RELATION OF BEET YELLOWS VIRUS TO THE PHLOEM AND TO MOVEMENT IN THE SIEVE TUBE

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ABSTRACT

In minor veins of leaves of *Beta vulgaris* L. (sugar beet) yellows virus particles were found both in parenchyma cells and in mature sieve elements. In parenchyma cells the particles were usually confined to the cytoplasm, that is, they were absent from the vacuoles. In the sieve elements, which at maturity have no vacuoles, the particles were scattered throughout the cell. In dense aggregations the particles tended to assume an orderly arrangement in both parenchyma cells and sieve elements. Most of the sieve elements containing virus particles had mitochondria, plastids, endoplasmic reticulum, and plasma membrane normal for mature sieve elements. Some sieve elements, however, showed evidence of degeneration. Virus particles were present also in the pores of the sieve plates, the plasmodesmata connecting the sieve elements with parenchyma cells, and the plasmodesmata between parenchyma cells. The distribution of the virus particles in the phloem of *Beta* is compatible with the concept that plant viruses move through the phloem in the sieve tubes and that this movement is a passive transport by mass flow. The observations also indicate that the beet yellows virus moves from cell to cell and in the sieve tube in the form of complete particles, and that this movement may occur through sieve-plate pores in the sieve tube and through plasmodesmata elsewhere.

INTRODUCTION

Previously we reported on the occurrence, distribution, and arrangement of particles of the beet yellows virus in various leaf parenchyma cells of *Beta vulgaris* L. (sugar beet) (11,18). We were able to identify the inclusion bodies discernible with the light microscope as aggregates of particles typical of the beet yellows virus and to relate certain degenerative changes in the host cells to the presence of the virus particles in these cells.

The prevalence of the virus particles in parenchyma cells of the phloem agrees with the view that the beet yellows virus belongs to the category of viruses termed phloem-limited (8, 13, 14). The beet yellows virus, however, is not so strictly limited to the phloem as are certain other yellows viruses (curly top and aster yellows, in particular). In later stages of infection in leaves the beet yellows inclusions may be found not only in the phloem but throughout the mesophyll and in the epidermis. The characteristics of its transmission from plant to plant and translocation within the plant indicate that this virus occupies a position intermediate between those of the restricted yellows viruses and the nonrestricted mosaic viruses with regard to the degree of dependence on the phloem tissue.

Experiments on translocation show clearly, nevertheless, that the establishment of systemic infection by the beet yellows virus involves its transport through the phloem and that this trans-

port occurs at comparable velocities (e.g., 30 cm per hr) and in the same direction as that of the photosynthates (8). Studies of development of internal symptoms, on the other hand, have demonstrated that the inclusions and the degenerative changes observable with the light microscope appear in differentiating shoots or roots only after the first sieve tubes mature. Moreover, the first symptoms are localized in cells contiguous to the sieve elements (14). Thus, the mature sieve tubes appear to be the conduits through which the virus reaches the uninfected differentiating parts.

The concept that plant viruses move specifically in the sieve tubes has been held to be acceptable for some time (cf. 26), but the form of virus that may be moving in these conduits continues to be a matter of speculation. The idea about the form in which the virus passes from cell to cell is equally uncertain. Our present communication deals with observations indicating that the virus may be moving through sieve tubes and passing from cell to cell in the form of complete particles.

MATERIALS AND METHODS

The present paper describes observations made mainly with the electron microscope. The material and techniques used were the same as were reported previously (11, 18). Inoculated and noninoculated control seedlings of *Beta vulgaris* L. were kindly supplied by Dr. C. W. Bennett of the United States Agricultural Research Station at Salinas, California. *Myzus persicae* was used as the vector for inoculating the plants, and the virus was one of the virulent strains isolated by Dr. Bennett (isolate No. 5, Brawley strain). The leaf material containing small veins was killed and fixed in 6% phosphate-buffered glutaraldehyde for 2 hr, washed in buffer for 3 hr, and postfixed in phosphate-buffered 2% osmium tetroxide. The material was dehydrated through a series of acetone solutions and embedded in epoxy resin. Sections were cut with a diamond knife and stained with uranyl acetate and lead. Observations were made with a Siemens Elmiskop I.

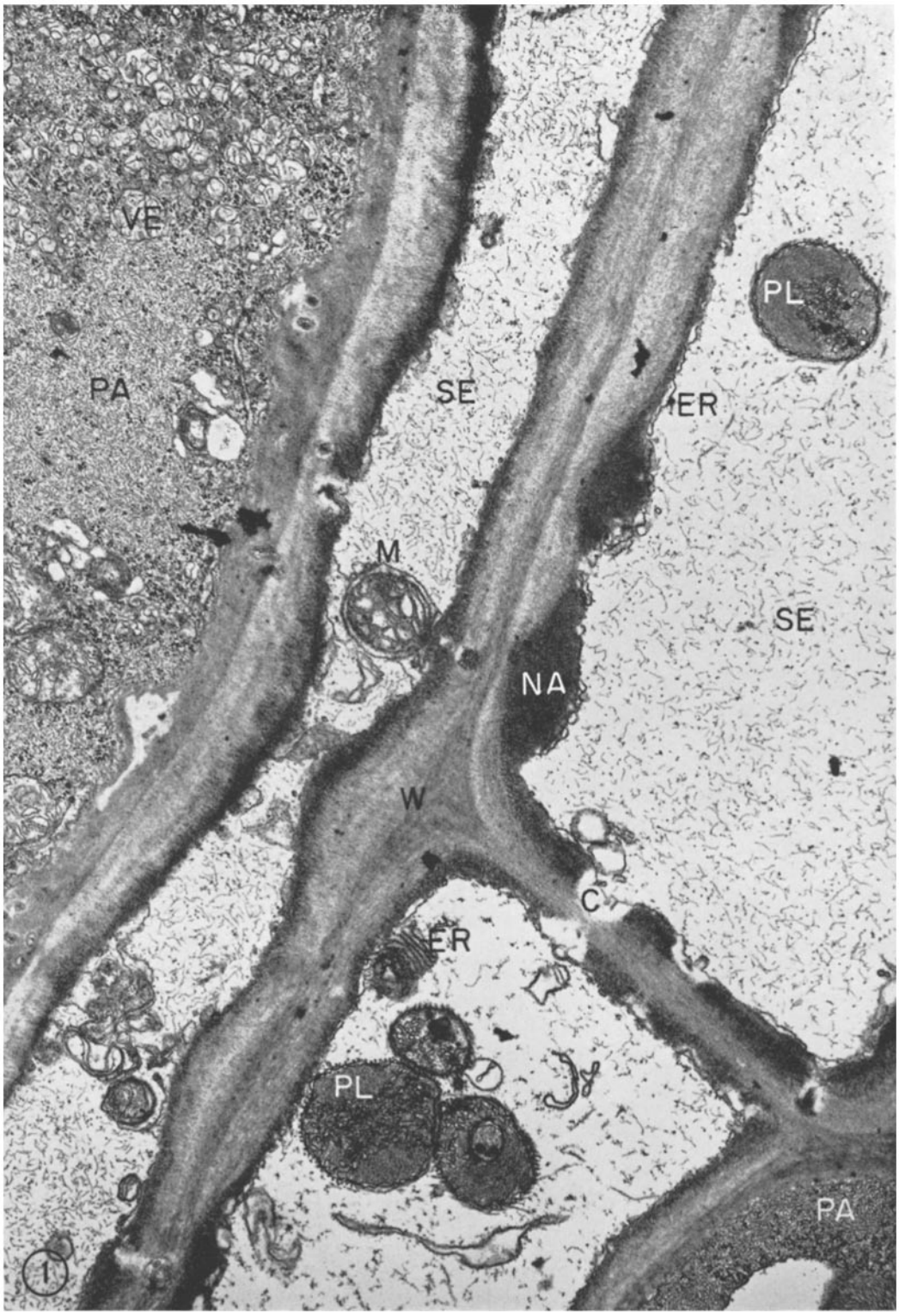
RESULTS

In leaves of the sugar beet infected with the beet yellows virus, particles of this virus were readily recognized in parenchyma cells of the veins and in mesophyll cells adjacent to the veins. With prolonged search, similar particles were found in sieve elements. These elements were mature, that is, they were enucleate and had no vacuole delimited by a tonoplast. Immature sieve elements that could be positively identified as such were not encountered.

Fig. 1 gives an example of sieve elements and parenchyma cells containing virus particles. Sections of three sieve elements (*SE*) with particles dispersed in their protoplasts are shown. Similar particles (*PA*) appear in larger and smaller aggregations in the adjacent parenchyma cell. The smaller aggregations are interspersed with cytoplasmic components such as mitochondria (below) and groups of vesicles (*VE*) typical of infected parenchyma cells (11). In the sieve elements, the particles occur together with plastids, mitochondria, and some endoplasmic reticulum (*ER*). The deeply stained part of the cell wall is probably the nacreous layer (*NA*; cf. 9). The section did not pass through the pores of the sieve plate but it exposed some of the callose (*C*) associated with two of the pores.

The dispersal of the virus particles throughout the protoplasts of the sieve elements results from the absence of vacuoles in these cells. In parenchyma cells, in which vacuoles are present and are delimited by tonoplasts, the virus particles are generally confined to the cytoplasm (very occasionally virus particles were recognized in vacuoles). Fig. 2 illustrates the contrast between the sieve element with dispersed particles (right) and the parenchyma cell with particles present in the

FIGURE 1 Longitudinal section through three sieve elements (*SE*) and associated parenchyma cells of small vein from *Beta vulgaris* leaf infected with beet yellows. Dense aggregations of virus particles (*PA*) in parenchyma cells; scattered particles in the sieve elements. The sieve plate not sectioned through pores but shows some callose (*C*). Darkly stained wall material (*NA*) is probably the nacreous layer. Groups of vesicles (*VE*), characteristic of beet yellows virus infection, in parenchyma cell to the left. Details: *C*, callose; *ER*, endoplasmic reticulum; *M*, mitochondrion; *NA*, nacreous wall layer; *PA*, beet yellows virus particles; *PL*, plastid; *SE*, sieve element; *VE*, vesicles associated with beet yellows infection; *W*, cell wall. × 16,000.


VE
PA
SE
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ER
M
SE
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C
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1

cytoplasm and absent in the three visible vacuoles (*VA*).

The particle content of the sieve elements varied greatly. In some, the particles were spread rather thinly (Figs. 1, 3, 5) or were discernible only in small numbers mainly at the sieve plate. In others, the particles were numerous (Figs. 2, 7, 8, 10), and in still others, they formed a solid packing (Fig. 11). In the dense aggregations, the particles in some regions showed an orderly spacing (Fig. 11, arrows).

The common components of mature sieve elements of *Beta* were present in the infected elements and were mostly free of any abnormalities. There were mitochondria (Figs. 1–3, 7) and plastids (Figs. 1, 3, 4, 7). The endoplasmic reticulum appeared in two forms, as stacks of membranes (Figs. 1, 3, 5) and as a reticulum closely associated with the plasma membrane (seen in sectional view as elongated vesicles in Figs. 2, 3, 5). The proteinaceous inclusion called slime (cf. 9, 15–17) was present likewise (Figs. 5, 7–9, *SL*). The plasma membrane was identifiable in many sections as a unit membrane (Fig. 3, 5, 8, 12, *PM*).

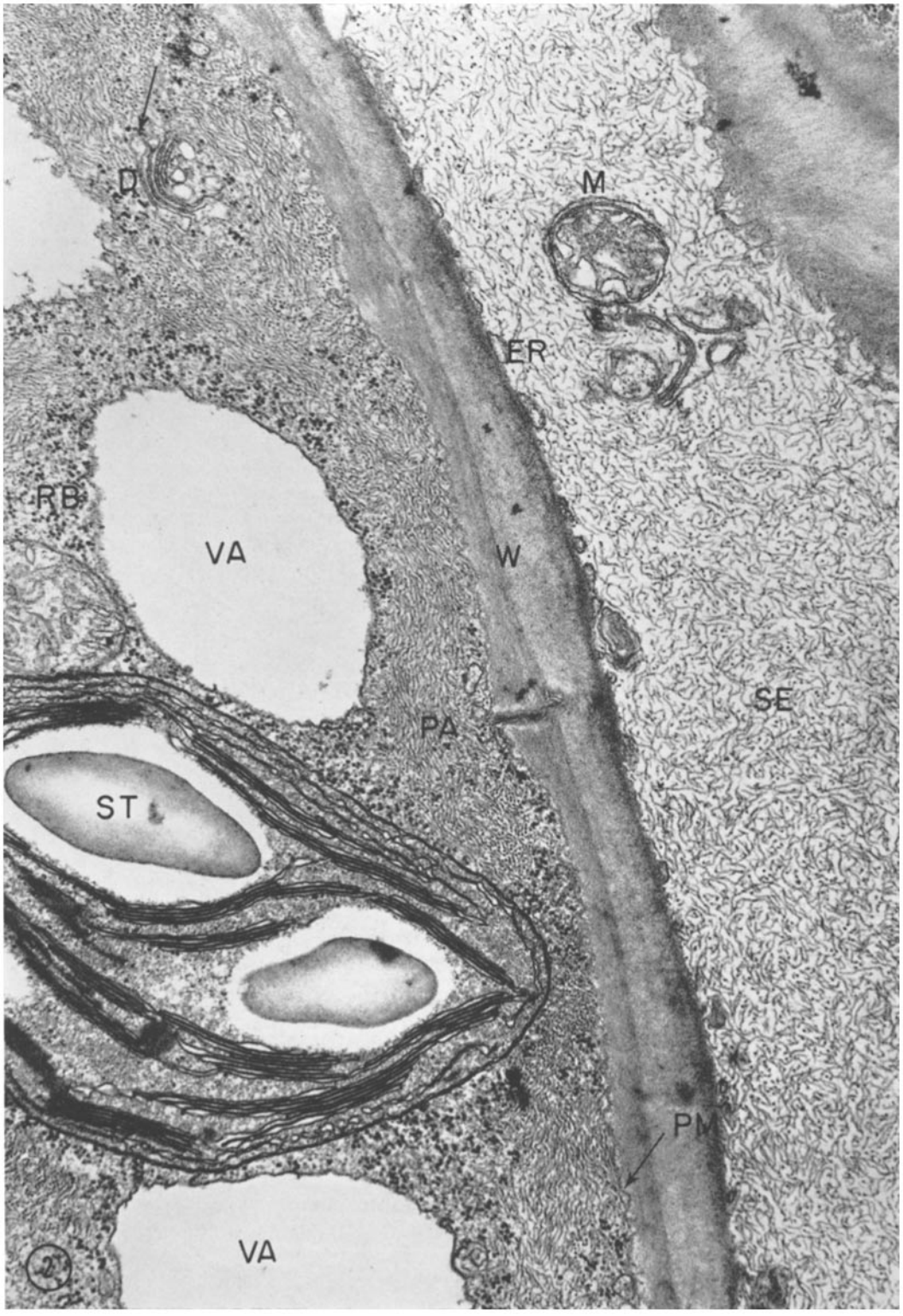
In some sieve elements, the organelles appeared in partially degenerated state; in others, the entire contents of the sieve element were disorganized and only the virus particles remained intact (Fig. 10). If a degenerated sieve element occurred next to one in which the normal cell components were still intact, large masses of callose were present on the common sieve area (Fig. 10). Significantly, no sieve element was found that contained the peculiar grouped vesicles which constitute such a prominent feature in the infected parenchyma cells (Figs. 1, 12, *VE*). We have previously suggested that these vesicles may be derived from the dictyosomes (11). In Fig. 2, the dictyosome at *D* is associated with vesicles one of which (arrow) resembles the vesicles in the parenchyma cell in Fig. 1. The cell in Fig. 2 appeared to be at an earlier stage of infection than that in Fig. 1 and vesicle formation may have just begun.

The apparent absence of vesicles in the infected sieve element protoplast cannot be explained without further developmental studies of the infection process. If the virus were to enter an immature sieve element, the protoplast of such an element would be expected to react as do those of the infected parenchyma cells. It would have the cytoplasmic components necessary for virus multiplication and the dictyosomes that could form the vesicles. Mature sieve elements, on the other hand, have no nuclei, no ribosomes, and no dictyosomes. The absence of vesicles in the infected sieve elements encountered in this study may mean that the virus did not multiply in these particular cells but entered them from the parenchyma cells.

The undeniable similarity between the particles in the sieve elements and those previously interpreted as beet yellows virus particles in parenchyma cells (11, 18) would seem to be a strong enough evidence that the particles in the sieve elements represent the virus. These particles show the same form and dimensions, and produce the same type of crystalline pattern when in dense aggregations (Fig. 11) as do the particles in the parenchyma cells. At the same time, the virus particles and their distribution in the sieve elements have a considerable similarity to fibrils of slime and their distribution. We were concerned about the possibility of confusing the two kinds of structures especially in view of the indication that the components of the slime are not solid fibrils but are tubular (Fig. 9). The virus particles also may appear tubular, for they show an electron-transparent center. However, the dimensions of the tubules of slime in the sieve elements of *Beta* are conspicuously larger than those of the particles of beet yellows virus in both longitudinal (Figs. 5, 8, *SL*) and cross-sectional (Fig. 9) views.

In our present preparations, the filaments asso-

FIGURE 2 Longitudinal section through one sieve element (*SE*) and a parenchyma cell of small vein from *Beta vulgaris* leaf infected with beet yellows. In parenchyma cell, virus particles (*PA*) in dense aggregations in cytoplasm and absent in vacuoles (*VA*). In sieve element (*SE*), particles scattered throughout cell. Part of plasmodesma opposite *PA* contains virus particles. Details: unlabeled arrow, dictyosome vesicle resembling those in parenchyma cell in Fig. 1; *D*, dictyosome; *ER*, endoplasmic reticulum; *M*, mitochondrion; *PA*, beet yellows virus particles; *PM*, plasma membrane; *RB*, ribosomes; *SE*, sieve element; *ST*, starch in chloroplast; *VA*, vacuole; *W*, cell wall. × 33,000.


D
M
ER
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VA
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SE
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VA

ciated with the sieve element plastids in *Beta* (cf. 16) proved also to be tubular in structure (Fig. 4) and highly similar to the tubules of slime in their dimensions. When exposed longitudinally in a tangential cut through a plastid (Fig. 3, *PL*) they could be easily mistaken for the tubules of slime, but their confusion with virus particles is unlikely because of discrepancy in dimensions.

The previous report on the absence of inclusion bodies of the beet yellows virus in sieve elements (13), as seen with the light microscope, and the present recognition of virus particles in these elements made it necessary to reexamine the beet yellows infected material with the light microscope. As before, no inclusion bodies were seen in the sieve elements, but in two instances abnormalities were detected in sieve elements of minor veins. In one, a sieve element was filled with darkly stained material which showed no structural details at the resolution of the light microscope, in the other, fibrils resembling those previously seen in parenchyma cells (cf. 18) were faintly discernible in a sieve element.

Virus particles were recognized not only in the lumina of the sieve elements but also in the pores of the sieve plates (Figs. 3, 5, 11) and in the plasmodesmata connecting the sieve elements with the contiguous parenchyma cells (Figs. 12, 13; no reference is made here to companion cells because in the minor veins the distinction between companion cells and parenchyma cells is highly uncertain). In sieve plates in which only the margins of the pores appeared in the sections the virus particles were seen inserted into the pores (Figs. 3, 5). The particles were in a nearly parallel alignment and, therefore, transverse sections through the pores also cut the virus particles crosswise (Fig. 6). Pores sectioned through their entire depth indicated that the particles extended from cell to cell (Fig. 11). The pores were lined with callose (*C* in Figs. 3, 5, 8, 11). The sieve plates containing virus particles in the pores have a remarkable resemblance to those with pores filled with slime as are so often seen in noninfected sieve elements (cf. 17). The plasma membrane could be recognized in some sections to line the pores (Fig. 3, *PM*, shown only part way into the pore).

The plasmodesmata between a sieve element and a parenchyma cell in a minor vein are branched, as they typically are in walls between sieve elements and companion cells. The branching occurs on the side of the parenchyma cell, but on the sieve element side there is only one pore. Fig. 12 shows part of a branched plasmodesma with two of several branches in view. In Fig. 13 several branches—all probably pertaining to one plasmodesma—appear in a transverse section. In Fig. 12, virus particles extend from the sieve element through the plasmodesmatal branches into the parenchyma cell, and in Fig. 13 are seen in cross-sections in the pores. The plasma membrane (*PM*) is discernible in some of the pores.

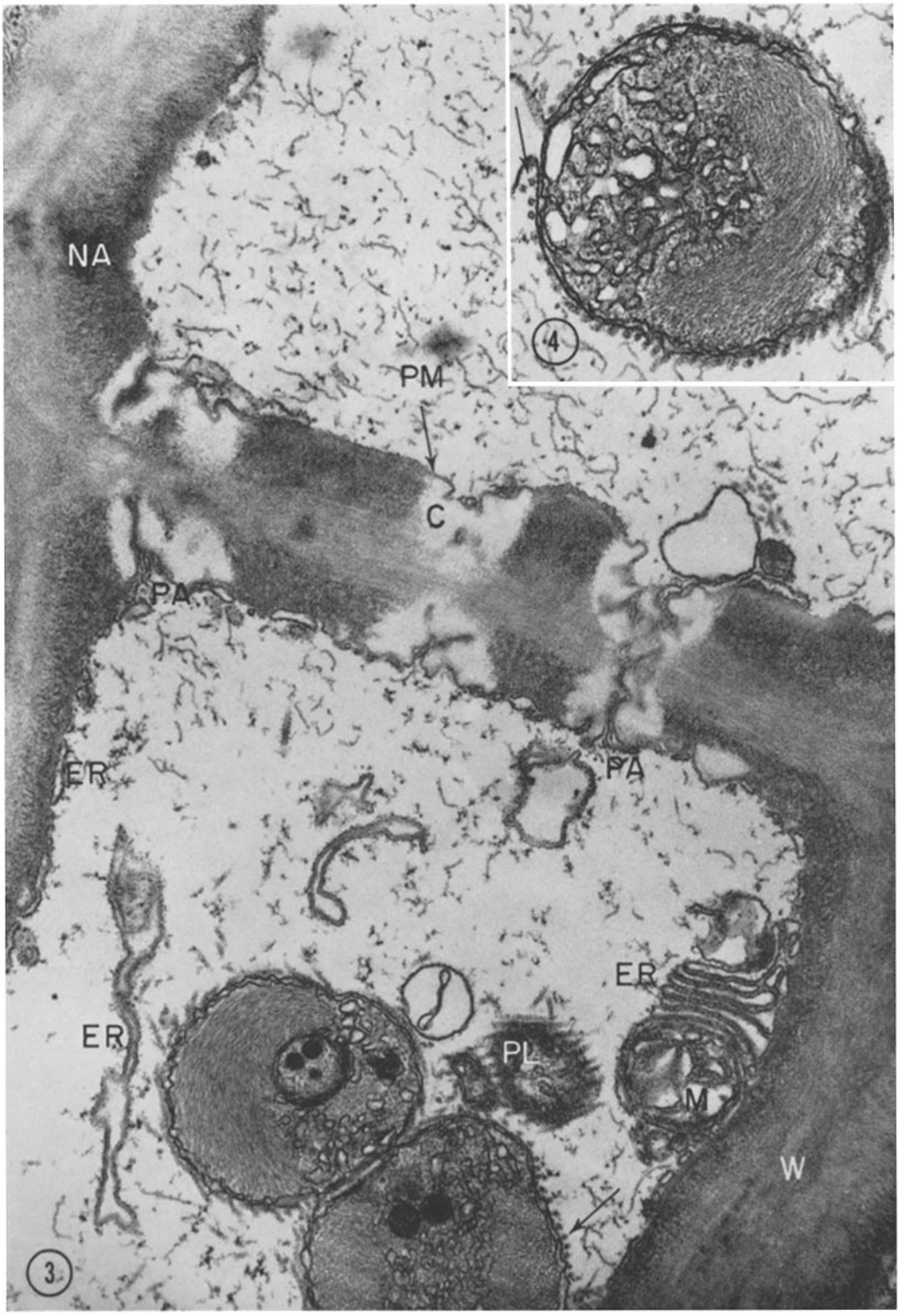
Virus particles were detected also in plasmodesmata between parenchyma cells. As is evident from Figs. 14 and 15, the plasmodesmata in parenchyma cells are large enough to accomodate a number of virus particles, and these occur in parallel alignment as they do in the larger pores in the sieve element walls in Figs. 11–13. Fig. 15 shows the plasma membrane (*PM* at arrow) bending into the pore of the plasmodesma.

DISCUSSION

The recognition of particles of the beet yellows virus in sieve elements supports the assumption that this virus is distributed in the plant through the phloem tissue. The concept of virus transloca-

FIGURE 3 Longitudinal section through sieve plate and two sieve elements of a small vein from *Beta vulgaris* leaf infected with beet yellows. A few scattered virus particles in the lumina of sieve elements, and some (at *PA*) are visible at entrance to sieve-plate pores (these are not cut through entire depth). The plastids bear fibrils which are seen in longitudinal section at *PL*. These fibrils appear to be tubular, for they have an electron-transparent core (unlabeled arrow; see also Fig. 4). Details: *C*, callose associated with sieve-plate pores; *ER*, endoplasmic reticulum; *M*, mitochondrion; *NA*, nacreous wall layer; *PA*, virus particles; *PM*, plasma membrane; *W*, cell wall. × 41,000.

FIGURE 4 Similar to Fig. 3. Enlarged view of sieve-element plastid to show tubular structure of filaments attached to plastid (arrow). × 57,000.


NA
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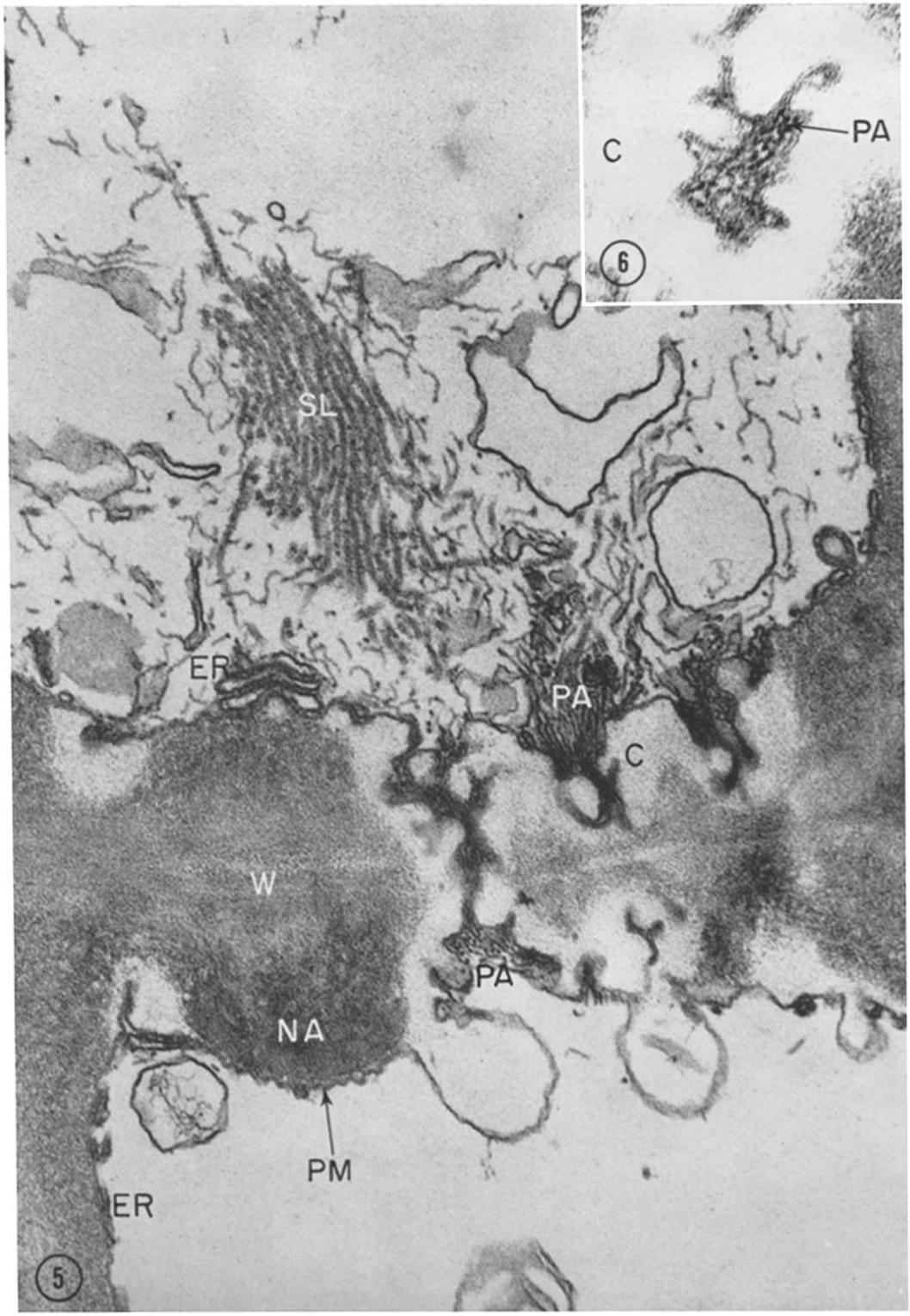


FIGURE 5 Longitudinal section through sieve plate of sieve tube of small vein from *Beta vulgaris* leaf infected with beet yellows. The sieve plate pores are not cut through entire depth. Virus particles (*PA*) are inserted into pores. They differ in size from fibrils of sieve-tube slime (*SL*). Plasma membrane (*PM*) partly lifted off from sieve plate in callosed region. Details: *C*, callose; *ER*, endoplasmic reticulum; *NA*, nacreous wall layer; *PA*, beet yellows virus particles; *SL*, slime; *W*, cell wall. × 62,000.

FIGURE 6 Material similar to that in Fig. 5. Transverse section of a sieve-plate pore near its entrance. Some virus particles (*PA*) are seen in transection. Callose (*C*) surrounds the pore. Compare with Fig. 5. × 114,000.

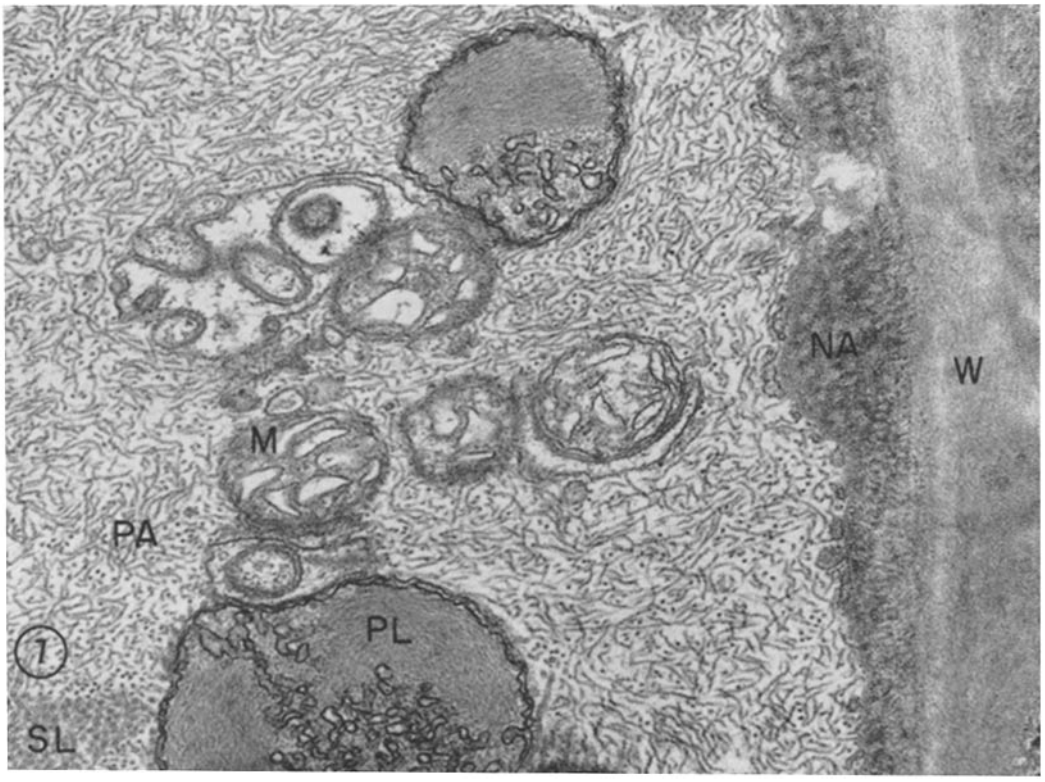


FIGURE 7 Longitudinal section through sieve element of small vein in leaf from *Beta vulgaris* infected with beet yellows. Mitochondria (*M*) and plastids (*PL*) are surrounded by virus particles (*PA*). At *SL*, slime fibrils in transection have larger cross-sectional diameter than virus particles next to them. Details: *M*, mitochondrion; *NA*, nacreous wall layer; *PA*, beet yellows virus particles; *PL*, plastid; *SL*, slime; *W*, cell wall. × 41,000.

tion in the phloem was formulated when it became apparent that the movement of viruses and that of organic materials had certain similarities. In some experiments, for example, mosaic viruses that were introduced into a mature leaf in median position on a tomato plant moved first to the root then upward toward the growing shoot parts (10, 25). The presence of developing fruit trusses modified the direction of movement, in that the downward movement was combined with an upward movement directed toward the trusses. This pattern of virus translocation parallels that obtained with ^{14}C-labeled sugar (29, pp. 509–510). The linear velocities recorded in some studies on virus movement—e.g., curly top virus, 75–150 cm per hr; maize streak virus, 20 cm per hr—are also similar to those established for the transport of organic solutes in the phloem. Bennett's (4–6) studies on effects of shading, defoliation, ringing, and grafting on the spread of different viruses in plants likewise have clearly related virus movement to the phloem tissue and to carbohydrate translocation.

The anatomic observations that in the phloem-related viruses the initial symptoms occur in the phloem tissue (cf. 15) agree well with the concept of virus movement in the phloem. Moreover, the dependence of internal symptom development on the presence of mature sieve elements points directly to these elements as the source of the infective agent that brings about the systemic spread of the virus through the plant. The results of our study on the beet yellows virus indicate that this infective agent is probably a complete virus particle.

The repeated observations that, during the initial postinoculation spread of the virus through the phloem, noninfected regions of the stem occur

between those that are infected (3, 10, 20–22, 24, 25, 31) are in harmony with the concept that complete virus particles are translocated. Most of the authors interpret the discontinuous infection as proof of a passive flow of the virus in the sieve tube. The translocation stream carries the virus along with the solute and, as the amount of virus is small in the initial stages of infection, virus particles fail to be retained in some parts of the path. Possibly the egress of the particles from the sieve element into the adjacent parenchyma cells depends on the state of the local equilibrium between the sieve element and the associated parenchyma cells with regard to the relative sugar concentration. As Weatherley (30) suggests, sugar probably passes back and forth between the sieve element and the parenchyma cells in accordance with the relative levels of their sugar content. The virus particles could leave the sieve element in which sugar was moving toward the parenchyma cell and they probably would continue on their path through the sieve tube at levels at which no sugar was leaving the conduit.

The occurrence of virus particles of beet yellows in the sieve elements of the sugar beet raises the question of origin of these particles. We do not know whether this particular complement of virus was merely translocated in the sieve elements or whether it had been formed in these cells from some few particles initially introduced by the insect vector or by migration from a parenchyma cell. The absence of a nucleus and the scarcity of ribosomes in mature sieve elements make it seem unlikely that the virus could replicate itself in such cells. However, the virus could enter an immature sieve element in which it would find all the entities required for its multiplication.

If the virus were found to be multiplying in immature sieve elements, the next question to be asked is whether the infected sieve element would continue on its normal course of differentiation and become enucleate and devoid of a tonoplast, or whether it would degenerate. The sieve elements which were found containing virus particles in our material were apparently mature, for neither tonoplast nor nucleus was identifiable in them. In most views, the plastids and mitochondria had a normal appearance, but they were obviously degenerated in some cells. Moreover, some sieve elements were completely degenerated and were walled off by heavy deposits of callose from the better preserved ones. If the degenerative changes were induced by the virus, the variations in intensity of these changes may have resulted from differences in timing of the infection with regard to the stage of differentiation of the cells. Severe degeneration may mean an early infection associated with virus multiplication in the cells.

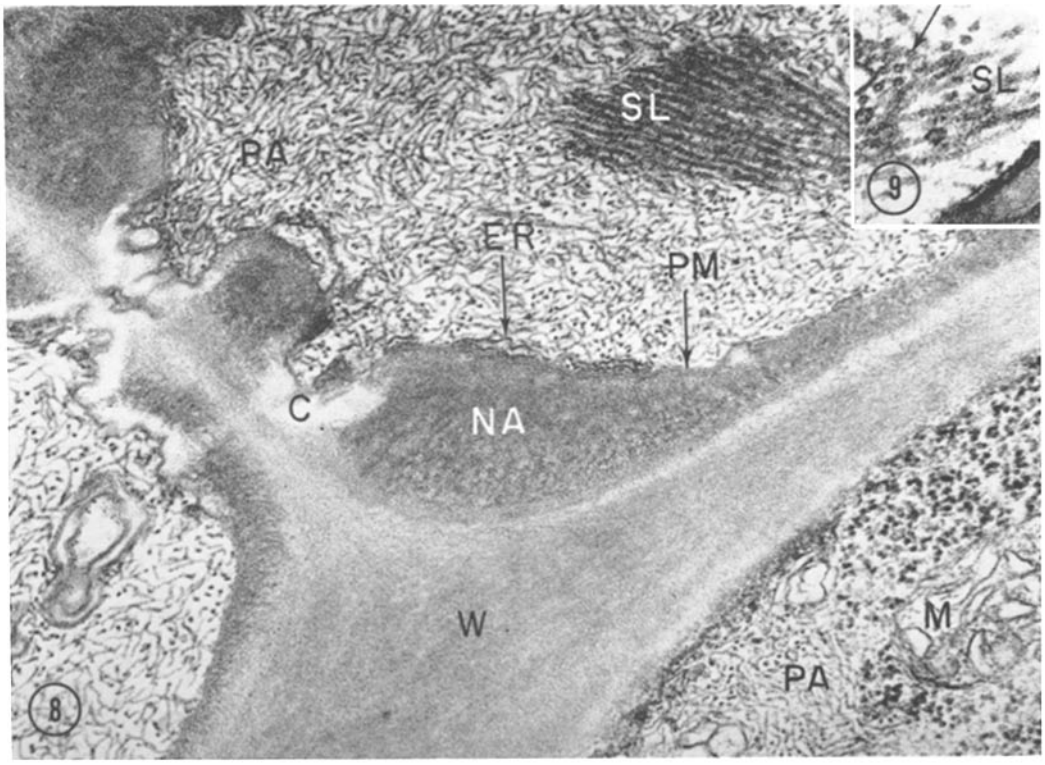
It seems significant that no sieve element with virus particles in its protoplast contained the masses of vesicles which are so characteristic of infected parenchyma cells. If these vesicles are derived from dictyosomes, as suggested by Cronshaw et al. (11), then their absence in the sieve elements would support the view that such elements were mature when the virus entered them, for dictyosomes disappear during the differentiation of sieve elements.

The amounts of virus in the sieve elements were highly variable. If the virus did not multiply in

FIGURE 8 Longitudinal section through two sieve elements and a parenchyma cell (corner at right) of small vein from *Beta vulgaris* leaf infected with beet yellows. Sieve plate with callose (*C*) between the sieve elements. Virus particles (*PA*) have much smaller dimensions than the fibrils of slime (*SL*), and their transectional areas are smaller than sections of ribosomes. Details: *C*, callose; *ER*, endoplasmic reticulum; *M*, mitochondrion; *NA*, nacreous wall layer; *PA*, beet yellows virus particles; *PM*, plasma membran *SL*, slime; *W*, cell wall. × 43,000.

FIGURE 9 Material similar to that in Fig. 8. Slime fibrils (*SL*), some in transection (arrow), showing electron-transparent core. × 66,000.

FIGURE 10 Longitudinal section of two laterally contiguous sieve elements of small vein from *Beta vulgaris* leaf infected with beet yellows. Wall (*W*) between the two elements bears massive callose (*C*) on a lateral sieve area. Dense aggregation of virus particles (*PA*) in upper sieve element. Completely degenerated protoplast with virus particles (*PA*) in lower sieve element. Particles in orderly arrangement at unlabeled arrow. × 41,000.


SL
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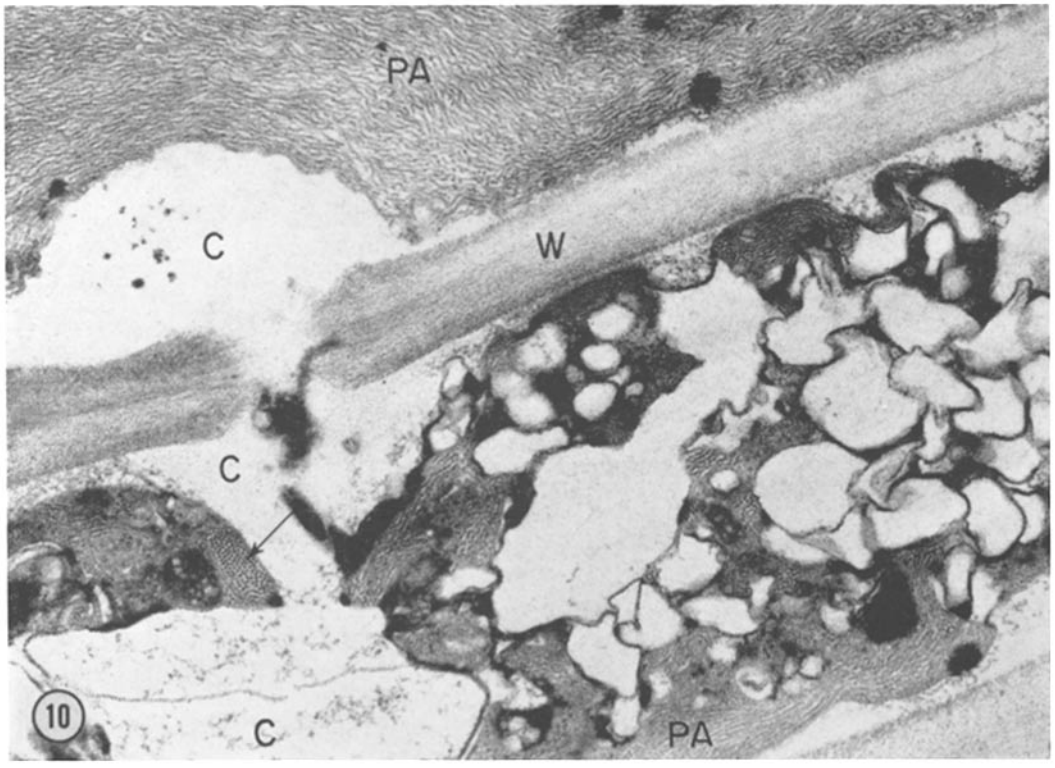
PA
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the sieve elements, the dense accumulations seen in some sieve elements must have been derived by migration from adjacent parenchyma cells. The leaves used in our study were younger than the leaf through which the plant was inoculated, and the virus in these leaves was a product of the systemic infection. Moreover, one would hardly expect that the vector could introduce large amounts of virus.

It is important to bear in mind that beet yellows virus particles were present in some sieve elements but not in others. In the light-microscope survey for evidence of virus in sieve elements, no organized inclusion bodies were seen in these cells, but some fibrous material was detected in one sieve element and dark amorphous material in another. Both sieve elements were located in minor veins. In a similar survey for inclusion bodies in tobacco infected with tobacco mosaic virus (TMV), striated material (crystalline TMV) was found in all kinds of cells including immature sieve elements, but their distribution was not general (12). They were most common in severely affected regions within the minor veins that were embedded in the mesophyll. It is possible that virus is more likely to occur in the sieve elements of the minor veins—that is, at the site of initial entry of the photosynthate into the conducting system—than in the larger veins and petioles in which the solute is translocated on its way out of the leaf.

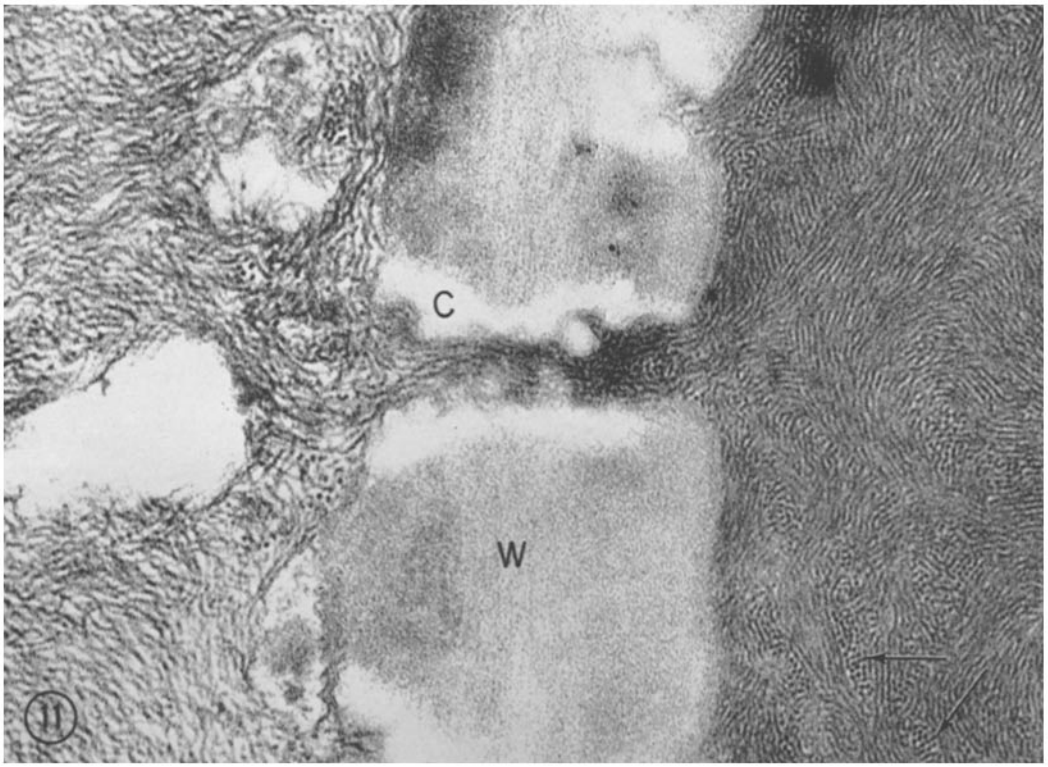
References to virus inclusions in sieve elements are few. Smith and McWhorter (28) found alveolate inclusions, resembling the X-bodies in TMV infections, in immature sieve elements of *Vicia faba* infected with a strain of tomato ringspot virus. The authors were aware of the possibility of confusion of the virus inclusions with slime bodies characteristic of immature sieve elements and indicated the difference between the two structures. Presence of virus in sieve elements may be deduced from the observations of Worley as reported by Schneider (26). By using a fluorescent antibody, Worley identified the antigen of southern bean mosaic virus in the sieve tubes of Black Valentine bean plants systemically infected with the virus. The occurrence of virus in sieve elements of systemically infected plants agrees with the concept that insect vectors feed on the contents of sieve elements and pick up the virus with the food.

A particularly significant aspect of our study is the recognition of virus particles in the sieve plate pores, the plasmodesmata between sieve elements and parenchyma cells, and the plasmodesmata between contiguous parenchyma cells. Questions about the form of virus moving from cell to cell and the channels utilized in this movement are frequently raised in discussions of virus translocation in the plant (cf. 1, 23, 26, 27). As Bawden (1, pp. 242) has pointed out, it can be considered established that, soon after having been inoculated into a plant cell, viruses change their form and become unstable. They probably separate into nucleic acid and protein (2, 23). A related concept is that the intercellular movement of a virus occurs in the form other than a complete particle (26, 27). Beet yellows virus appears bo be moving from cell to cell as a complete particle.

According to a generally accepted view, viruses move from cell to cell through plasmodesmata (7, 26, 27). This assumption was questioned by Kassanis et al. (19) who recorded movement of TMV in tobacco tissue culture without finding any plasmodesmata between the cells. The size relation between virus particles and plasmodesmatal pores has also been discussed, but electron microscopy has shown that plasmodesmata are

FIGURE 11 Longitudinal section through two sieve elements of small vein from *Beta vulgaris* leaf infected with beet yellows. Wall (*W*) between the two elements exhibits one sieve-plate pore lined with callose (*C*) and containing virus particles. Masses of virus particles in both cells but particularly abundant in cell to the right. At unlabeled arrows the particles are in orderly arrangement. × 66,000.

FIGURE 12 Longitudinal section of parenchyma cell (above) and sieve element (*SE*, below) of small vein from *Beta vulgaris* leaf infected with beet yellows. Wall (*W*) between the two cells bears a branched plasmodesma containing virus particles (*PA*). Vesicles (*VE*) and ribosomes (*RB*) present in parenchyma cell, absent in sieve element. Details: *PA*, beet yellows virus particles; *PM*, plasma membrane; *RB*, ribosomes; *SE*, sieve element; *VE*, vesicles associated with beet yellows infection; *W*, cell wall. × 78,000.


C
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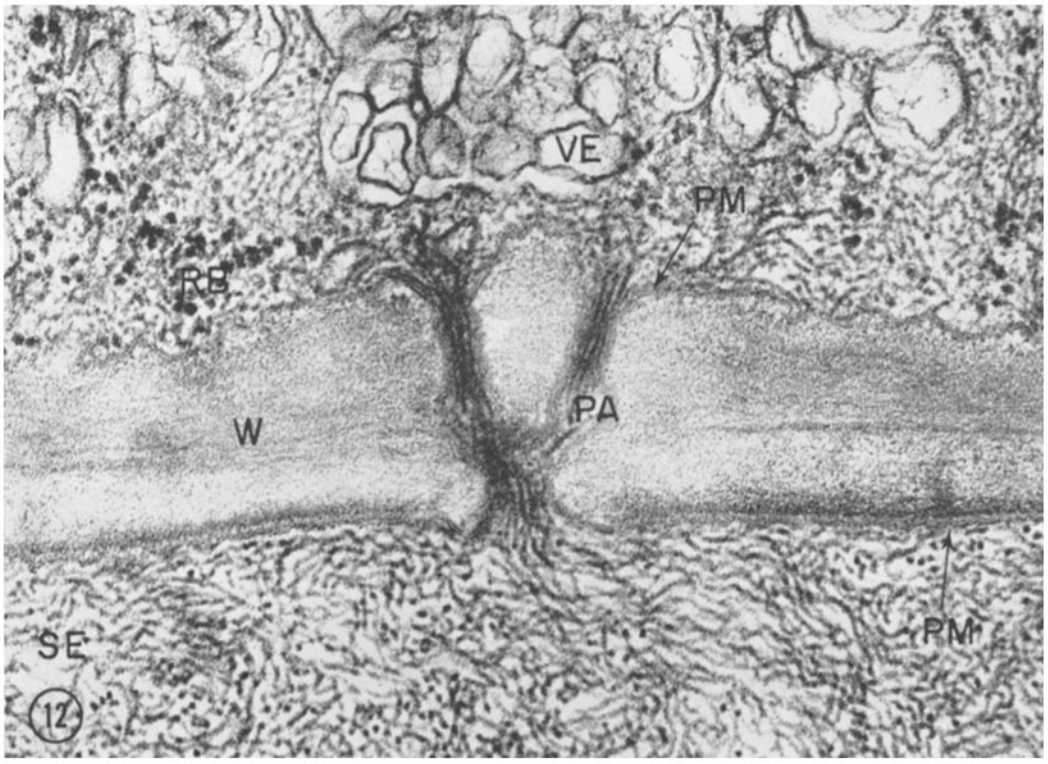
11


VE
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12

wide enough to permit passage of virus particles (cf. 26). It could also appear to constitute a problem that virus particles having the shape of rods or filaments would have to become properly oriented in order to pass through the plasmodesmata.

Our study shows that beet yellows virus particles occur in plasmodesmata and that the latter are large enough to accommodate a number of particles at one time. Their parallel arrangement in the plasmodesmata suggests that the particles become oriented as they enter a plasmodesma.

Conceivably, viruses could move through the wall directly. In the preceding paper on the beet yellows virus (11) a possibility of a close association of the virus with the cell surface was suggested. One could visualize viruses causing a destruction of the plasma membrane and then passing through the wall as complete or incomplete particles.

The results of the present study are obviously significant with reference to the problem of interpreting translocation in the phloem in relation to sieve tube structure. In view of the recent discussion of this problem by one of us (17), a speculation on the relation of virus movement to the translocation in the sieve tube should suffice here.

Regardless of whether the virus recognized in the sieve elements multiplied in situ or entered these elements from parenchyma cells, one can assume that the virus moves in both directions between the sieve tube and the associated parenchyma cells. At one site, the virus enters the conduit from a parenchyma cell in which it was multiplying; at another, it egresses from the sieve element to start a new infection in a parenchyma cell still free of virus.

The movement of virus particles between the sieve elements and the parenchyma cells can be visualized as determined by the source-and-sink relation between the conduit and the associated cells. Release of sugar into the sieve elements—presumably a dominant phenomenon in the minor veins in which also the virus appears to be so abundant in the sieve elements—is followed by a flow of water into the conduit. Virus particles are carried along. According to the pressure-flow concept of translocation, the reverse movement of sugar, from sieve elements to parenchyma cells (when these cells function as a sink), involves release of water from the conduit. Thus, the movement of the virus particles from the sieve elements to a parenchyma cell could rest on the same mechanism as the movement in the reverse direction.

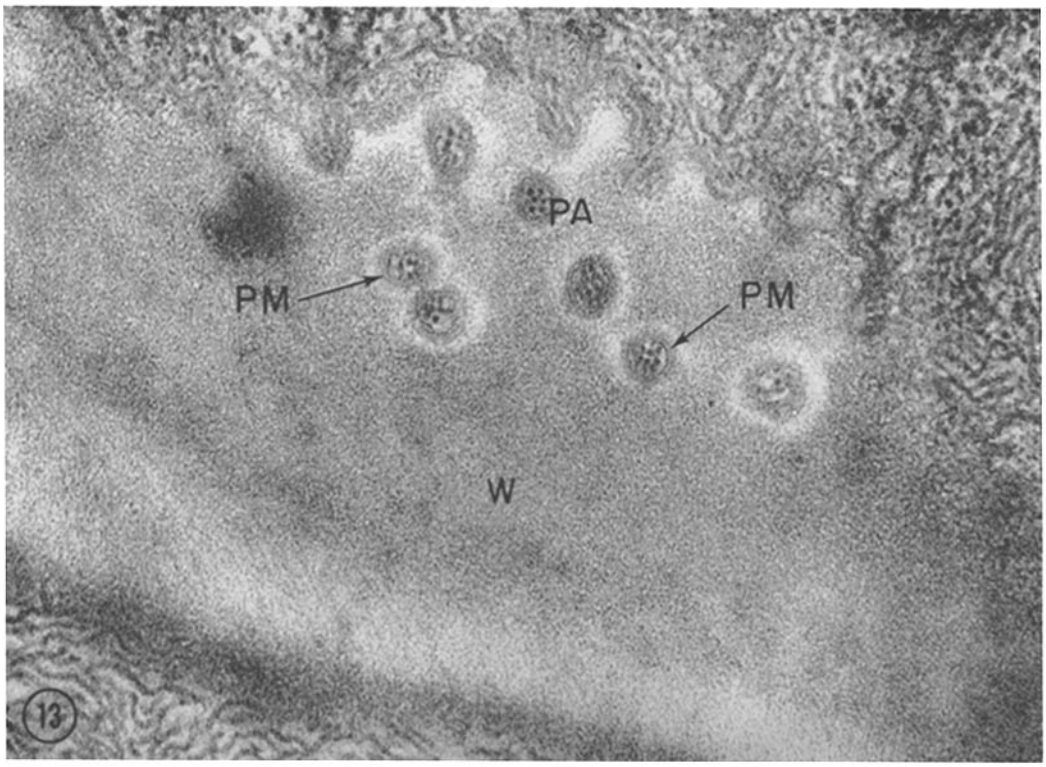
The distribution of the virus particles in the sieve element is as it would be expected to be in a cell lacking a vacuole delimited by a tonoplast. The particles are spread throughout the cell. This distribution in conjunction with the occurrence of particles in the pores of the sieve plates is compatible with the idea that the particles are passively carried through the conduit with the translocation stream.

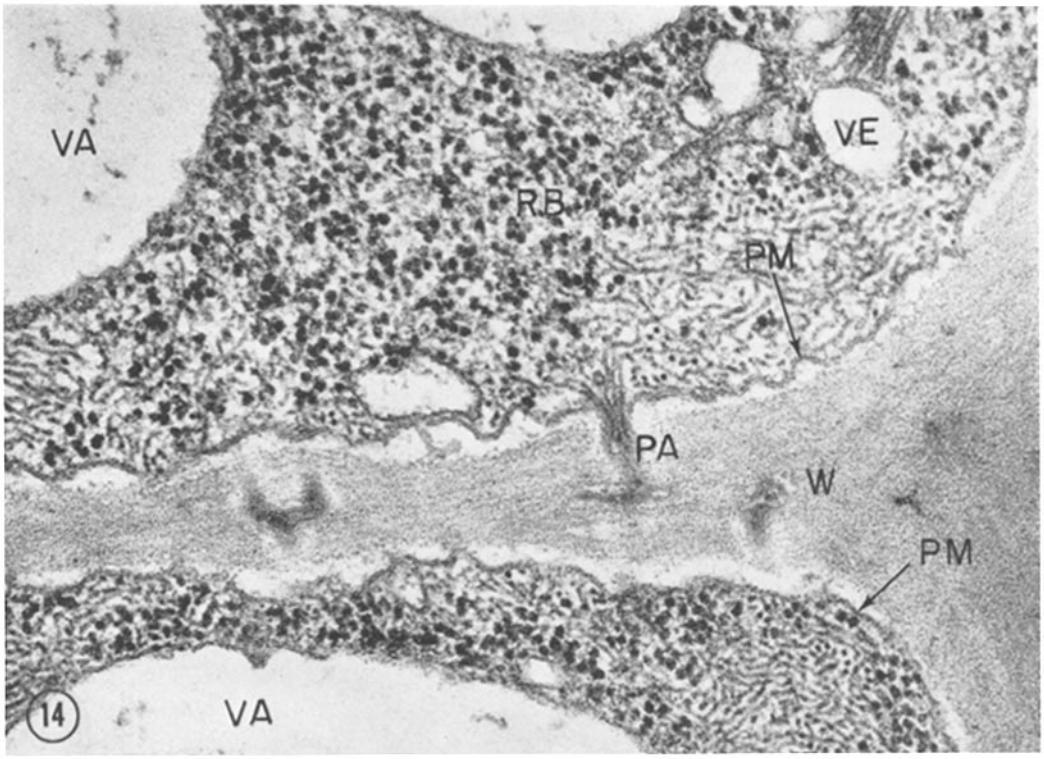
One should not assume, however, that all of the virus aggregations discovered in the sieve elements were in the process of being transported in the sieve tube. Some aggregations were so dense that they appeared to plug the lumen and the pores. Conceivably, virus particles could accumulate in such amounts that normal movement would be interrupted.

The movement of virus particles between parenchyma cells through plasmodesmata constitutes a distinct aspect of virus translocation. We have no clear conception of the mechanism of interchange of materials across plasmodesmata in a parenchyma tissue. Possibly this interchange depends on pressure gradients similar in kind, but perhaps not in degree, to those occurring

FIGURE 13 Oblique section through wall (*W*) between parenchyma cell (above) and sieve element (below) of small vein from *Beta vulgaris* leaf infected with beet yellows. The branches of plasmodesma connecting the two cells are cut transversely. Each contains virus particles (*PA*) and is lined with plasma membrane (*PM*). × 83,000.

FIGURE 14 Longitudinal section of two parenchyma cells of small vein from *Beta vulgaris* leaf infected with beet yellows. The wall (*W*) between the two cells shows part of a plasmodesma containing virus particles (*PA*). Ribosomes (*RB*) are larger than the transectional views of virus particles. No virus particles are present in vacuoles (*VA*). Details: *PA*, beet yellows virus particles; *PM*, plasma membrane; *RB*, ribosomes; *VA*, vacuole; *VE*, dictyosome vesicle. × 69,000.


PA
PM
PM
W
13


VA
VE
RB
PM
PA
W
PM
14
VA

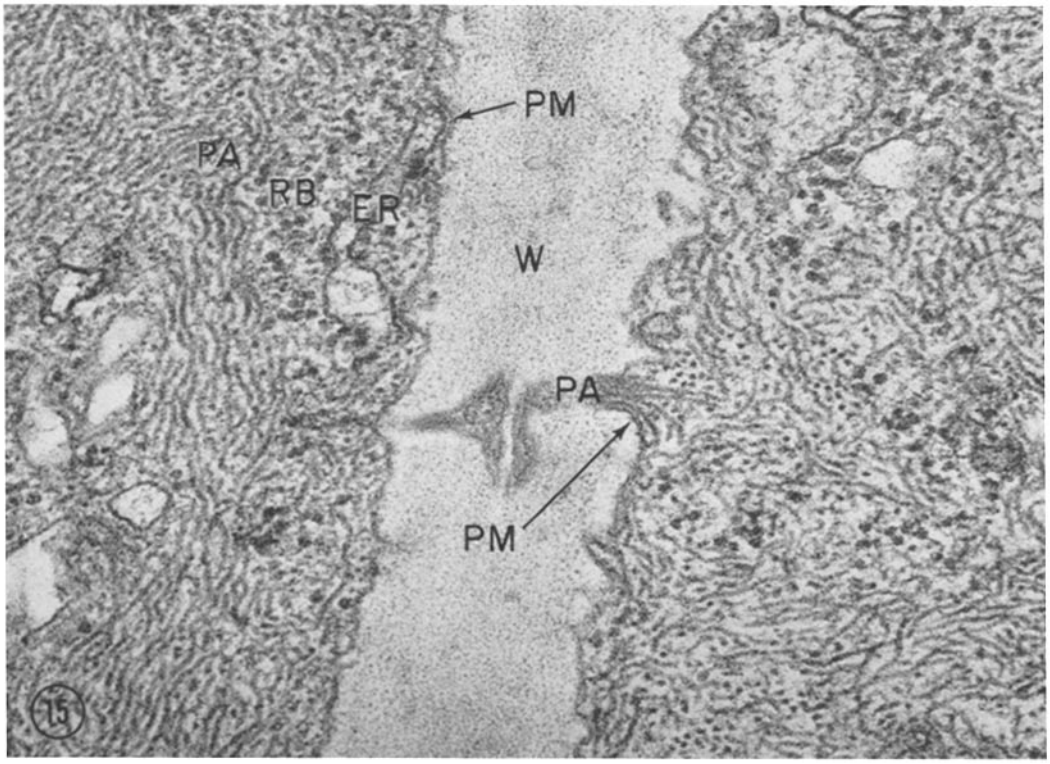


FIGURE 15 Longitudinal section of two parenchyma cells of small vein from *Beta vulgaris* leaf infected with beet yellows. The wall (*W*) between the two parenchyma cells shows part of a plasmodesma (with part of a median nodule) containing virus particles (*PA*). The plasma membrane (*PM*) is visible at entrance into plasmodesmatal pore. Both cells contain numerous virus particles (*PA*) and some ribosomes (*RB*). Details: *ER*, endoplasmic reticulum; *PA*, particles of beet yellows virus; *PM*, plasma membrane; *RB*, ribosomes; *W*, cell wall. × 88,000.

between the sieve elements and the parenchyma cells. One could think also of a propagation of the cytoplasmic streaming from one cell to another. A complicating factor in this respect is the presence of a core within the plasmodesma that restricts the lumen of the canal to a considerable extent. (In our material no core was visible in plasmodesmata that contained virus particles.) Theoretically, this core would seem to present an impediment to the passage of virus particles, but it is possible that virus infection modifies the structure of the plasmodesma or that the particles mechanically push the obstruction aside. Whatever the relation may prove to be, the discovery of the beet yellows virus particles in the plasmodesmata in itself considerably clarifies our thinking about the progress of the virus in the plant.

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