 3), *Trifolium*, *Medicago*.

Protuberances may be observed on all walls of a transfer cell, but they are usually less abundant on the wall contiguous with one particular sieve element, possibly its sister cell (Figs. 1, 3, 5, and 8). They may be opposed to the bundle sheath, to xylem elements, to the other type of phloem parenchyma, to other transfer cells and sieve

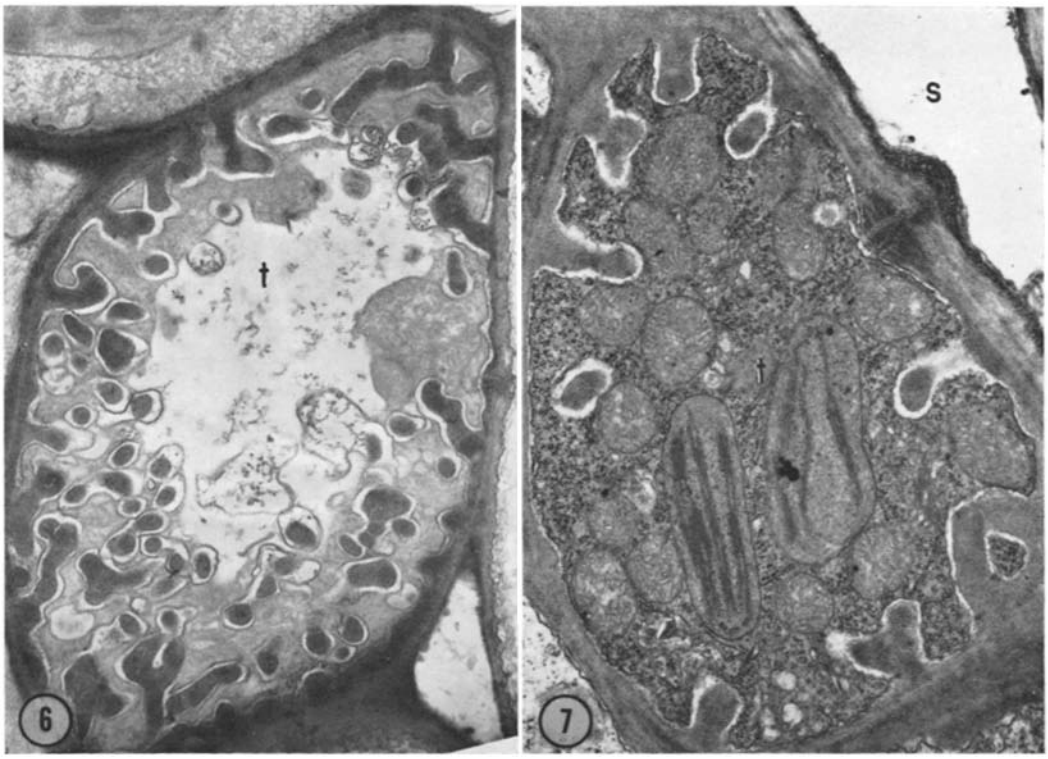

FIGURE 6 *Pisum arvense* transfer cell (*t*). × 12,000.

FIGURE 7 *Lupinus* transfer cell (*t*) and sieve element (*s*). × 14,000.

elements, and even to intercellular spaces (Fig. 1). We suggest that the transfer cells with their large area of plasma membrane and abundant mitochondria function in the accumulation of solutes from the cell wall fluids. Other studies testify to the presence of solutes in this "free space" (12), to the possibility that walls might provide a pathway for sugar movement (15), and to the capacity of leaf vein tissues to absorb solutes against steep concentration gradients (2, 16). More specifically, Esau (5) has noted the marked ability of companion cells to accumulate neutral red.

Radioautographic experiments demonstrate that transfer cells of *Pisum arvense* can absorb and incorporate into insoluble components radioactivity derived either from labeled carbon dioxide fixed in the leaf by photosynthesis or from a labeled amino acid supplied through the transpiration stream. The latter type of experiment (Fig. 12) simulates the way in which the transpiration stream of *Pisum* normally delivers amino acids such as leucine from roots to the leaves. A leaf, if mature, is then capable of reexporting a proportion of such compounds to other parts of the plant (20). In the present context the experiment demonstrates uptake and assimilation by transfer cells of what is, at least initially, an extracytoplasmic solute and suggests that a normal function of the transfer cell may be in the retrieval of solutes arriving in the transpiration stream. The possibility of enzymatic transformations of solutes within the protuberant wall of transfer cells must not be overlooked. This wall is a site of intense activity of acid phosphatase (Fig. 13), an enzyme long associated with phloem (17) and suggested to be involved in the acceptance of sugars into cells of the phloem (1).

There is therefore every indication that the transfer cells are specialized for an uptake function. A light microscope survey of their development and distribution reveals, in addition, an association with export of materials. In $^{14}CO_2$-feeding experiments, it has been shown that the appearance of wall protuberances in transfer cells

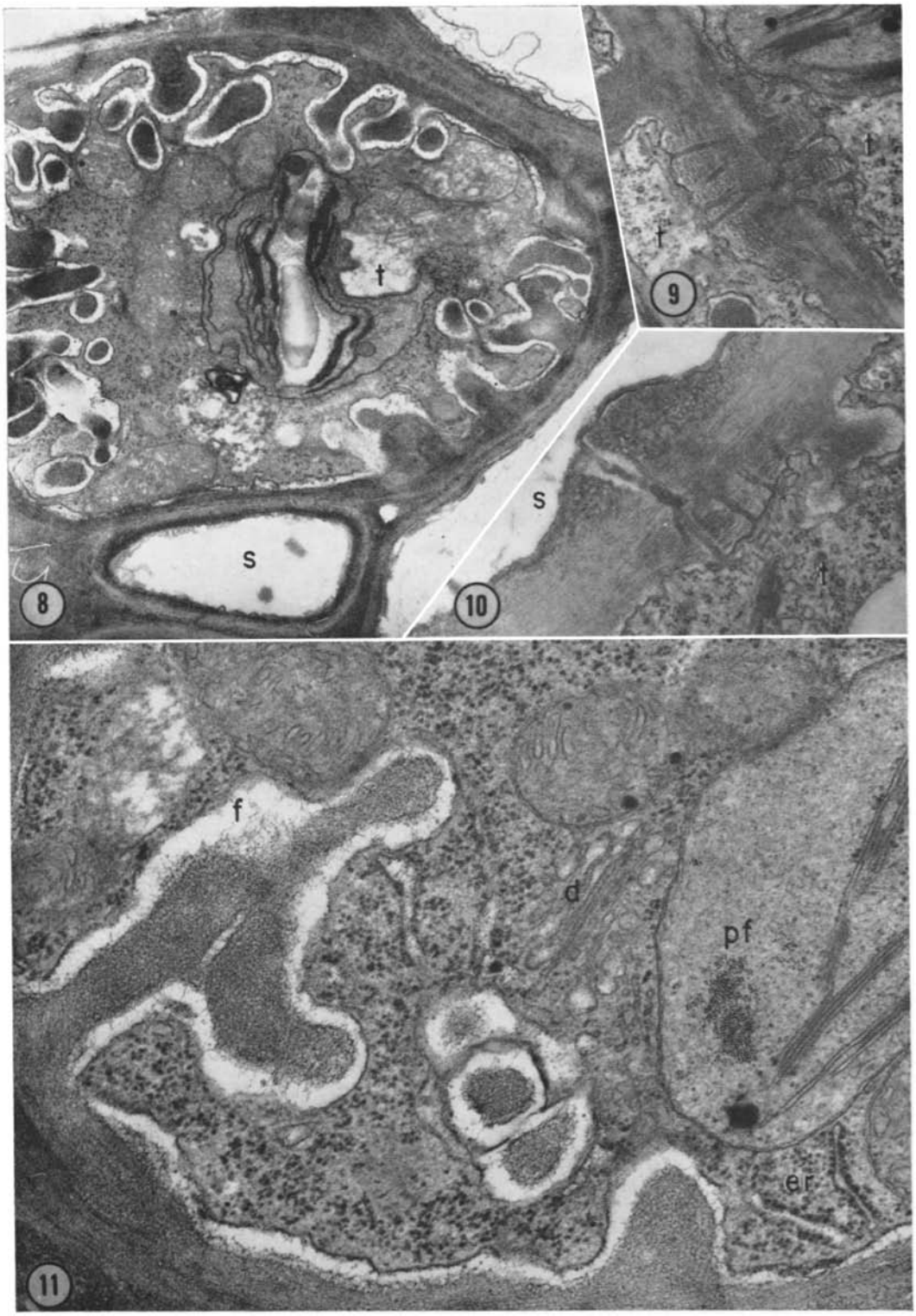

FIGURE 8 *Pisum arvense* transfer cell (*t*) and sieve element (*s*). × 13,000.

FIGURES 9 and 10 Compound plasmodesmata in *Lupinus* minor vein phloem. Fig. 9, between two transfer cells (*t*); Fig. 10, between transfer cell (*t*) and sieve element (*s*). × 22,500.

FIGURE 11 Part of a *Lupinus* transfer cell. *f*, microfibrillar texture of wall protuberance; *d*, dictyosome, *pf*, phytoferritin; *er*, rough endoplasmic reticulum. × 37,500.

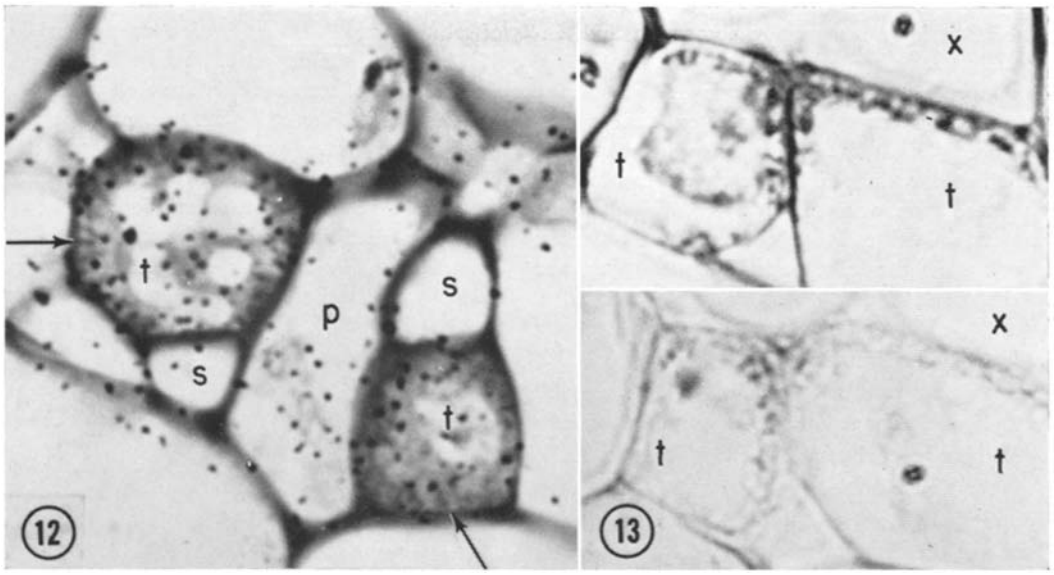

FIGURE 12 Radioautograph showing localization of insoluble products of incorporation of 4,5-T leucine, administered through the petiole of a detached but otherwise intact mature leaf by transpirational uptake. *t*, transfer cells; *s*, sieve elements; *p*, unspecialized phloem parenchyma; arrows indicate protuberances. Toluidine blue. × 2,800.

FIGURE 13 Acid phosphatase in walls of *Pisum arvense* transfer cell. 3-μ sections of tissue were incubated in complete reaction mixtures with glycerophosphate as substrate (10). Top, unheated section; bottom, section heated to 100°C for a few minutes. Minus-substrate controls gave neagtive results. *p*-formaldehyde fixation, low temperature embedding in glycol methacrylate (Feder, N. Personal communication). *x*, tracheary elements; *t*, transfer cells. × 3,000.

of a young leaf (*Pisum*) coincides with the commencement of export from the leaf, and that their subsequent outgrowth parallels the buildup in export activity as the leaf matures. Significantly, they fail to enlarge in leaves left in darkness after they have been given sufficient light for leaf expansion. Their development, early in germination, in underground cotyledons (*Pisum*, *Vicia*) as well as in cotyledons born above ground (*Ulex*, *Genista*) suggests a role in the mobilization of stored reserves.

In *Pisum arvense*, wall protuberances are well developed in minor vein transfer cells of stipules and pods but not in nonphotosynthetic organs such as roots, petals, and scale leaves. In general, they are most prominent in minor veins, less so in main veins, and not easily detectable with the light microscope in the petiole and stem. The "trabeculae" found by electron microscopy of stem secondary phloem in *Pisum sativum* (23) presumably represent a persistent, albeit sparse and reduced, form of the protuberances. Certainly, we find them well developed in minor veins of this species. A single section of the winged stem (cladode) of *Genista sagittalis* neatly displays the reduction in development of protuberances from minor veins of the photosynthetic wings to the central, longitudinally-conducting vascular tissue.

Whatever the nature of the uptake and accumulation mechanisms in minor veins, transfer of solutes to sieve tubes must precede export. If accumulation within transfer cells realizes concentrations of the same order of magnitude as those in the sieve tube, it is unlikely that passage to the latter would constitute a rate-limiting step in translocation. Here, the potential for symplastic transfer exists; compound plasmodesmata, of the kind described for other companion cells (8, 24), traverse the wall between transfer cells and their associated sieve elements (Figs. 7, 10). Plasmodesmata also interconnect the transfer cells (Fig. 9).

In the system we propose, these specialized transfer cells comprise an interconnected collecting apparatus in the minor vein, presenting to its

exterior a large surface for absorption and, internally, direct and easy access to the sieve tubes. We have suggested that they function in active uptake of extracytoplasmic solutes; their protuberances, the phosphatase activity in their walls, their mitochondria, and their close association in time and space with the export of assimilates all support this contention. However, this mechanism may not be the only, or indeed the major, one whereby solutes gain access to the sieve tubes. A route involving plasmodesmata between transfer cells and the bundle sheath is not excluded, although we have as yet failed to observe such cytoplasmic connections. Also, the role of the remaining parenchyma in the vein is unknown.

Clearly, it would be premature to assign too specific a function to the transfer cells. In some instances their main activity may be the retrieval of solutes from the transpiration stream (see Fig. 12), in others, the export of photosynthates or the mobilization of reserves. We are well aware that minor vein transfer cells with wall protuberances are not universally present in higher plants. A preliminary survey involving 37 families has shown them to be very common in the *Leguminosae*; and positive identifications have been made in the *Balsaminaceae*, *Valerianaceae*, *Rubiaceae*, *Plantaginaceae*, *Dipsacaceae*, *Scrophulariaceae* (see Fig. 3), and *Compositae* (Fig. 5). Just how widespread and significant they are remains to be seen.

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REFERENCES

1. BAUER, L. 1953. *Planta.* **42**:367.
2. BIELESKI, R. L. 1966. *Plant Physiol.* **41**:447.
3. DE MORRETES, B. L. 1962. *Am. J. Botany.* **49**:560.
4. DORR, I. 1967. *Naturwissenschaften.* **17**:474.
5. ESAU, K. 1934. *Am. J. Botany.* **21**:632.
6. ESAU, K. 1965. Plant Anatomy. John Wiley & Sons, Inc., New York. 2nd edition.
7. ESAU, K. 1967. *Proc. Am. Phil. Soc.* **111**:219.
8. FALK, H. 1964. *Planta.* **60**:558.
9. FISCHER, A. 1884. Untersuchungen über das Siebröhren-System der Cucurbitaceen. Gebrüder Borntraeger, Berlin.
10. GOMORI, G. 1952. Microscopic Histochemistry. University of Chicago Press, Chicago, Ill.
11. HABERLANDT, G. 1914. Physiological Plant Anatomy. The Macmillan Company, London.
12. HAWKER, J. S. 1965. *Australian J. Biol. Sci.* **18**:959.
13. JENSEN, W. A. 1965. *Am. J. Botany.* **52**:238.
14. KASTEN, F. H. 1959. *Histochemie.* **1**:466.
15. KRIEDEMANN, P. 1967. *Planta.* **73**:175.
16. KURSANOV, A. L. 1963. *Adv. Botan. Res.* **1**:208.
17. LESTER, H. H., and R. F. EVERT. 1965. *Planta.* **65**:180.
18. LÜTTGE, U. 1966. *Naturwissenschaften.* **53**:96.
19. MAIER, K. 1967. *Planta.* **77**:108.
20. PATE, J. S. 1968. Physiological aspects of inorganic and intermediate nitrogen metabolism. *In* Aspects of Nitrogen Metabolism and Utilization in Plants. Academic Press Inc., New York. In press.
21. RENAUDIN, S. 1966. *Bull. Soc. Botan. France.* **113**:379.
22. THOMSON, W. W., and L. L. LIU. 1967. *Planta.* **73**:201.
23. WARK, M. C. 1965. *Australian J. Botany.* **13**:185.
24. WOODING, F. B. P., and D. H. NORTHCOTE. 1965. *J. Cell Biol.* **24**:117.
25. ZIEGLER, H. 1965. *Deut. Botan. Ges. Ber.* **78**:466.