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Multinucleate Transfer Cells Induced in Coleus Roots by the Root-Knot Nematode, *Meloidogyne arenaria*

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With 23 Figures

Summary

The occurrence and position of wall protuberances in giant cells induced in coleus roots by the root-knot nematode *Meloidogyne arenaria* is described, and the structure and function of giant cells is compared with that of syncytia induced by cyst-nematodes.
Extensive protuberance development occurs on walls of giant cells adjacent to xylem vessels. Protuberances are less well developed next to sieve elements, and almost absent next to parenchyma cells. On walls between giant cells they occur on both sides or only one side. The formation of protuberances indicates that giant cells are multinucleate transfer cells. The position of protuberances marks the wall area where solutes enter the cell. Solutes are obtained from xylem and phloem elements, and the position of protuberances at the junction between giant cells and vascular elements indicates an extensive flow of solutes along cell walls. The observations support the hypothesis that wall protuberances form as a result of selective solute flow across the plasmalemma.
No cell wall dissolution was observed, although wall gaps may occur between giant cells as a result of breakage during rapid cell expansion.

1. Introduction

Larvae of root-knot nematodes invade susceptible host plant roots and induce the formation of giant cells at the site where they become sedentary (CHRISTIE 1936, DROPKIN and NELSON 1960, DROPKIN 1969). The larvae obtain nutrients from the giant cells, which enlarge as the nematodes grow. Although BIRD (1961), OWENS and SPECHT (1964), and others have reported that considerable cell wall breakdown occurs in the formation of giant cells HUANG and MAGGENTI (1969 a and b) have shown that giant cells induced by *Meloidogyne javanica* form by repeated mitosis accompanied by cell expansion but without subsequent cytokinesis. Irregular wall ingrowths in giant cells have been reported (BIRD 1961, HUANG and MAGGENTI 1969 a and b, PAULSON and WEBSTER 1970), but their localization has not been described. We have confirmed (JONES and NORTHCOTE 1972) that in potato roots the potato cyst-

nematode (*Heterodera rostochiensis*) induces syncytia that are formed by cell wall dissolution rather than by repeated endomitosis and that they have similar irregular ingrowths. We described the localization of the wall ingrowths and suggested that syncytia induced by *H. rostochiensis* are a form of transfer cell.

The present work describes the location of wall ingrowths in mature giant cells induced in coleus roots by *Meloidogyne arenaria* and compares the giant cells induced by this nematode with syncytia induced by *H. rostochiensis* with regard to their formation and function.

2. Material and Methods

Coleus (*Coleus blumei* Benth.) plants were infected with larvae of *M. arenaria*. Root galls were harvested at different stages of development, fixed with 5% glutaraldehyde, post-fixed with 1% osmium tetroxide, dehydrated with cellosolve, and then embedded in araldite (JONES and NORTHCOTE 1972). Sections 0.5–1.5 μ thick were cut with glass knives, floated onto 15% ethanol and transferred with a loop of platinum wire onto a glass slide. These sections were stained with 1% methylene blue and 1% Azur II in 1% borax for 5 minutes at 100 °C, and examined by a Zeiss Ultraphot II. Sections (50–100 nm) for examination by electron microscopy were cut with glass knives, floated onto 15% ethanol, and collected on uncoated 400 mesh electron microscopy grids. For study by light and electron microscopy sections were cut with a Sorvall Porter-Blum MT2 ultramicrotome. The electron microscope sections were stained in saturated uranyl acetate in 50% ethanol followed by alkaline lead (MILLONIG 1961) and examined by an AEI EM6B at 60 kV.

3. Results

The nematode larva and the giant cells were typically surrounded by thin walled, vacuolate cells of the gall. At the distal and proximal ends of the giant cell complex (relative to the root tip) the cells were surrounded by xylem vessels (Fig. 1), but where the giant cells were wider the xylem vessels were displaced to one or more discrete regions (Fig. 2). Frequently xylem elements were present in the deep indentations between the giant cells and

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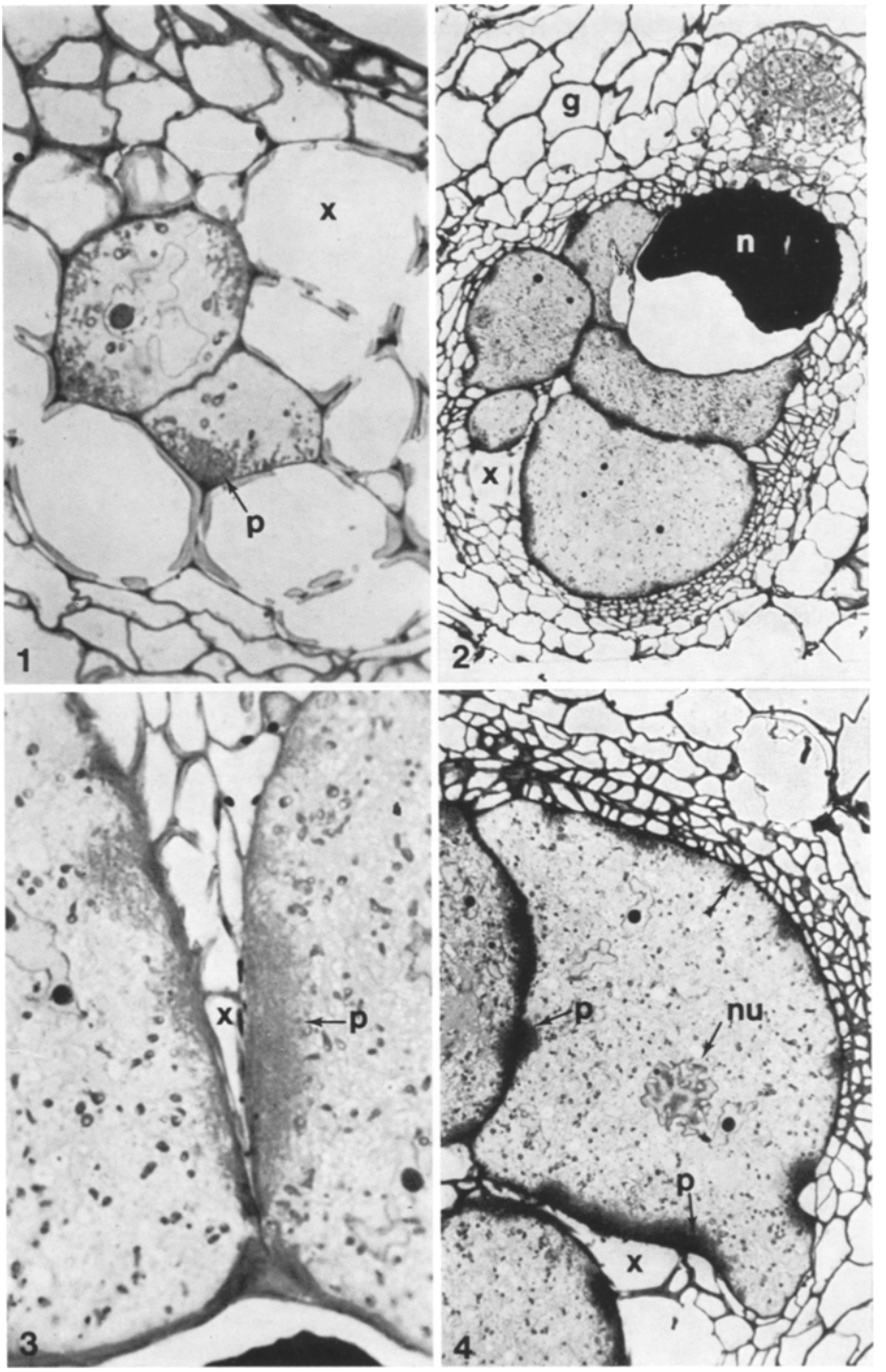
Fig. 1. At the distal and proximal ends of the complex, giant cells are surrounded by xylem vessels (*x*). Protuberances (*p*) occur projecting into the cells next to most xylem vessels. ×430

Fig. 2. Xylem vessels (*x*) are localized in discrete regions, frequently at the junction between giant cells. Next to the nematode (*n*) a lateral root meristem is present. The complex is surrounded by large parenchyma cells of the gall (*g*). ×200

Fig. 3. Wedge shaped xylem vessels (*x*) occur in the deep indentations between giant cells. Protuberances (*p*) are present on walls adjacent to these vessels. ×1,050

Fig 4. Small cells surround a giant cell where xylem vessels are absent. Protuberances (*p*) are found on the giant cell wall where a number of walls of adjacent cells converge onto it (tailed arrow). Next to xylem vessels and in localized places between giant cells the protuberances are well developed. A hypertrophied nucleus (*nu*) with an amoeboid profile is evident. ×425



Figs. 1–4

here they often assumed an abnormal wedge shape (Fig. 3), elsewhere the cells immediately surrounding the giant cells were usually small and thin walled and consisted of parenchyma, sieve elements and companion cells (Figs. 4 and 5). The parenchyma cells were larger than those of the phloem (Fig. 5). Near the head of the nemathode, the giant cells often surrounded the larva (Fig. 6), but the main body of the nematode was usually displaced to one side of the giant cell complex (Fig. 2).

Protuberances were present projecting inwards on all the outer limiting walls of the giant cells adjacent to xylem vessels (Figs. 7, 8, and 9) and also on the walls adjacent to some sieve elements (Fig. 10). Small ingrowths were also frequently present on outer walls adjacent to companion cells and sometimes on those adjoining parenchyma cells as well (Fig. 5) especially where a number of walls of the adjacent cells converged onto a small area of that of the giant cell (Figs. 4 and 11). Fig. 12 shows a structure between a sieve element and a giant cell that may have been a pore lined with callose with profiles of endoplasmic reticulum in the adjacent giant cell cytoplasm. However considerable study of other similarly placed sieve elements revealed no open connections so that if they did occur they cannot contribute significantly to the transport of nutrients to the giant cells. Localized ingrowths of the wall were also present between neighbouring giant cells, either on both sides (Fig. 17) or mainly on one side of the wall, and both patterns could occur fairly close together on the same wall (Figs. 13 and 14). The walls between giant cells were of variable thickness, and plasmodesmata but no ingrowths occurred where the walls were very thin (Figs. 15 and 16). Where a xylem or phloem element was present at the junction between giant cells wall ingrowths adjacent to the elements were less well developed than those found elsewhere (Fig. 18). However on both sides of the wall separating the giant cells there were more extensive wall ingrowths near the cell junction and a gradual decrease away from it (Fig. 19). The extent and complexity of wall protuberance development was greatest on walls adjacent to xylem elements (Figs. 4, 7, and 9) and also locally on walls between giant cells (Figs. 4, 13, 14, and 17), although next to sieve elements the ingrowths were not so pronounced (Fig. 10).

Examination of stained sections of the wall protuberances indicated that they had a composition similar to normal unlignified secondary walls. The

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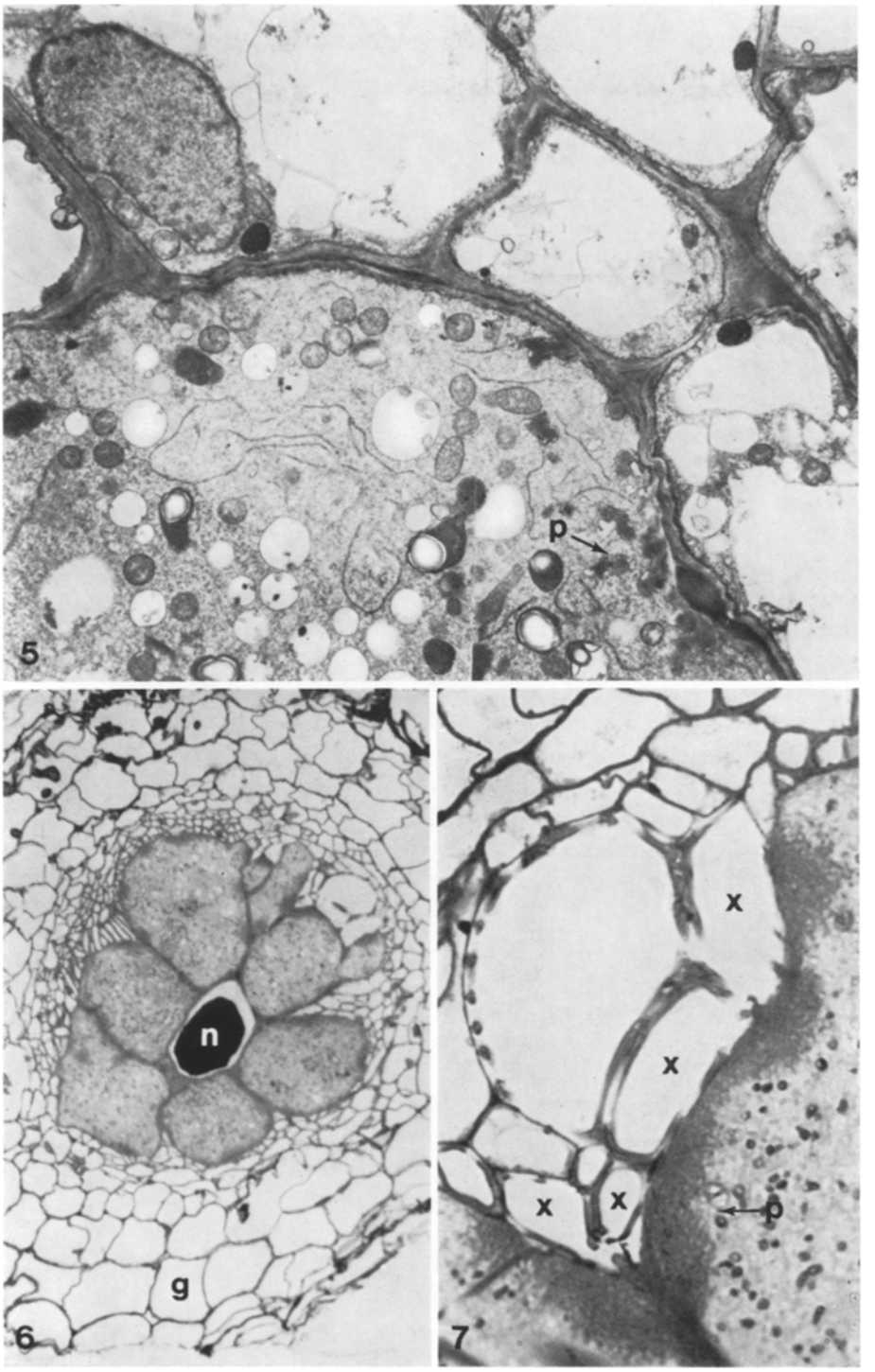
Fig. 5. Parenchyma cells adjacent to a giant cell. Small wall protuberances (*p*) occasionally form next to these cells. ×6,600

Fig. 6. The giant cells surround the nematode (*n*) near its head. Around the complex cells have been stimulated to divide, resulting in a ring of small cells surrounded by the gall cells (*g*). ×170

Fig. 7. Extensive development of wall protuberances (*p*) occurs adjacent to xylem vessels (*x*). ×1,250



Figs. 5–7

plasmalemma followed the irregular ingrowths and occasionally "boundary formations" were present (ESAU, CHEADLE, and GILL 1966, HUANG and MAGGENTI 1969 b, JONES and NORTHCOTE 1972), these were usually associated with those protuberances that had penetrated furthest into the cytoplasm (Fig. 20). The cellulose microfibrils appeared to be randomly distributed within the protuberances whereas those of normal secondary walls were parallel to one another (Fig. 20).

No cell wall stubs similar to those found in syncytia induced by the potato cyst-nematode (JONES and NORTHCOTE 1972) were observed in giant cells, although under the light microscope regions where the wall was not resolved were occasionally apparent (Fig. 21). These regions may correspond to positions at which plasmodesmata could be seen with the electron microscope (Figs. 15 and 16). Alternatively small wall gaps between giant cells may be present at the periphery of the complexes.

The giant cell cytoplasm was similar to that reported by BIRD (1961) and PAULSON and WEBSTER (1970). Since it contained numerous organelles, amoeboid hypertrophied nuclei, and short elements of smooth endoplasmic reticulum (Figs. 4, 5, 13, 14, and 17) it evidently had an extremely active metabolism. Crystalline bodies surrounded by an elaborate smooth membrane system (PAULSON and WEBSTER 1970) were present (Fig. 22). This may be the feature reported in light microscope studies as long narrow rods measuring up to 70 μ long and 1 μ wide (DROPKIN and NELSON 1960). Orientation of organelles indicating mass cytoplasmic flow was not observed and lignin was not found in the peripheral giant cell walls. In this respect the giant cells were different from the syncytia induced by the potato cyst-nematode (JONES and NORTHCOTE 1972).

4. Discussion

The appearance of giant cells induced in coleus roots by *M. arenaria* suggests that they form solely by the expansion of single cells with nuclear endomitosis (HUANG and MAGGENTI 1969 a). No cell wall gaps between giant cells were observed under the electron microscope, although it is possible that occasionally they may occur towards the outside of a complex, probably by breakage of the wall as a result of the considerable and rapid expansion of the giant cells. They probably do not contribute to the formation of giant cells.

The plasmalemma follows the invaginations of the protuberances and thus its surface area is greatly increased. Cells with wall ingrowths have been called "transfer cells" (GUNNING, PATE, and BRIARTY 1968, GUNNING and PATE 1969, PATE and GUNNING 1969), and their function has been described as intensive selective transport over short distances.

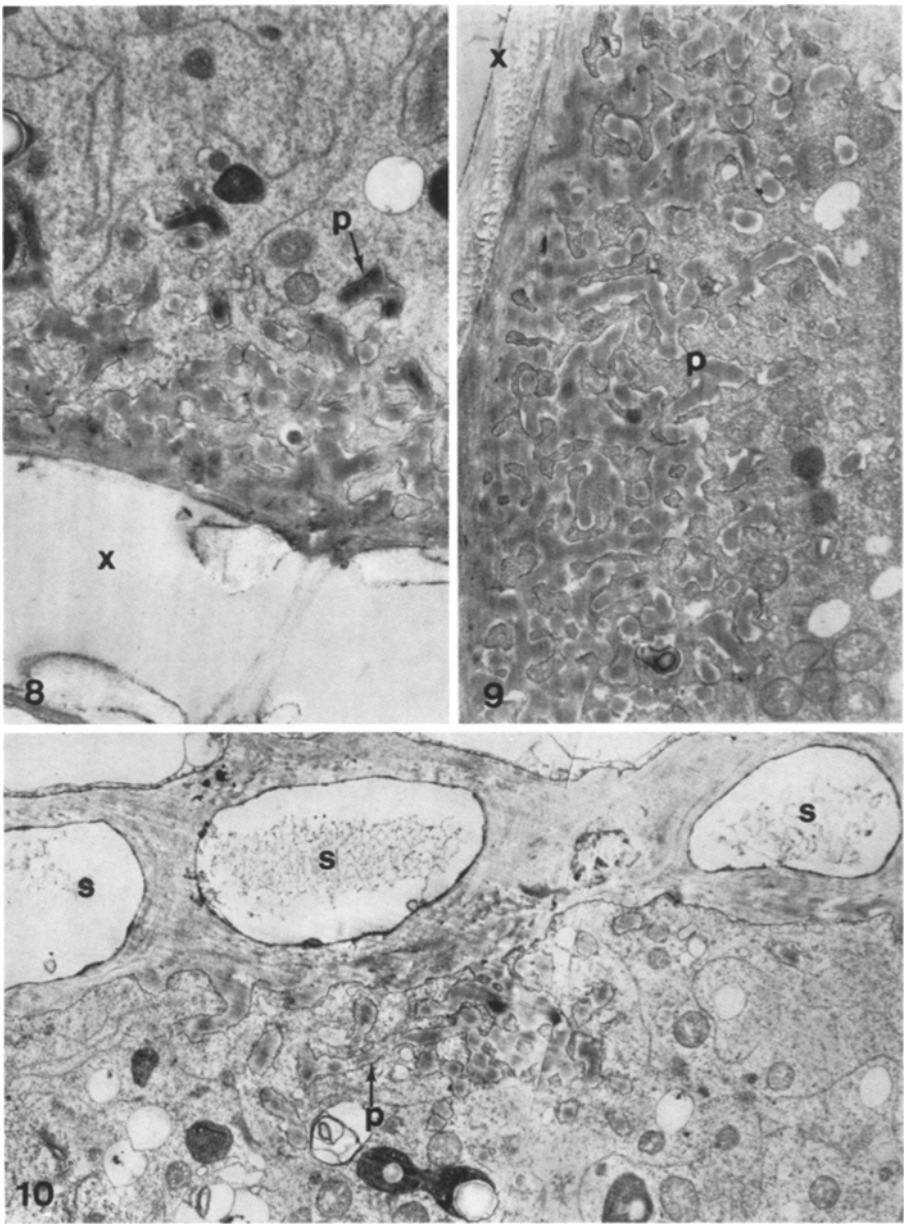


Fig. 8. The staining of lignified xylem thickenings is different from that of protuberances (*p*), which appear similar to non-lignified secondary walls. A hydrolysed cross-wall is present in the xylem element (*x*). ×9,000

Fig. 9. Anastamosing protuberances (*p*) adjacent to a xylem vessels (*x*). ×9,000

Fig. 10. The distribution of protuberances (*p*) next to sieve elements (*s*) is not constant, as the ingrowths are not present against every element. ×7,500

They are found where there is a large movement of solute with a minimum flow of solvent or where adverse surface: volume relationships exist between donor and receptor compartments. We have suggested (JONES and NORTHCOTE 1972) that syncytia induced by the potato cyst-nematode (*H. rostochiensis*) are a form of multinucleate transfer cell and this also is true for the giant cells induced by root-knot nematodes. We proposed that wall ingrowths form as a direct response to the flow of solutes and this results in a steep chemical gradient across the plasmalemma at these positions.

The presence of wall protuberances thus provides a marker to the site where the bulk of the nutrients required by the giant cell or syncytium are crossing the plasmalemma, and their degree of complexity may be directly related to the rate of solute transfer and the length of time the flow is maintained across the plasmalemma. In syncytia induced by *H. rostochiensis* wall protuberances were found to be much more extensive adjacent to xylem vessels than to sieve elements. Protuberances similarly occurred adjacent to the xylem in giant cells, but here their development next to sieve elements was much more pronounced than that in the syncytia. Thus giant cells obtain solutes both from the xylem and phloem elements for a considerable time, whereas solutes are only obtained at the extremities of syncytia from the sieve elements for the short while that these remain functional. However, the elongated syncytia induced by cyst-nematodes are in contact with a much greater surface area of xylem vessels and probably obtain a greater proportion of solutes from xylem sap than do giant cells. The difference in the functional activity between sieve elements adjacent to syncytia and those adjacent to giant cells must result from the different modes of expansion of the two types of complexes. Giant cells form in the centre of the root, behind the tip (CHRISTIE 1936), in the region of differentiating xylem elements (PAULSON and WEBSTER 1970) and are thus free to expand in every direction (Fig. 23). The expansion in any one direction is therefore considerably less than that of the syncytial complex which forms after the xylem areas have become

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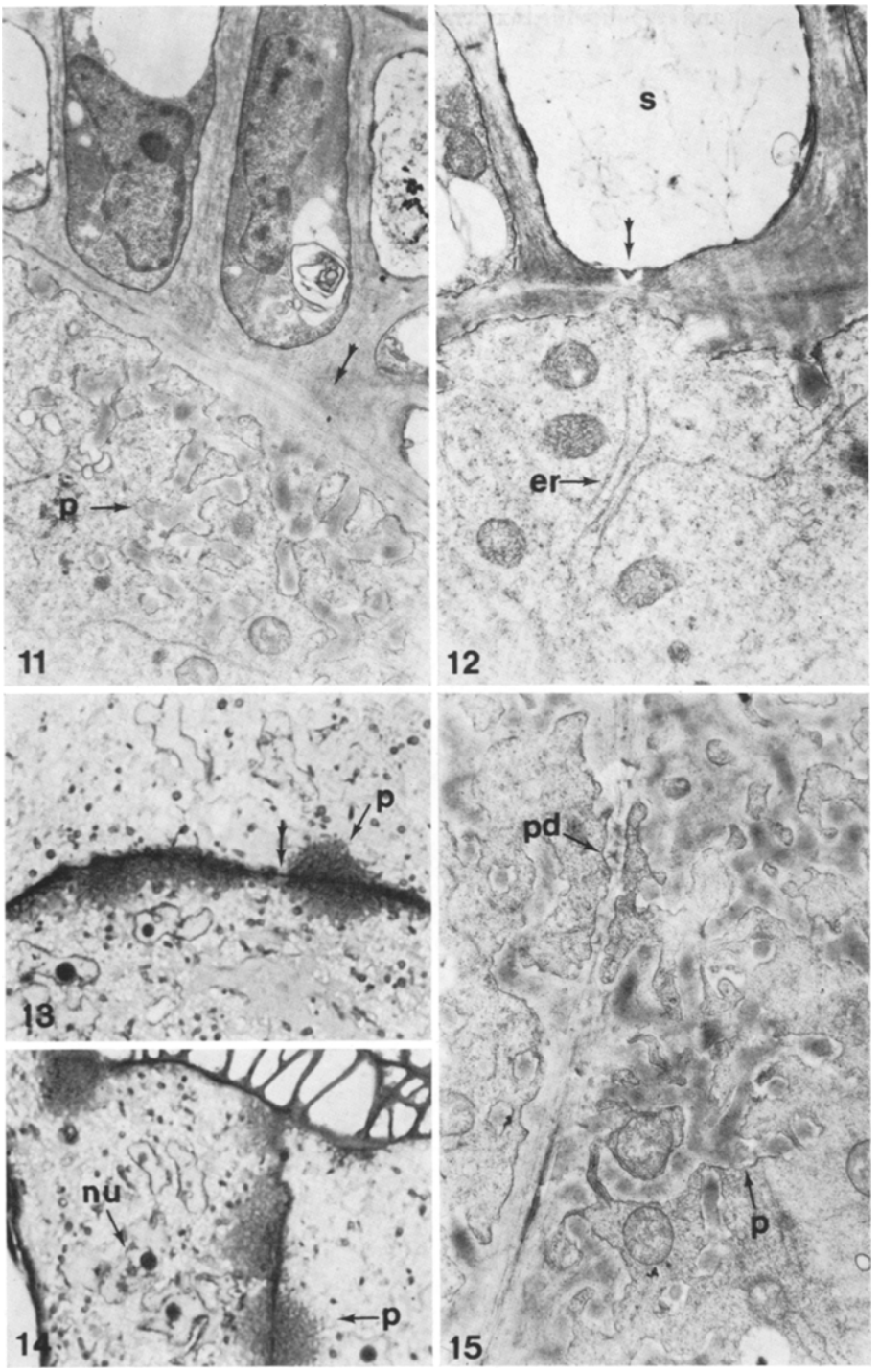
Fig. 11. The position of protuberances (*p*) where a number of walls of cells outside a giant cell converge into it suggests solutes flow along these walls as indicated by the tailed arrow. ×9,000

Fig. 12. The structure (tailed arrow) between the sieve element (*s*) and giant cell is possibly a pore lined with callose. Profiles of endoplasmic reticulum (*er*) are present in the giant cell over the structure. ×12,000

Figs. 13 and 14. Wall ingrowths (*p*) may occur on both sides or mainly on one side of the wall separating giant cells. Plasmodesmata are probably present at the narrow wall areas (tailed arrow). Nuclei (*nu*) in the giant cells have prominent nucleoli, and the cytoplasm is filled with small vesicles and organelles. Figs. 13 and 14 ×960

Fig. 15. Plasmodesmata (*pd*) are present in the areas where the wall between giant cells is narrow. Protuberances (*p*) occur on other regions of the wall. ×12,000



Figs. 11–15

established, and the lignified xylem limits the direction of its expansion towards the cortex. Sieve elements on the cortical side of syncytia then become crushed as the syncytia expand outwards and this renders the phloem non-functional (Fig. 23). The cells surrounding giant cells are stimulated to divide and this probably brings about the formation of new sieve elements. There is no comparative stimulation of cells adjacent to syncytia.

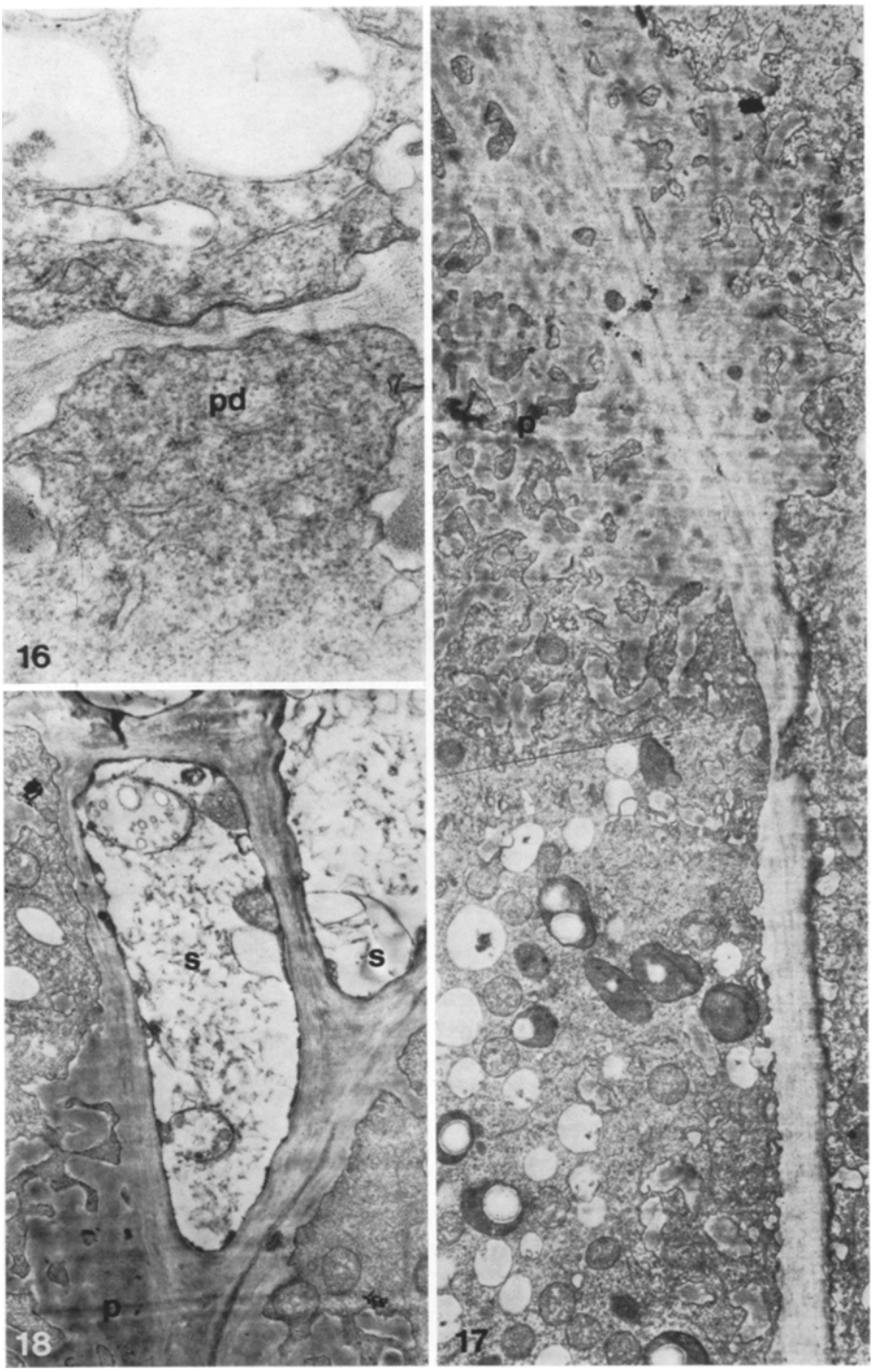
Although giant cells induced by root-knot nematodes and syncytia induced by cyst-nematodes are formed by different methods (endomitosis and cell wall dissolution respectively) the resultant complexes are functionally equivalent. In each case an extremely active metabolism is required to synthesize wall protuberances and cytoplasmic components and also to provide the energy for massive selective concentration of nutrients from the conducting vessels, despite withdrawal of cytoplasmic components by the nematode which acts as a nutrient sink. In each case the nematode has stimulated the differentiation of unspecialized cells to form cells with a specific physiological function. Furthermore, development of protuberances in both syncytia and in giant cells supports the theory that the wall protuberances form as a result of solute flow and that their formation is a secondary response independent of the mechanism of formation of the complexes.

HUANG and MAGGENTI (1969 b) suggested that the nematode larva might feed from intercellular spaces between the giant cells but from studies using the cyst-nematode, this appears unlikely since the orientation of organelles in syncytia implies a mass flow of the cytoplasm as a result of direct ingestion by the nematode. The discrete nature of giant cells suggests that root-knot nematodes must feed from individual giant cells in turn and this is supported by observations that root-knot larvae maintain a flexible neck region (LINFORD 1937) whereas cyst-nematode larvae do not (MANKAU and LINFORD 1960). An obvious orientation of organelles is therefore less likely to be found in a giant cell, as the larva may not have recently fed from it, and also because the total nutrient flow does not pass via wall gaps where any flow would be most obvious.

Fig. 16. The wall separating giant cells is slightly thicker at the point where plasmodesmata (*pd*) cross it, although the walls are much narrower than elsewhere and lack secondary thickenings. ×36,000

Fig. 17. The variable thickness of walls separating giant cells is evident. Extensive ingrowths (*p*) are localized to a region of wall whereas other regions are virtually free of ingrowths. ×16,000

Fig. 18. Sieve elements (*s*) at the junction between giant cells. Few protuberances are present adjacent to the elements, but extensive ingrowths (*p*) form on the wall between the giant cells. Solute probably flows along the walls towards the dividing wall shown at the bottom of the picture. ×9,000



Figs. 16–18

Since neighbouring giant cells provide a rich nutrient source for each other the hypothesis that protuberances form in response to solute flow is further supported by their occurrence on the walls separating such cells. Demand for solutes for a given giant cell depends on whether the nematode has recently ingested cytoplasm and also on the number of vascular elements that supply the translocated solutes. Thus, depending on demand, solute flow will occur between neighbouring giant cells, so that as a result of feeding by the nematode a flow of solutes occurs between the nematode and the giant cell and between the latter and the vascular elements or other giant cells. As a result of the gradient set up, protuberances will form at predictable places. Where solute flow first occurs protuberances form fastest, and these in turn allow an even greater flow of solute so that eventually a transport channel is established where relatively elaborate wall ingrowths are formed. At the narrow wall areas where plasmodesmata are found, solutes may move directly through the plasmodesmata and not across the plasmalemma, or there may be an inherent mechanism which prevents cell wall deposition around the plasmodesmata. The hypothesis also predicts that if wall gaps occur between neighbouring giant cells, no new wall protuberances will form on the remaining dividing wall.

Solutes which diffuse from conducting elements into the free space of the wall must be able to move along the walls to sites where cellular demand is greatest and therefore extracellular concentration is lowest. Hence wall protuberances develop considerably on the walls between giant cells when conducting elements are in contact with neighbouring giant cells. This wall that they have in common provides the best channel along which the translocated nutrients are drawn to both cells and serves to emphasize the conducting function of the plant cell wall.

The difference in the mechanisms of formation of giant cells and syncytia may well be reflected in the occurrence of galls associated with root-knot infections and the absence of galls with cyst-nematode infections. Presumably

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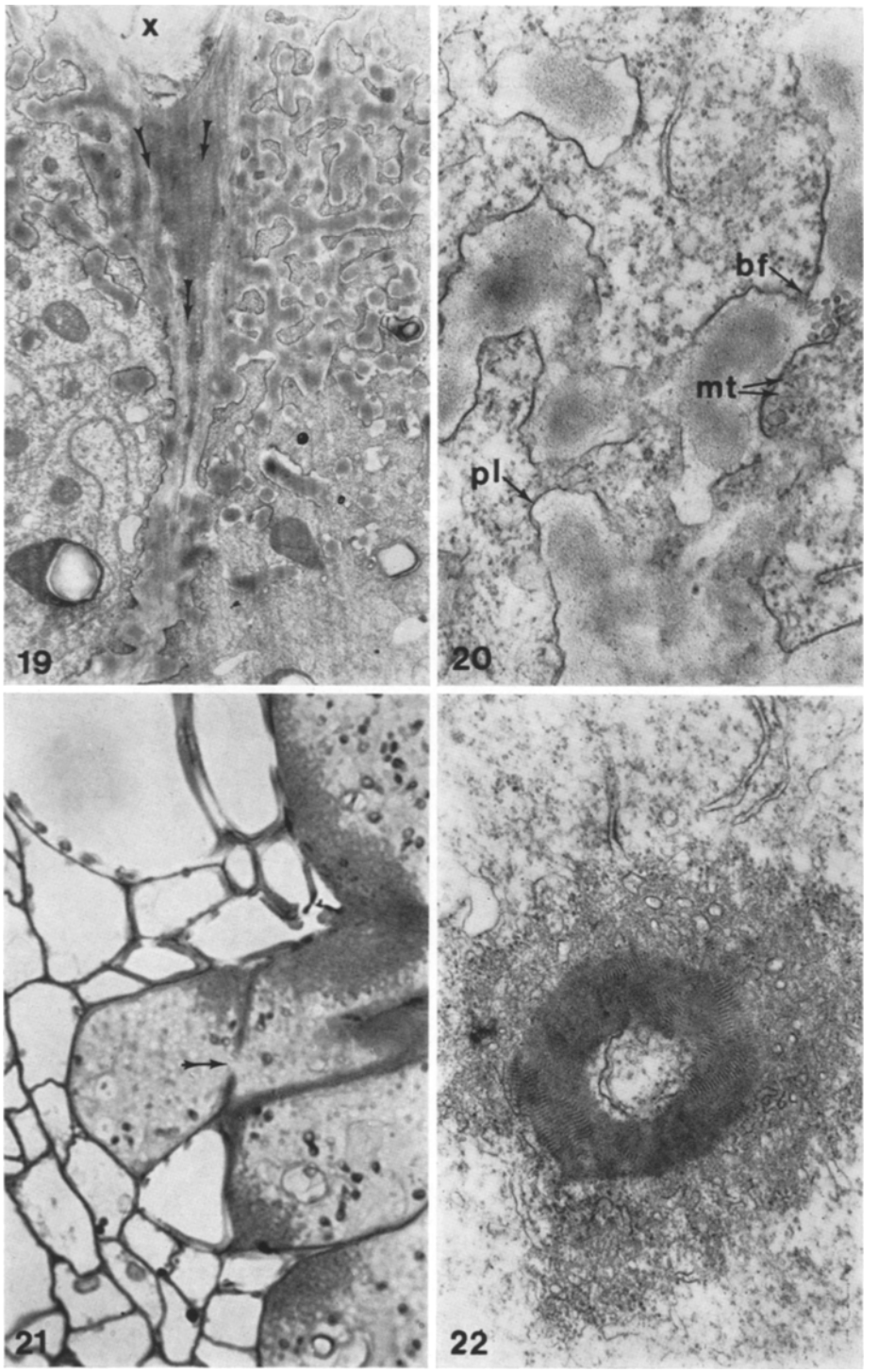
Fig. 19. At the junction of a xylem vessel (*x*) and giant cells the extent of wall protuberance development decreases away from the junction along the common wall between the giant cells. This suggests a considerable solute conduction (tailed arrows) along the wall. ×9,000

Fig. 20. The microfibrils in the protuberances are randomly distributed. The plasmalemma (*pl*) follows the irregular profile of the protuberances, and a "boundary formation" (*bf*) is present with microtubules (*mt*) in the cytoplasm close by. ×36,000

Fig. 21. The tailed arrow indicates an area where the wall if present between giant cells is not resolved in the light microscope. ×1,200

Fig. 22. A crystalline body, possibly proteinaceous, surrounded by an elaborate smooth membrane system. ×36,000



Figs. 19–22

the stimulus for mitosis induced by the root-knot nematode in giant cells diffuses to surrounding cells, but the factors preventing cytokinesis do not diffuse in sufficient quantity to be effective.

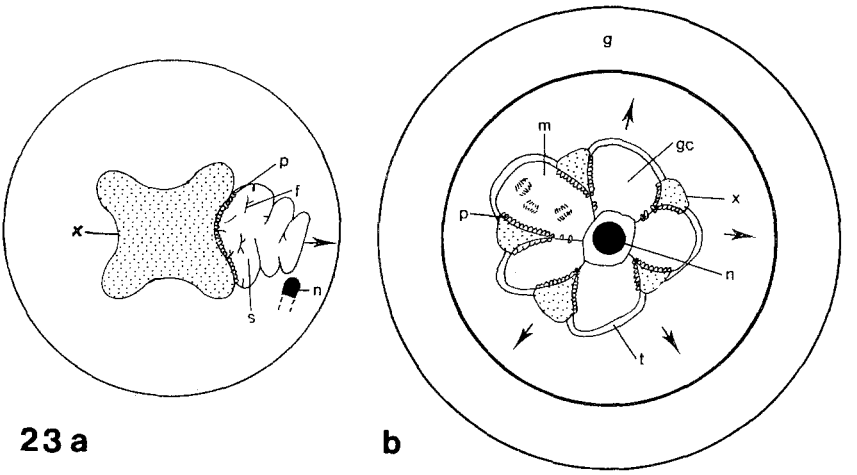


Fig. 23 *a*. Syncytia (*s*) induced by cyst-nematodes (*n*) usually form after the lignified xylem areas (*x*) have formed. Expansion is therefore limited towards the cortex alone (arrow), and cells including sieve elements on the cortical side of the syncytium are crushed. Protuberances (*p*) are only present on syncytial walls adjacent to the xylem elements. Wall fragments (*f*) occur within the syncytia

Fig. 23 *b*. Giant cells (*gc*) induced by root-knot nematodes (*n*) form in provascular elements before the xylem pattern is established. The cells are free to expand in all directions (arrows) so xylem elements (*x*) differentiate in localized regions. Surrounding thin walled cells (*t*) are stimulated to divide and some of these differentiate to sieve elements. A gall (*g*) is formed around the complex. A proportion of these elements may be damaged by giant cell expansion so protuberances (*p*) do not form adjacent to all of them. (Protuberances next to sieve elements are not indicated in this figure)

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