Early Stages of Nematode-induced Giant-cell Formation in Roots of Impatiens balsamina

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Abstract: Giant cells induced in roots of *Impatiens balsamina* by *Meloidogyne javanica* and *Meloidogyne incognita* have been examined by light and electron microscopy. The first sign of giant-cell formation was division of cells surrounding a larva. Cell plate alignment appeared to proceed normally, but cytokinesis was unsuccessful and binucleate cells formed subsequently. No wall breakdown was evident then or later. The number of nuclei appeared to increase by repeated mitosis without separation by cytokinesis. Although no holes in walls were observed, wall stubs were found, and mechanisms for their formation are suggested. *Key Words:* Root-knot nematode, *Meloidogyne javanica*, *Meloidogyne incognita*, electron microscopy, cytokinesis.

The events involved in the induction of giant-cells by root-knot nematodes (*Meloidogyne* spp.) have been the subject of controversy. The predominant view was that at initiation of giant-cell formation several adjacent members of a row of undifferentiated cells in the central cylinder coalesced through the dissolution of their walls, followed by a combination of cell-wall breakdown and mitosis without cytokinesis to bring about the increase in size (up to several hundred micrometers) and the multinucleate condition (2, 6, 10, 13, 24, 29, 30). Huang and Maggenti (16), however, counted chromosome numbers in giant cells in *Vicia faba* undergoing mitosis, and reported a ploidy sequence of 4, 8, 16, 32, and 64 n. They suggested that wall breakdown is not involved in giant-cell formation, but that these cells develop solely by repeated mitosis without cytokinesis. Bird (3, 4, 5) later provided evidence from serial 2-μm sections that wall breakdown may occur at later stages after induction, and that the DNA contents of giant-cell nuclei do not appear to fit a simple endomitotic sequence. He also repeated Huang and Maggenti's

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work on chromosome numbers, and was unable to find a fit with the ploidy sequence expected from repeated mitosis without cytokinesis. Examinations of giant cells by electron microscopy (2, 21, 32) were not directed solely to answering the question of what happens during the initial stages of giant cell induction, but the general impression emerged that the cells develop by repeated mitosis without cytokinesis.

From examinations of squash preparations of giant cells fixed, isolated after pectinase treatment, and stained with Feulgen reagent, Bird (5, 6) proposed that the initial event of giant-cell formation is wall breakdown which produces linear cells with two or more nuclei. Since such events are not clear at the light-microscope level, the present work was carried out by both light and electron microscopy to clarify the nature of the events during the first few days after induction. In conjunction with this work, the effects of caffeine on cytokinesis in uninfected root tips have been compared with the inhibition of cytokinesis in developing giant cells (unpublished observations). The developmental sequence during giant cell induction is discussed, and possible explanations are suggested to reconcile divergent views on the process.

MATERIALS AND METHODS

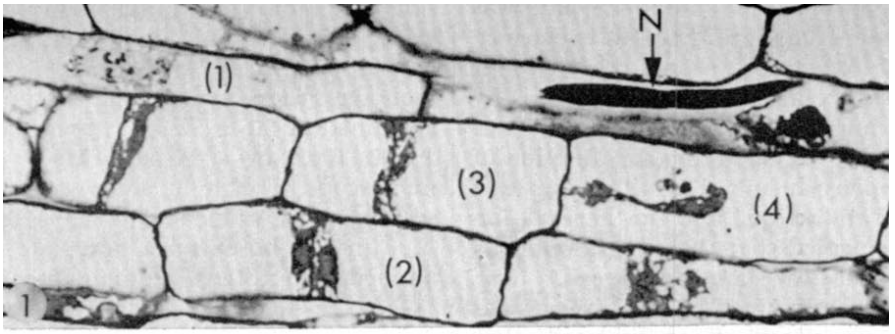
Roots of dwarf balsam seedlings (*Impatiens balsamina*) germinated on damp filter paper were inoculated (24-h exposure) with larvae of *Meloidogyne javanica* or *M. incognita* as described previously (22). The roots were then washed and the seedlings planted in vermiculite. Roots infected with *M. javanica* were fixed for microscopy 24, 48, or 72 h after planting, and those infected with *M. incognita* were fixed at various times after planting (3, 6, 10, 15, 22, 33 days). Infection and subsequent culture were in a growth chamber (day 25 C, night 20 C, 14-h day).

Preparation for microscopy after fixation in 3% glutaraldehyde or 3% gluturaldehyde plus 3% acrolein has been described (19). To ensure that whole giant cells could be viewed in the electron microscope without intervening grid bars, sections were examined on parlodion-coated 2-by-1-mm slot grids or coated 100-mesh (254-μm) grids, in addition to normal 200-mesh (127-μm) gilded grids, and complete montages were made. The sections were examined at 60 or 75 kV, respectively in a JEOL 100B or a Hitachi H500 electron microscope. For light microscopy, 0.5-μm sections were stained with 0.5% toluidine blue in 1% borax on a hot plate and examined with a Zeiss photomicroscope.

RESULTS

Migration of larvae: Larvae of *Meloidogyne incognita* and *Meloidogyne javanica* migrate intercellularly (14). Wall separation occurs along the middle lamella ahead of larvae. Wall breakage was not observed in balsam, although walls were distorted in some cases, presumably by pressure exerted by the larvae. The cytoplasmic density in cells next to larvae was frequently increased, a result of an increase in ribosomal numbers. (However, in nearly all stages of giant cell development the density of free ribosomes is less in the ground cytoplasm than in the cytoplasm of adjacent normal cells). The cell separation causes rupture of the plasmodesmata between previously joined cells, thus breaking their symplastic continuity. Together with the pressure exerted by the larva, this effect may cause a localised wound response contributing to the increased cytoplasmic density.

Presumptive giant cells 24 h after infection: In tissues examined 24 h after infection, no wall breakdown was observed although evidence of recent mitotic events was seen frequently in cells immediately adjacent to the nematode or one or two cell layers away. In Fig. 1, for example, one cell adjacent to a nematode is shown in anaphase (cell 1), and in two other neighboring cells vesicles which would form the cell plate under normal conditions are lined up across the cell (cells 2 and 3). In another cell, a phragmoplast has extended about half-way across the vacuole (cell 4). Details of the presumptive cell plates shown in Fig. 1 as seen in adjacent sections by electron microscopy are provided in Fig. 2, 3, 4. In cell 2 of Fig. 1, the row of vesicles stretches completely from one mother cell wall to the other (Fig. 2). A few micro-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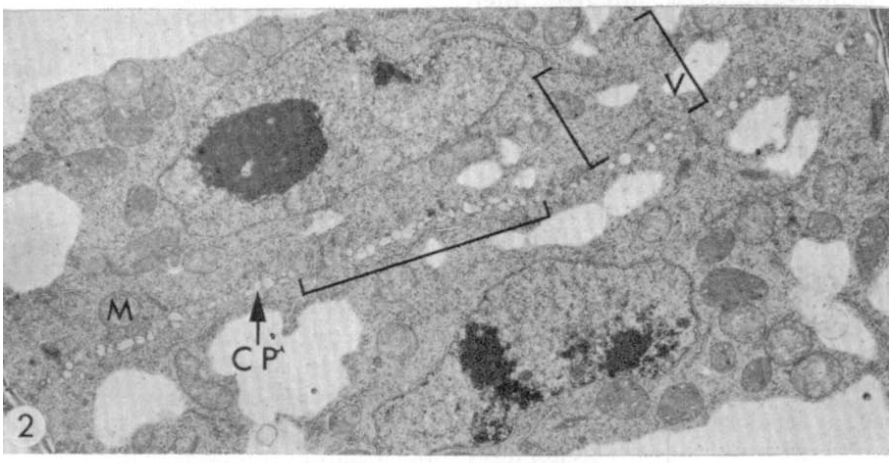
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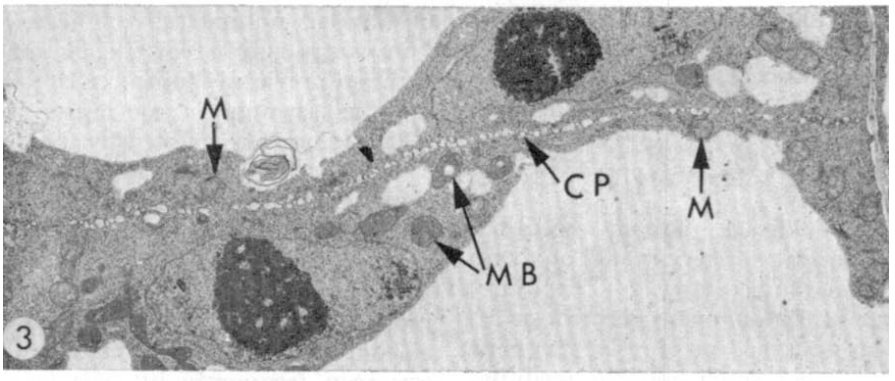
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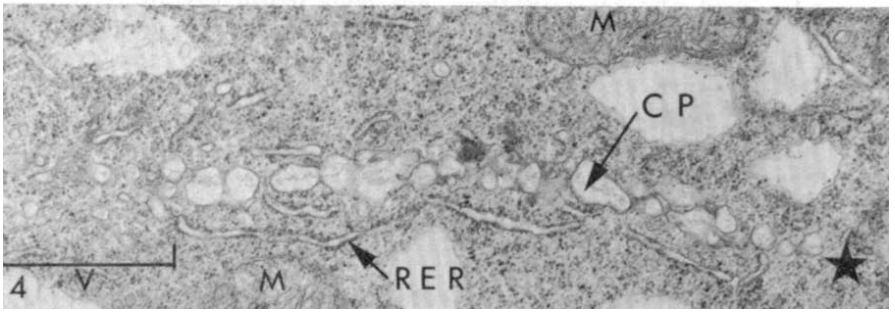
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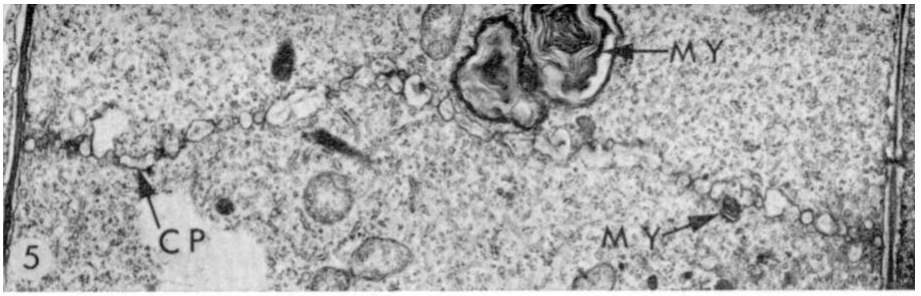
tubules are still to be found where the presumptive cell plate reaches the mother cell wall on one side. It is notable that Golgi bodies, vacuoles, mitochondria, and endoplasmic reticulum are to be found close to the row of cell-plate vesicles in the region between the two nuclei. In addition, numerous vesicles lie between the presumptive cell plate and one of the nuclei (Fig. 4). Slight aggregation of vesicles has occurred to about the same extent along the whole length of the presumptive cell plate, except at the edges near the mother cell walls. Cell 3 of Fig. 1 is in a similar state except that the daughter nuclei are no longer opposite one another. Cell organelles, again, approach the presumptive cell plate closely, in some places numerous vesicles occur near the plate, and myelin-like membranous figures are associated with it (Fig. 3). Mitochondria and plastids are close to the row of vesicles, a situation not encountered under normal circumstances until considerably more fusion of cell plate vesicles has occurred. Cell 4, Fig. 1, is not so advanced, and the presumptive cell plate which develops in a phragmosome across the vacuole has not reached either mother cell wall. Myelin figures and multivesicular bodies were seen also in presumptive cell plates next to other larvae (Fig. 5, 6). The lengths of cells in which rows of vesicles like those illustrated were seen next to the nematode varied from 50 to 90 μm. The cells were not obviously different in length from neighboring cells at this stage. The cells shown in Fig. 1-6 are all highly vacuolate, and in the absence of nematode infection it is unlikely that cells as far from the meristematic region as these would undergo division, or at least that as many adjacent cells would divide together.

The separation of cells adjacent to the nematode, along the middle lamella of the wall, was found frequently (Fig. 7). Convolutions of the plasmalemma often occurred in the cells bordering such cell separation.

Forty-eight hours after infection: Two binucleate cells whose nuclei were undergoing synchronous mitosis were observed at this stage. In the cell shown in Fig. 8 both nuclei exhibit metaphase plates. The metaphase plates of these two nuclei are perpendicular to one another, one being parallel to the long axis of the root and the other nearly at right angles to it. One of the nuclei in the other cell is shown at higher magnification in Fig. 9, and the metaphase plate is oriented at an angle to the long axis of the cell. Sectioning of a number of blocks did not reveal any cells in which cytokinesis was occurring. Cells with 2, 4, and 8 nuclei were also observed near nematodes. With the greater number of nuclei, these were directly counted only when the nuclei were lined up in the parietal cytoplasm, and numbers of nuclei intermediate between 4 and 8 were also seen. Since there was no sign of wall breakdown or wall stubs in any of these cells, it is assumed that not all the nuclei in such cells were within the plane of the section. In most cases, the multinucleate cells were of a similar dimeter or slightly wider than neighboring cells, but lengths of up to 180 μm were measured. The lengths were variable, however, and an apparently binucleate cell measuring only 30 μm was also observed (Fig. 13). Thus, maximum length can double between 24 and 48 h after infection, and one, two, or possibly more cycles of mitosis can occur within the 48 h. At this stage, the cells are dominated by the central vacuole, and nuclei are usually restricted to a parietal layer of cytoplasm (Fig. 10, 12).

The stimulation of mitosis coupled to normal cytokinesis continues in cells lying

FIG. 1-4. *1)* Light micrograph, 24 h after infection. Cells near second-stage larva (N) in clear stages of division are marked by numbers. Cell (1) is in anaphase, cells (2) and (3) in late telophase, and cell (4) in early telophase. *Meloidogyne javanica,* x550. *2)* Adjacent section to that of Fig. 1, showing the row of presumptive cell-plate (CP) vesicles of cell (2) which stretch from wall to wall across the cell. Between the cell plate and one of the nuclei, numerous vesicles (V) occur in the area indicated. Mitochondria (M) are found close to the cell plate: the central region is shown at higher magnification in Fig. 4. *M. javanica,* x7,200. *3)* Cell plate (CP) of cell (3) from Fig. 1. The daughter nuclei are no longer opposite each other. Slight fusion of cell-plate vesicles has occurred, mitochondria (M) and micro-bodies (MB) are situated near the cell plate. *M. javanica,* x4,850. *4)* Part of the cell plate (CP) from Fig. 2. A region where numerous individual vesicles (V) are found is indicated. Rough endoplasmic reticulum (RER) and mitochondria (M) are evident. The asterisk denotes a region where cell-plate vesicles are absent. *M. javanica,* x23,400.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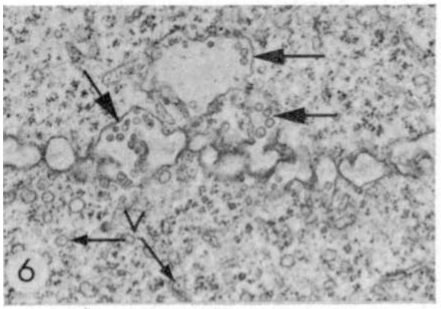
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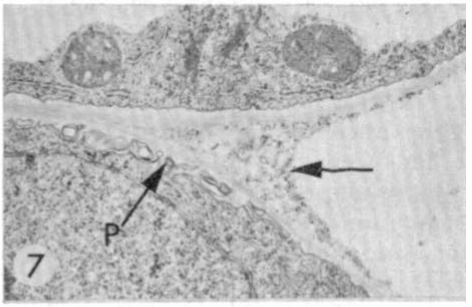
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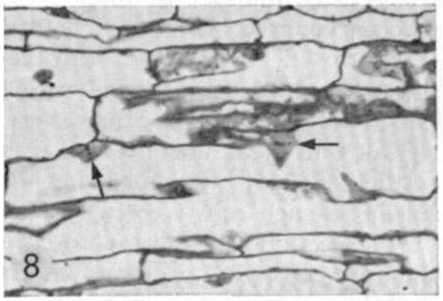
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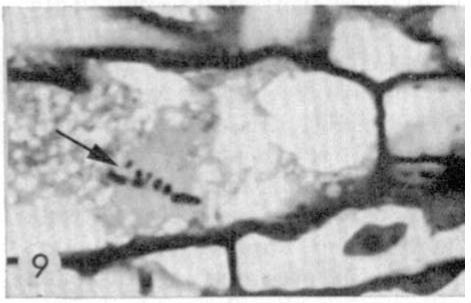
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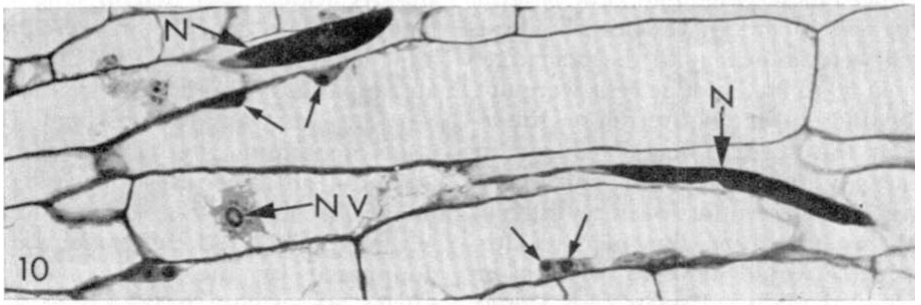
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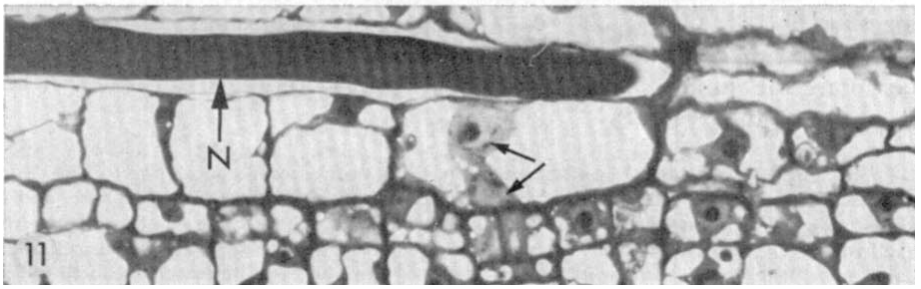
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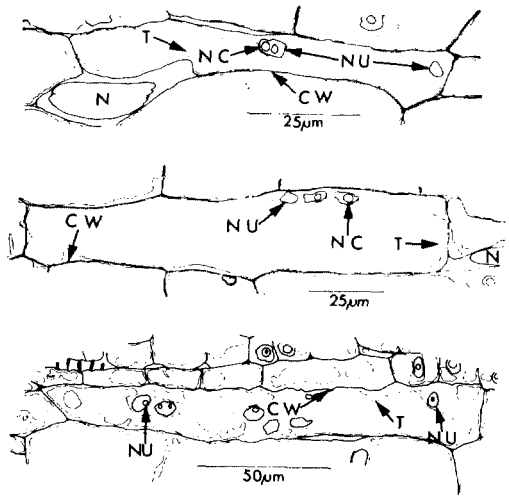
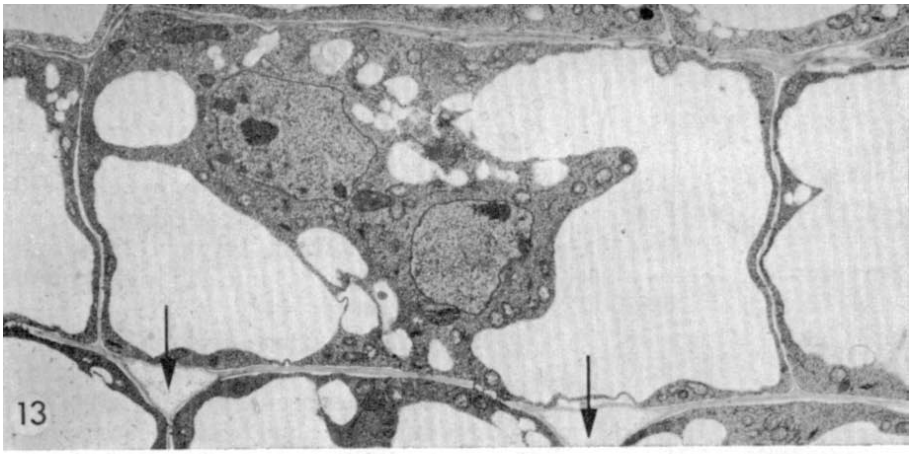


FIG. 12. Tracings from montages of developing giant cells photographed on slot grids, 48 h after infection. Cell walls and nuclei with or without nucleoli are depicted by thicker lines, and the tonoplasts with thinner lines. No evidence of wall breakdown is present. At junctions with other cell walls, extra material is sometimes deposited. The number of nuclei, or parts thereof, visible in the sections are 2, 3, and 7, respectively in the top, middle, and bottom cells. N = nematode, T = tonoplast, CW = cell wall, NU = nucleus, NC = nucleolus.

outside the developing giant cells. One giant cell may be flanked by up to 10 normal cells along one side. The normal cells are generally shorter in diameter and length than cells a few layers away (Fig. 11). The appearance of the developing giant cells is consistent with their formation from cells shown in Fig. 1-6, in which it is suggested that failure of the presumptive cell plate vesicles to fuse has generated binucleate cells, with subsequent repetition of this process. The increase in length can be accounted for by the fact that these cells occur in the zone of elongation behind the root tip, and would probably have differentiated into vascular elements under normal circumstances. Sieve tubes and xylem-vessel elements are normally considerably longer than other cells within the stele. In Fig. 12 tracings are provided of montages of developing giant-cells photographed in the electron microscope at magnifications between 2,700 and 3,300X. The nuclei, nucleoli and tonoplasts are shown.

Seventy-two hours after infection: Because not all nematodes locate the correct cells and begin inducing giant cells at the same time, and because conditions may vary in different roots, the range of sizes of giant cells is greater with increasing time after infection. By 72 h, developing giant cells were observed with 2 to 8 nuclei or more within individual sections. The maximum length measured at this stage was 400 μm. No wall breakdown was observed, and complete walls could be traced in montages of giant cells (Fig. 16). The walls were extremely thin where plasmodesmata were located, and could even bulge out slightly into a neighboring cell although remaining intact. The developing giant cells induced by *M. incognita* were slightly further advanced than those induced by *M. javanica*. This difference was most probably a consequence of slightly different culture conditions. The central cell vacuoles of giant cells induced by *M. incognita* were starting to be reduced in volume relative to that of the cytoplasm. The possibility of intrusive growth of giant cells (that is, the extension of giant cells by separation and growth between normal cells, particularly at either end of the complex) is suggested by observations exemplified in Fig. 14, 15,

FIG. 5-11. *5)* Presumptive cell plate (CP) next to larva 24 h after infection. Myelin figures (MY) are present, and numerous vesicles are scattered throughout the cytoplasm on both sides of the cell plate. *Meloidogyne javanica,* x8,625. *6)* Multivesicular bodies (unlabelled arrows) in another section of the cell plate in Fig. 5. Other vesicles, some with electron-dense cores (V) occur in the cytoplasm, although microtubules are absent. *M. javanica,* x17,140. *7)* Separation of cells next to a nematode along the middle lamella (arrow). Plasmalemmasome-like convolutions of the plasmalemma (P) occur in cells near sites of wall separation. *M. javanica,* x9,000. *8)* Binucleate cell 48 h after infection, in which both nuclei exhibit metaphase plates. The planes of these plates, indicated by arrows, are almost perpendicular to each other. *M. javanica,* x267. *9)* Metaphase plate of one nucleus in a binucleate cell 48 h after infection. The plane of the plate is indicated by the arrow. *M. javanica,* x730. *10)* 48 h after infection. The binucleate cells next to the nematodes (N), whose nuclei are indicated by arrows, are dominated by their central cell vacuoles. Cytoplasm is restricted to a parietal layer. A nucleus with a prominent nucleolar vacuole (NV) is also present. *M. javanica,* x330. *11)* Nematode (N) next to a binucleate (arrows) cell 48 h after infection. Numerous divisions in the next cell layer have produced small meristematic cells. *M. javanica,* x530.



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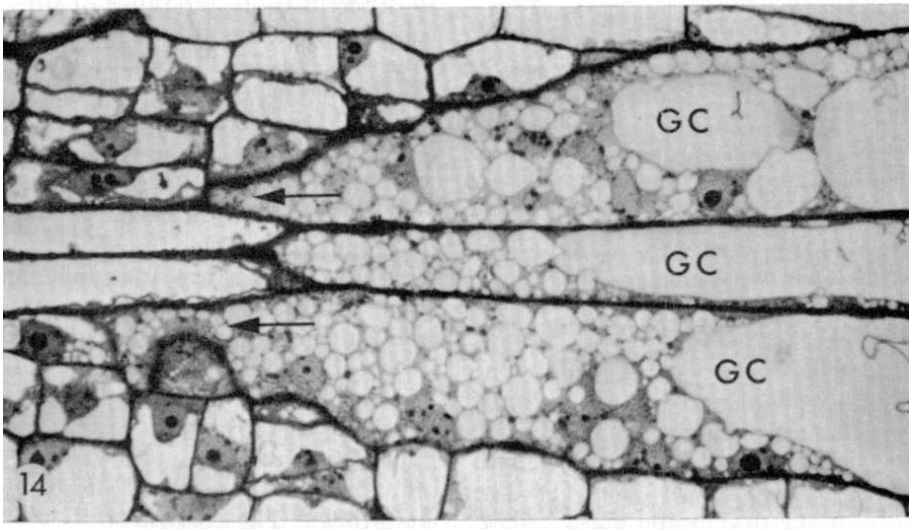

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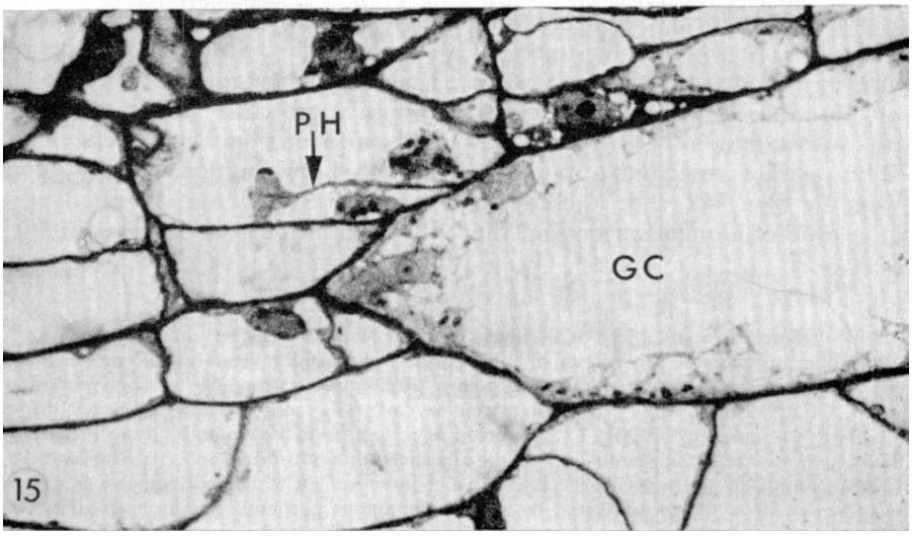

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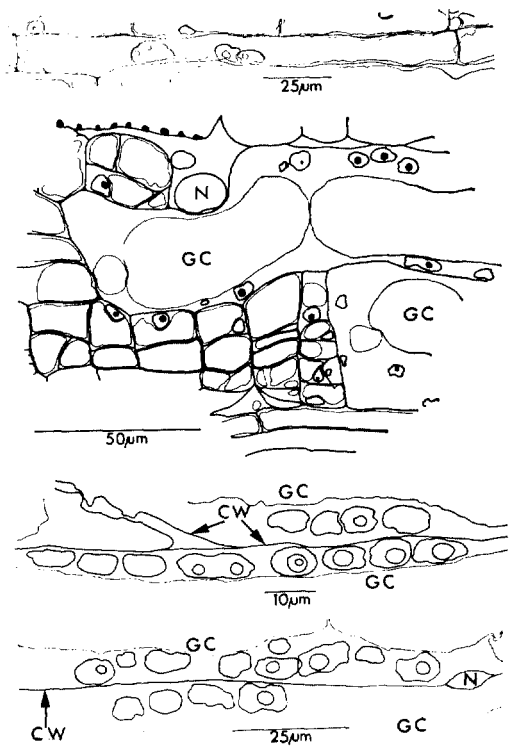


FIG. 16. Tracings of montages of developing giant cells 72 h after infection. Conventions and abbreviations as in Fig. 12. The top tracing shows a long thin cell with three nuclei, one larger than the other two and having two nucleoli. The upper middle trace shows that division of neighboring cells allows giant cells to expand without wall breakdown. The two lower traces show the junction between two giant cells, in which nuclei are lined up in the parietal cytoplasm. In both cases one of the cells has 4 nuclei, in the upper trace 8 nuclei occur in a row, and in the lower trace 9 nuclear profiles are present although the total number of nuclei may be less than this since they possess irregular outlines with cytoplasmic invaginations.

17. The regions indicated in Fig. 14 were examined by electron microscopy, and no wall breakdown was present. Nevertheless, there can be little doubt that the greatest contribution to giant-cell elongation is from the general elongation of all the cells behind the root tip. This observation does suggest, however, that giant cells will be longest when root extension growth is greatest. Factors which might retard this extension (adverse temperatures, differences in growth rates between plants, proximity of initial infection to the tip, multiple infections, etc.) will lead to shorter giant cells.

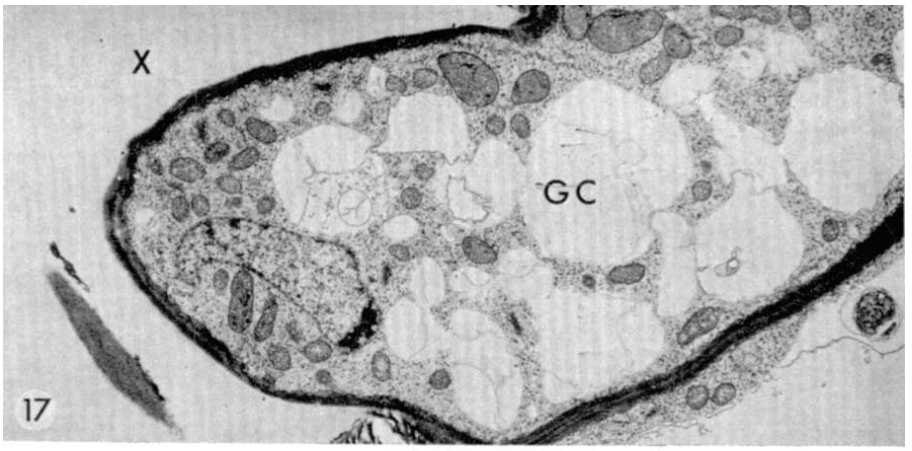
Although no gaps were observed in giant-cell walls, a wall stub was found (Fig. 18, 19). The whole giant cell was visible on a slot grid, and there was no corresponding wall fragment on the opposite wall to the stub. It will be noted that the stub is next to an air space, or separation, between two giant cells, and that two dark lines (arrows, Fig. 19) continuous with the *outside* of the giant-cell wall run up the stub. This image is consistent with formation of the stub by collapse of the space between the two giant cells and the compression and folding of the wall back on itself.

In Fig. 17, the cell wall and cytoplasm of a developing giant cell is seen to bulge like a partial tylose into the lumen of an abnormal xylem element. A different plane of section could have suggested that this "tylose" was not attached to the rest of the cell.

Within the nematode, the lipid droplets are surrounded by numerous glycogen rosettes (Fig. 20), a sign that the larva has received a positive signal from the plant that giant cell induction has been successful (12).

Six days after induction: By this time, lateral expansion of giant cells is considerable (Fig. 22, 24). Numerous nuclei are present within the cytoplasm of giant cells, the central cell vacuole is further reduced, and differentiation of abnormal vascular elements around the giant cells is advanced. A wall stub is evident in Fig. 24, attached to a giant-cell wall, but no breaks or gaps are found in the giant-cell walls. Again, there is no corresponding wall fragment

FIG. 13-15. *13)* Binucleate cell 48 h after infection. Separation of the wall along the middle lamella is indicated by the arrows. *Meloidogyne javanica,* x3,465. *14)* 72 h after infection. Parts of three giant cells (GC). The central cell vacuoles are much reduced in size. The wedge-shaped ends of the giant cells suggest that intrusive growth between the cells outside may occur in the direction of the arrows. These regions were examined by electron microscopy: no wall breakdown was evident. *Meloidogyne incognita,* x650. *15)* 72 h after infection. Intrusive growth by extension of the giant cell (GC) is suggested. Note the phragmoplast (PH), about two-thirds of the way across the neighboring cell. Such divisions may allow for expansion or extension of giant cells without crushing adjacent cells. *M. incognita,* x970.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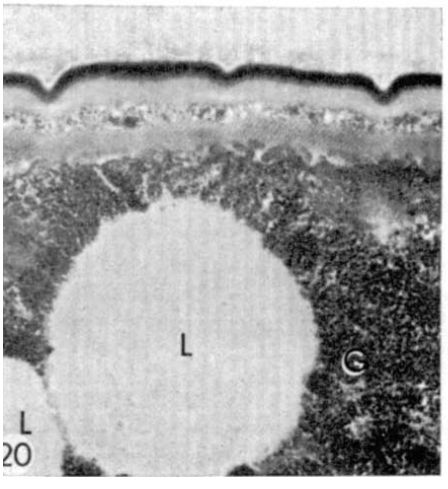
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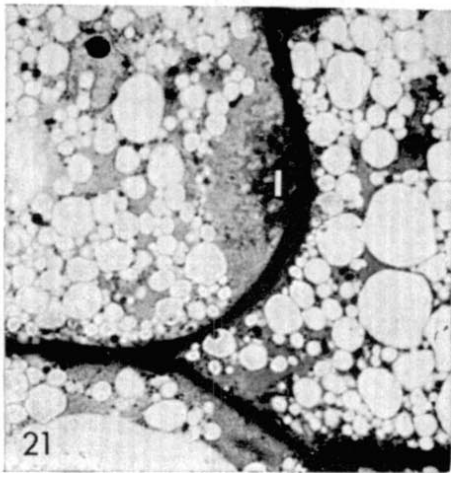
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elsewhere in the giant cell. The extensive division of cells around the giant cells, which compensates for their lateral expansion, is evident. Transfer cell-wall ingrowths are forming next to vascular elements, and it is remarkable that cytoplasmic vacuoles are absent from such regions (Fig. 21, 23). Numerous mitochondria, profiles of rough endoplasmic reticulum, and Golgi bodies occur in these regions, and numerous coated vesicles are found. Deposition of polysaccharides over wall regions where plasmodesmata are absent can become quite extensive. In Fig. 25, which illustrates a junction between three giant cells, the thin wall of the pit fields which is traversed by plasmodesmata is in marked contrast to the evenly thickened areas between the pit fields.

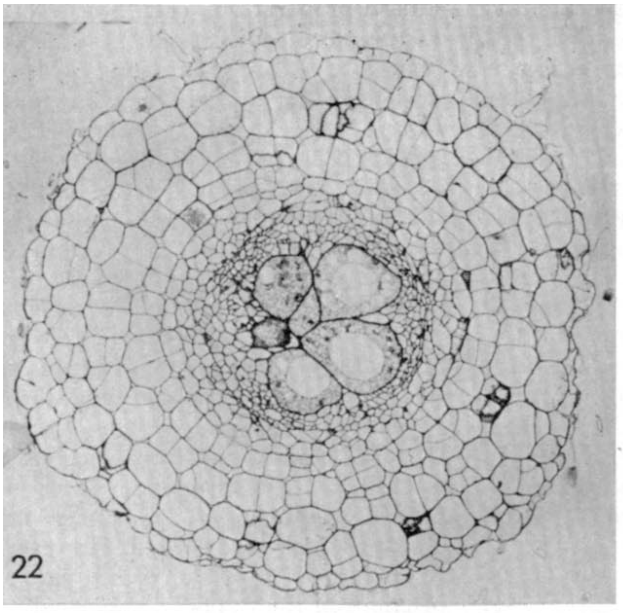
DISCUSSION

The major objections raised by previous workers to the hypothesis that giant cells induced by *Meloidogyne* spp. are formed by repeated mitosis without cytokinesis, without the participation of wall breakdown, include: the rapid elongation of developing giant-cells during the first 3 days after induction, lack of an explanation of how the developing giant-cells may expand without damaging or crushing surrounding cells, the apparent observations of gaps in walls, possible anomalies in measuring DNA content per nucleus, and chromosome counts which apparently do not fit the required ploidy sequence. These points are considered here in light of the present results.

After fixation, hydrolysis in HCl, staining with Schiff's reagent, treatment with pectinase, and dissection from the plant, Bird (5) examined giant-cells from *Vicia faba* and claimed that both cell fusion and cell-wall breakdown could be observed in squash preparations (Fig. 3 of reference 5). Examination of material at similar stages in the electron microscope provided no evidence of such wall breakdown and cell fusion. Indeed, the thickness of the cell plate and the cell wall where plasmodesmata occur, is close to the limit of resolution of the light microscope. A comparison with the effects of caffeine on cytokinesis (unpublished observations 31) has given little cause to doubt that the binucleate cells observed after 48 h of infection with *Meloidogyne incognita* and *javanica* larvae form by the same mechanism. Although the alignment of cell-plate vesicles is apparently normal they do not fuse, and they subsequently become dispersed. The control of spindle alignment at 24 h after infection does not appear abnormal. However, when the nuclei of binucleate cells divide, spindle orientation does appear abnormal, a point which has been emphasised in relation to mitoses observed at later stages as well (16, 30). Because we did not detect telophase nuclei in tetranucleate cells or at subsequent stages we cannot say whether cell-plate vesicles again line up and disperse. In no stage of giant-cell development have we seen "wall gaps"—that is, holes in a continuous wall. This type of wall gap is preserved in other situations where wall breakdown occurs, in nematode-transformed cells (e.g., *Heterodera* spp, 20; *Rotylenchulus* spp., 34; *Nacobbus* spp., 23), under nonpathological situations where wall breakdown occurs (1, 8, 36), or where incomplete walls form, such as in the guard cells or stomata of grasses (37). The occasional wall stubs having no apparent continuity with the wall on the opposite side of the cell may be explained by a process of 'build-up' rather than 'breakdown'.

In cells treated with methyl xanthines such as caffeine, cell plate vesicles either

FIG. 17-21. *17)* 72 h after infection. Part of a giant cell (GC) bulging into the lumen of a xylem element (X). *Meloidogyne incognita*, x5,200. *18)* 72 h after infection. An air space separates two giant cells (GC), and from the border of the space a folded wall stub projects into one cell (unlabelled arrow). The wall on the opposite side of the cell was also examined, and it lacked such a structure. *M. incognita*, x2,665. *19)* Enlargement of wall stub from Fig. 18. Note the dark lines running up its centre (arrows), apparently continuous with the wall by the air space. This suggests that the stub formed by collapse of the air space, with folding back of the wall onto itself. *M. incognita*, x17,000 *20)* Part of the nematode 72 h after infection. The lipid droplets (L) are surrounded by numerous glycogen rosettes (G). *M. incognita*, x25,500. *21)* Six days after infection. At this stage, wall ingrowths (I) are forming, in this case on a wall between two giant cells. The cytoplasm next to the ingrowths is free of vacuoles. *M. incognita* x820.



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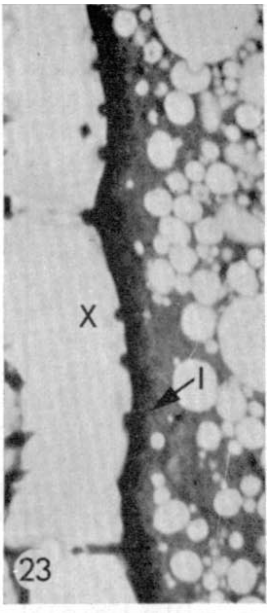

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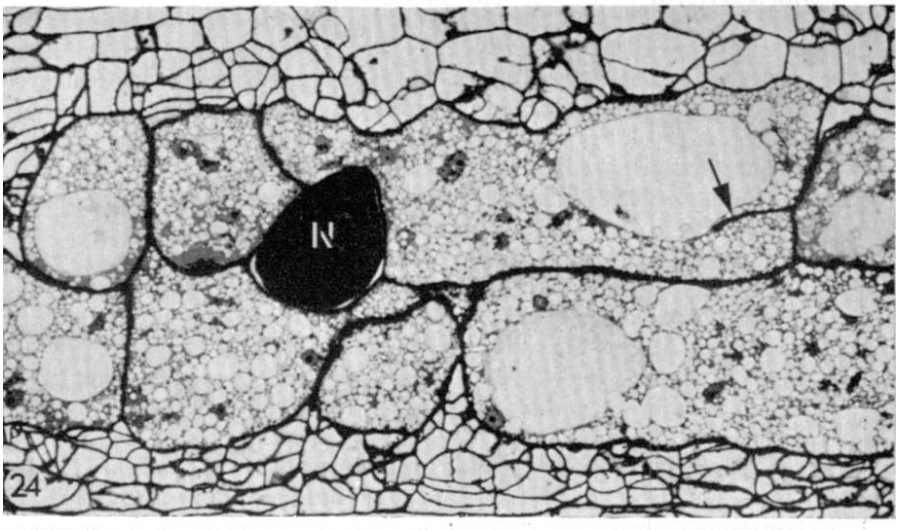

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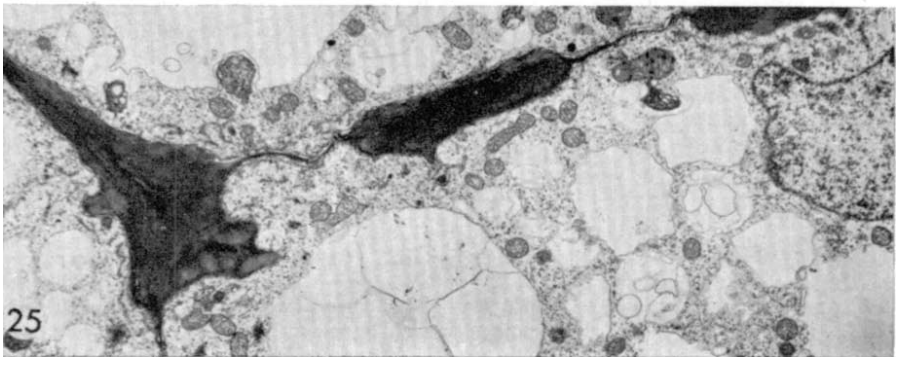

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fail to fuse or fuse partially to produce wall stubs (31). Subsequent polysaccharide deposition may consolidate and thicken such stubs. Thus, we explain the stub illustrated in Fig. 24 as having formed after telophase by partially successful fusion of cell plate vesicles between two daughter nuclei, probably located in the peripheral cytoplasmic layer. The cell wall may provide an anchor point for only one edge of the fusing vesicles. In three dimensions the stubs are probably flanges, and may be the structures described as crosswalls seen by scanning electron microscopy (Fig. 5 of reference 18).

The apparent occurrence of wall gaps has been reported (3, 21) from examination of serial sections of resin-embedded giant cells by light microscopy. We now believe that the interpretation of such micrographs is equivocal. The irregular outline of individual giant cells at many sites, perhaps contributed to by intrusive growth, but without wall breakdown and cell fusion, creates tylose-like out-growths with unusual shapes. Some other reports, in which pit fields between neighboring giant cells are indicated as wall gaps, are incorrect (29).

Rohde and McClure (35) exposed cotton seedlings to *M. incognita* larvae for 24 h and then transferred the seedlings to a solution containing ^{3}H-methyl-thymidine for subsequent growth. The occurrence of binucleate cells with unlabelled nuclei after subsequent autoradiography 24 h after infection was thought to support the contention that the cells must have arisen by wall breakdown and fusion of two cells. However, they do point out that this state may be explained by DNA replication prior to thymidine treatment. The latter explanation is consistent with our observations. As in previous studies (10, 13, 29, 30), Rohde and McClure (35) claim that wall breakdown occurs. One limitation of wax sections, however, is that they are prone to breaks in cell walls, and another is that when the orientation of a very thin wall changes from vertical to horizontal within a section it may apparently disappear. Such phenomena might well be interpreted as wall breakdown.

Cellulase production by root-knot larvae (7) need not be taken to support the hypothesis that giant cells form by wall breakdown: it is entirely possible that the enzyme is concerned with opening the pathway of intercellular migration for the invading nematode.

The rapid elongation of giant cells behind root tips is explicable from their position within the zone of elongation. Under our growth regime, roots of *Impatiens* increased in length by about 2.5 cm over 3 days. As mentioned previously, factors which might reduce elongation will therefore result in shorter giant cells. Intrusive growth, which may also contribute toward giant-cell elongation, has been documented in a number of situations, such as the formation of non-articulated laticifers, fibers and sclerieds (9) or in protophloem fibers (33). After 72 h when most elongation has occurred, lateral expansion begins. The rapid division of cells around the giant-cells is oriented in such a way as to minimise stress; no crushing of cells occurs, and increased wall deposition may occur at the ends of giant-cells, where stress may be greater. The giant-cells can be compared to an embryo sac cushioned in a compression-free space by the disposition of the surrounding walls (25, 26, 27). The concept of stress as a morphogenetic trigger seems applicable to the arrangement of those cells immediately outside giant-cells and to the patterns of division within the surrounding gall. The formation of numerous thin-walled meristematic cells next to the end of a giant-cell (see Fig. 15, 16) must be related to requirements for lateral ex-

FIG. 22-25. *22)* Cross section of a 6-day-old gall. Note the pattern of divisions in the gall cells which allow for expansion of the giant cells at its center. *Meloidogyne incognita,* x85. *23)* Wall ingrowths forming at the junction between a six-day-old giant cell and xylem elements. *M. incognita,* x958. *24)* Longitudinal section of giant cells within a 6-day-old gall, and associated nematode (N). The central cell vacuoles are greatly reduced, and the cytoplasm is filled with small vacuoles. A single wall stub (arrow) is present. Note also the irregular disposition of walls of cells outside the giant cells. *M. incognita,* x233. *25)* The junction between three 6-day-old giant cells. The variation in wall thickness is striking: the thin regions are pit fields where the wall is traversed by numerous plasmodesmata. *M. incognita,* x4,875.

pansion. Again, there is no wall breakdown at such sites, and the diagram of Dropkin (11) needs revising in this respect.

Measurements of the DNA content of giant-cell nuclei by microphotometer were found to be variable, and not to follow a ploidy sequence (4). The question of how this irregularity in DNA content may be explained (6) has many answers, including the obvious one that the ploidy of nuclei may in fact be irregular. Because of the lack of orientation of the mitotic spindles, chromosomes from different metaphase plates can fuse. Metaphase plates with different numbers of chromosomes have been documented (16). Aneuploidy resulting from chromosome connections at anaphase could cause some nuclei not to have DNA contents which fit an expected ploidy number (16). Measurements of DNA content of nuclei in the 'S' phase will give the same result, as will the possible amplification of specific genes such as those for ribosomal RNA. During later stages, the staining of giant-cell nuclei can be variable within one cell, so that there may indeed be more than one class of nucleus present. After mitoses have finished, the possibility also exists of variable endo-replication of DNA. A particularly good demonstration of how nuclei with different DNA contents may be obtained is provided by Hervás (15). Treatment of cells with γ-hexachlorocyclohexane during the start of mitosis causes a loss of spindle polarity, with formation of multipolar spindles. Subsequent treatment with caffeine prevents cytokinesis (see 15, Fig. 3) thus giving one cell with a variable number of nuclei of different sizes. The total chromosome count of such a cell is 4n, with, for example, 3 or 4 aneuploid nuclei. Since spindle orientation is somewhat random in developing giant-cells, and since cytokinesis does not occur, it is suggested that the failure to fit DNA contents of nuclei to an exact ploidy sequence does not mean that wall breakdown and cell fusion has occurred during their development.

Possibly the most difficult result to explain without invoking some cell-fusion events is the failure of Bird (5) to find, from chromosome counts of mitotic giant-cells, numbers of chromosomes which in one giant-cell add up to a ploidy number consistent with a repeated mitotic sequence. The explanation may be that it is difficult to obtain accurate ploidy counts with such high chromosome numbers. Also, the limits of individual giant cells may not have been accurately determined in squash preparations.

We have seen nothing at the electron-microscope level during the first 72 h after giant cell induction which would indicate that cell fusion has occurred. The relative area of wall that can be studied by thin section is necessarily limited, but study of giant-cell wall surfaces by scanning electron microscopy (18) of stages from 72 h after infection has not revealed wall gaps like those easily visible in syncytia induced by cyst-nematodes (17) or *Nacobbus* (23). The sequence of giant-cell development that we envisage to occur up to about 72 h after induction is provided in Fig. 26. Nuclei within the developing giant-cells arise solely by mitosis without cytokinesis. Between 3 and 6 days after induction, vascular elements differentiate outside the giant-cells, and transfer cell ingrowths are formed next to these (18). Mitosis continues within

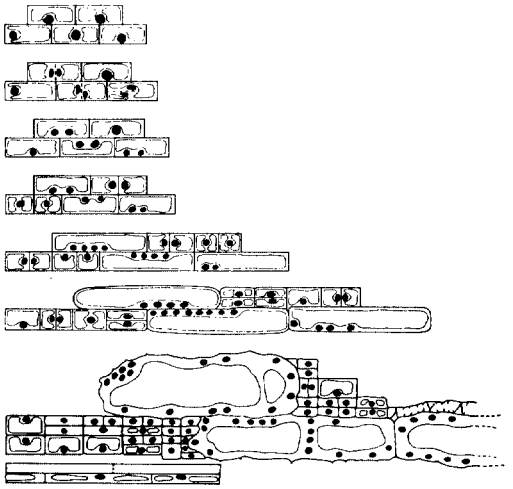

FIG. 26. Diagram of the events which occur up to about 72 h after the induction of three giant cells by *Meloidogyne* spp. Multinucleate cells develop after the failure of cytokinesis. Synchronous mitosis occurs within the same cell, though not necessarily at the same rate as in other cells. The cells are situated in the zone of extension behind the root tip; most of the elongation of the giant cells occurs before expansion begins. Vascular elements start to differentiate outside the giant cells, and rapid division of neighboring cells allows for their expansion.

giant-cells up to the time of nematode moulting (5), about 10 days.

The possibility cannot be totally ruled out that cell-wall breakdown occurs after 3 to 6 days from induction, but we now feel that if it occurs it is a much rarer phenomenon than previously thought. We agree with Huang and Maggenti (16) that the basic mechanism of development of giant cells involves repeated mitosis without cytokinesis.

Although plant cells usually have a single nucleus, a number of exceptions are found in normal plant development, closely resembling nematode-induced giant cells. For example, the replication of nuclei without subsequent cytokinesis occurs in the endosperm of many plants. Similarly developing metaxylem elements, as in the fern *Marsilea,* frequently grow to many times the volume of the surrounding cells within a few hundred microns of the initials, and their nuclei may undergo simultaneous divisions to contain 2, 4, 8, or 16 nuclei (28). The mechanisms which cause cell expansion and mitosis but prevent cytokinesis may be related in some way, and the hypothesis that an inhibitor of cyclic AMP phosphodiesterase is involved (such as caffeine and its analogues), which would raise levels of cyclic AMP, with all the attendant consequences, is attractive. However, there is no evidence to support that hypothesis. Further discussion on possible mechanisms of giant-cell formation, including the possible involvement of hormones and the effect of nutrient removal by the nematode, is provided by Jones and Dropkin (18).

LITERATURE CITED

1. BAL, A. K., and J. F. PAYNE. 1972. Endoplasmic reticulum activity and cell wall breakdown in quiescent root meristems of Allium cepa L. Z. f. Pflanzenphysiol. 66:265-272.
2. BIRD, A. F. 1961. The ultrastructure and histo-chemistry of a nematode-induced giant cell. J. Biophys. Biochem. Cytol. 11:701-715.
3. BIRD, A. F. 1972. Cell wall breakdown during the formation of syncytia induced in plants by root-knot nematodes. Int. J. Parasitol 2: 431-432.
4. BIRD, A. F. 1972. Quantitative studies on the growth of syncytia induced in plants by root-knot nematodes. Int. J. Parasitol 2:157-170.
5. BIRD, A. F. 1973. Observations on chromosomes and nucleoli in syncytia induced by Meloidogyne javanica. Physiol. Plant Path. 3:387-391.
6. BIRD, A. F. 1974. Plant response to root-knot nematode. Annu. Rev. Phytopathol. 12:69-85.
7. BIRD, A. F., W. J. S. DOWNTON, and J. S. HAWKER. 1975. Cellulase secretion by second stage larvae of the root-knot nematode (Meloidogyne javanica). Marcellia 38:165-169.
8. BOWES, B. G. 1972. Fine structural observations on necrotic adventitious root apices in Glechoma hederacea L. Protoplasma 74:41-52.
9. CARR, D. J. 1976. Plasmodesmata in growth and development. Pages 243-289 *in* B. E. S. Gunning, and A. W. Robards, eds. Intercellular Communication in Plants: Studies on Plasmodesmata. Springer-Verlag.
10. CHRISTIE, J. R. 1936. The development of root-knot nematode galls. Phytopathology 26: 1-21.
11. DROPKIN, V. H. 1969. Cellular responses of plants to nematode infections. Annu. Rev. Phytopathol. 7:101-122.
12. DROPKIN, V. H., and J. ACEDO. 1974. An electron microscope study of glycogen and lipid in female Meloidogyne incognita (Root-knot Nematode). J. Parasitol. 60:1013-1021.
13. DROPKIN, V. H., and P. E. NELSON. 1960. The histo-pathology of root-knot nematode infections in soybeans. Phytopathology 50: 442-447.
14. ENDO, B. Y., and W. P. WERGIN. 1973. Ultrastructural investigation of clover roots during early stages of infection by the root-knot nematode, Meloidogyne incognita. Protoplasma 78:365-379.
15. HERVAS, J. P. 1976. Multinucleate plant cells. I. Aneuploidy in proliferating population. Exp. Cell Res. 97:203-212.
16. HUANG, C. S., and A. R. MAGGENTI. 1969. Mitotic aberrations and nuclear changes of developing giant cells in Vicia faba caused by root-knot nematode, Meloidogyne javanica. Phytopathology 59:447-455.
17. JONES, M. G. K., and V. H. DROPKIN. 1975. Scanning electron microscopy of syncytial transfer cells induced in roots by cyst-nematodes. Physiol. Pl. Path. 7:259-263.
18. JONES, M. G. K., and V. H. DROPKIN. 1976. Scanning electron microscopy of nematode-induced giant transfer cells. Cytobios. 15: 149-161.
19. JONES, M. G. K., and B. E. S. GUNNING. 1976. Transfer cells and nematode induced giant cells in Helianthemum. Protoplasma 87: 273-279.
20. JONES, M. G. K., and D. H. NORTHCOTE. 1972. Nematode-induced syncytium—a multinucleate transfer cell. J. Cell Sci. 10:789-809.
21. JONES, M. G. K., and D. H. NORTHCOTE. 1972. Multinucleate transfer cells induced in coleus roots by the root-knot nematode, Meloidogyne arenaria. Protoplasma 75:381-395.
22. JONES, M. G. K., A. NOVACKY, and V. H.

DROPKIN. 1975. Transmembrane potentials of parenchyma cells and nematode-induced transfer cells. Protoplasma 85:15-37.

23. JONES, M. G. K., and H. L. PAYNE. 1977. Scanning electron microscopy of syncytia induced by Nacobbus aberrans in tomato roots. Nematologica 23:172-176.
24. KOSTOFF, D., and J. KENDALL. 1930. Cytology of nematode galls in Nicotiana roots. Zentralbl. Backteriol. Parasitenk. Hyg. Abt. II 81:86-91.
25. LINTILHAC, P. M. 1974. Differentiation, organogenesis, and the tectonics of cell wall orientation. II. Separation of stresses in a two-dimensional mode. Am. J. Bot. 61:135-140.
26. LINTILHAC, P. M. 1974. Differentiation, organogenesis, and the tectonics of cell wall orientation. III. Theoretical considerations of cell wall mechanisms. Am. J. Bot. 61:230-237.
27. LINTILHAC, P. M., and W. A. JENSEN. 1974. Differentiation, organogenesis, and the tectonics of wall orientation. I. Preliminary observations on the development of the ovule in cotton. Am. J. Bot. 61:129-134.
28. LIST, A. 1963. Some observations on DNA content and nuclear volume growth in the developing xylem cells of certain higher plants. Am. J. Bot. 50:320-329.
29. LITTRELL, R. H. 1966. Cellular responses of Hibiscus esculentus to Meloidogyne incognita acrita. Phytopathology 56:540-544.
30. OWENS, R. G., and H. N. SPECHT. 1964. Root-knot histo-genesis. Contrib. Boyce Thompson Inst. 22:471-489.
31. PAUL, D. C., and C. W. GOFF. 1973. Comparative effects of caffeine, its analogues and calcium deficiency on cytokinesis. Exp. Cell Res. 78:399-413.
32. PAULSON, R. E., and J. M. WEBSTER. 1970. Giant cell formation in tomato roots caused by Meloidogyne incognita and Meliodogyne hapla (Nematoda) infection, A light and electron microscopy study. Can. J. Bot. 48: 271-276.
33. PIZZOLATO, T. D., and C. HEIMSCH. 1975. A striking example of intrusive growth in protophloem fibers of Pelargonium. Bull. Torrey Bot. Club. 102:53-54.
34. REBOIS, R. V., P. A. MADDEN, and B. J. ELDRIGE. 1975. Some ultrastructural changes induced in resistant and susceptible soybean roots following infection by Rotylenchulus reniformis. J. Nematol. 7:122-139.
35. ROHDE, R. A. and M. A. McCLURE. 1975. Autoradiography of developing syncytia in cotton roots infected with Meloidogyne incognita. J. Nematol 7:64-69.
36. SASSEN, M. M. A. 1965. Breakdown of the plant cell wall during the cell fusion process. Acta. Bot. Neerl. 14:165-196.
37. SRIVASTAVA, L. M., and A. P. SINGH. 1972. Stomatal structure in corn leaves. J. Ultrastruct. Res. 39:345-363.