

THE ULTRASTRUCTURE AND HISTOCHEMISTRY OF A NEMATODE-INDUCED GIANT CELL

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ABSTRACT

The development of giant cells induced by the nematode *Meloidogyne* in tomato roots has been followed under controlled growth conditions and the ultrastructure and histochemistry of these structures have been examined. Entry of the nematode larvae into the roots took place within 24 hours; giant cell formation started on the 4th day and involved breakdown of the cell walls accompanied by thickening of a surrounding giant cell wall and an increase in density and area of the cytoplasm. The nuclei increased in number by simultaneous mitosis throughout a single giant cell. The peak of cytoplasmic density was reached after moulting and during egg production. The rate of protein synthesis in the giant cell is correlated with the rate of growth of the nematode. The giant cell wall is a thick, irregularly surfaced structure which contains all the normal polysaccharide components of a cell wall. The cytoplasm is rich in protein and RNA and contains mitochondria, proplastids, Golgi bodies, and a dense endoplasmic reticulum. The nuclei are large and irregular in shape and contain large nucleoli and a number of Feulgen-positive bodies scattered irregularly along the nuclear envelope. The nucleolus contains RNA and fat as well as Feulgen-positive granules which are revealed after treatment with ribonuclease. It consists of a dense outer cortex surrounding a much lighter central core and is connected at times with the Feulgen-positive bodies in the nucleus. Speculation is provided on the role of these bodies in cytoplasmic protein synthesis.

INTRODUCTION

The giant cells described in this paper are multinucleate structures which usually develop in the vascular tissues of plants parasitized by nematodes of the genus *Meloidogyne*. The term giant cell used in this context is perhaps a little confusing as this cell in no way resembles some other types of giant cells which have been described, for instance, those induced in tissue cultures after treatment with x-rays (46). Nevertheless, because this term has been used repeatedly in the literature and is familiar to plant nematologists, it has been retained in this paper, although perhaps the term syncytium or coenocyte would be more accurate.

These structures were first described by Treub (48) 75 years ago and have been investigated numerous times since then (12-14, 17-19, 26, 27, 35, 37, 39, 40, 47, 48). The combined researches of these investigators have led to the discovery of much data about the development and histology of giant cells which may be summarized as follows:

Infection of roots takes place within 24 hours of exposure. The larvae migrate through the cortex and usually come to lie with their bodies in this region and their heads in the vascular cambium. Generally, only cells from the stele are transformed into giant cells but they have been found in the

cortex and will develop in stems and leaves under experimental conditions (31). Cell multiplication followed by cell-wall breakdown gives rise to large multinucleate giant cells which have thick walls and are filled with a dense granular cytoplasm; their nuclei and nucleoli are enlarged and the nuclei are irregularly shaped. Giant cells contain more protein, amino acids, and nucleic acids than normal cells.

Previous investigators differ on certain aspects of giant cell development, particularly with regard to the method of formation of the large nuclei and to the components of the dense cytoplasm.

In the present study the ultrastructure and histochemistry of giant cells grown under controlled conditions are examined and observations are made on the influence of the nematode stimuli on protein synthesis in these structures.

MATERIALS AND METHODS

All seeds in each experiment came from a single susceptible tomato plant of the Pan America variety. They were sterilized, washed, and germinated as previously described (6). Plants used in the cytophotometric analysis were from a single clone.

Germinating seeds were planted in rows in sterile "perlite" and when the seedlings were 1 to 2 inches in height with several lateral roots, freshly hatched *M. javanica* larvae were introduced around their roots. Plants were exposed to infection for 24 hours in a constant environment of 12 hours of light and 12 hours of darkness, a day temperature of 24°C ($\pm 1^\circ\text{C}$) and a night temperature of 18°C ($\pm 1^\circ\text{C}$). The plants were then removed, washed in distilled water, transplanted into perlite in sterile pots and grown under the above-mentioned conditions of constant environment.

Material for microscopic study was obtained from two series of experiments in which infected seedlings were grown in perlite using a nutrient solution previously described (6). The plants were grown for 30 days in the first experiment, and for 51 days in the second. Plants were harvested each day for the first 9 days and subsequently twice weekly to the 30th day and then at weekly intervals to the 51st day. Their roots were fixed, embedded, sectioned, stained, and examined.

Light Microscopy

The fixatives used were:

Zenker's (without acetic), Bouin's (3), Carnoy's, and formol-calcium. Ethanol was used as the dehydrating agent, benzene as a clearing agent, and the

specimens were embedded in paraffin wax with a m.p. of 58°C. Mayer's albumin (2) or gelatin (25) was used as an adhesive. Serial sections were cut on a Spencer microtome at 10 μ .

The following staining methods and histochemical procedures were used:

The gallocyanin technique (20)

The Feulgen reaction (16)

The toluidine blue reaction (43)

The mercuric-bromophenol blue method (7)

The periodic acid-Schiff extraction technique (25)

The Sudan black B method for lipids in paraffin sections (43)

The ribonuclease technique (7)

The perchloric acid technique (21)

The trichloroacetic acid technique (44)

Cytophotometry

A comparative estimation of the protein content of different giant cells was made by measuring the percentage light transmission at a wavelength of 610 $m\mu$ through 10 μ sections stained with mercuric-bromophenol blue (7), using a Lison histophotometer. Great care was taken to ensure that the areas of cytoplasm used for light transmission measurements were free of nuclei. In addition, sections cut before and after the one on which measurements were made had to be nuclei-free in the same region. Five readings were taken through the cytoplasm of each giant cell of each section. Numerous sections from different galls were examined.

Electron Microscopy

Pieces of root 2 to 3 mm long containing the nematodes were cut from a fresh plant and quickly placed in fixative. The fixatives and stains used in these experiments were 1 per cent buffered osmium tetroxide (veronal-acetate, pH 7.2) at 5°C for 30 minutes and 2 per cent and 5 per cent potassium permanganate in distilled water at 5°C or 25°C for 30 minutes (36). The material was then washed repeatedly in Ringer's isotonic plant solution or water for 30 minutes and dehydrated in either ethanol or acetone (33). In some cases, the material was treated before or during dehydration with ferric chloride (5), phosphotungstic acid, or uranyl acetate (33). The last stages of fixation and dehydration and the early stages of embedding in methacrylate or Araldite were carried out *in vacuo*.

Sections were cut to give a silver interference colour (41) using a diamond knife and a Si-Ro-Flex ultramicrotome. They were mounted on nitrocellulose-coated grids and viewed under either a Philips 100 Kv Metalux or a Siemens Elmiskop 1 electron microscope.

OBSERVATIONS

1. *Growth and Development of Giant Cells*

Under the controlled conditions of these experiments infection was well established within 24 hours of the nematodes being introduced around the roots. Other workers (12, 19, 35) have reported similar rates of entry of *Meloidogyne* larvae into roots.

Plants harvested immediately after exposure to infection showed that entry could be through a single aperture, presumably made by the first larva to enter the root, through which others appeared to follow, as has been described by Peacock (42). However, other roots from this first harvest contained several larvae partly embedded in the root and grouped close together, as well as single larvae in the process of entering the root. Thus, entry could occur through many separate apertures. When these were close together cell breakdown sometimes resulted in a fairly large aperture through which subsequent infection occurred (Fig. 1).

Hypertrophy of the cortical cells occurred within 24 hours of the nematode's entry and gave rise to the characteristic galling associated with this genus. However, this symptom does not occur in all species of plants, as Treub (48) and many others have since noted. Also, this reaction can be initiated merely by surface feeding on the root without subsequent entry (32).

Under the conditions of these experiments giant cell formation was first observed on the 4th day (Fig. 2). Breakdown of several cell walls occurred in the region adjacent to the nematode's head, and several large, irregular nuclei, each containing a large strongly stained nucleolus, became apposed to each other. The nuclei contained gallocyanin-positive bodies scattered along their membranes. Both the nuclei and nucleoli were much larger than normal. Their dimensions remained the same during subsequent development of the giant cell although they increased in numbers. Giant cell development involved further breakdown of cell walls accompanied by thickening of the giant cell wall and an increase in density and area of the cytoplasm.

Serial sections of giant cells harvested at 9 and 13 days showed that within this period the giant cell was close to the average maximum size that it would reach, *i.e.*, about 250 μ . However, the cytoplasm at 13 days (Fig. 3 *a*) was still vacuolated

and less dense than at 30 days (Fig. 4). Mitosis within a giant cell was observed at nine days and at thirteen days (Fig. 3 *b*). It is interesting to note that in both cases all the nuclei present in one giant cell of the group influenced by a single nematode were undergoing mitosis, whilst all the nuclei in the adjacent giant cells were in the resting stage with intact nuclear membranes.

Examination of numerous serial sections showed that the giant cells in a particular group associated with one nematode were connected with each other, as has been observed and described previously (12, 35). However, these connections usually appeared to be at the extremities of the giant cells and did not appear to result in close physiological co-ordination because of the individual manner in which mitosis occurred within one giant cell of a group. Each giant cell may possibly be individually stimulated by the parasite, perhaps shortly after feeding. The method of feeding in this genus has been described (29, 30) and it appears that the nematode feeds in turn on the giant cells within reach of its mobile head.

The cytoplasm in giant cells was most dense after the nematode had moulted and was starting to produce an egg sac and eggs, *i.e.*, when the demands of the nematode were greatest. In these experiments moulting was observed from the 20th to the 27th days. Full-grown female nematodes were observed on the 30th day.

2. *Nematode Growth and Protein Synthesis in the Giant Cell*

Attempts were made to culture giant cells in the absence of the nematode, using the synthetic medium described by Cutter (15). These experiments were unsuccessful, although various workers (49, 50) have succeeded in growing members of the *Heteroderidae* in sterile root cultures, presumably with normal giant cell formation. Apparently, either the method used was unsuitable, *e.g.*, the giant cell might have been damaged when the parasite was removed, or the stimulus from the nematode might be essential for giant cell development. Because the tissue culture experiments were tedious, time-consuming, and only significant if they could show that nematode-free giant cell tissue could be cultured *in vitro*, they were not repeated. Instead, the following experiments were undertaken in an attempt to discover whether the nematode stimulus is essential for giant cell formation.

Three plants of a clone of the Pan America variety were used. One was left untreated and two were exposed to nematode infection for 48 hours so that a heavy infestation was built up. All three were then grown in water cultures on full nutrient in a cooled glass house. The plants and nematodes grew rapidly. Some roots were taken from the infected plants on the 14th day and the roots of one of them were immersed in water at 50°C for 10 minutes, after which the plant was returned to the water culture. This treatment killed all the nematodes and retarded growth and water uptake of the plant. However, all plants were healthy when harvested on the 22nd day; some of the younger roots had been injured, but the older roots containing the galls looked healthy and sections of the root taken on either side of the gall showed no cellular abnormality. The material was fixed in formol-calcium, dehydrated, and embedded. Longitudinal serial sections, 10 μ thick, were cut and then stained with mercuric-bromophenol blue (7). This stain, which has been used for quantitative estimation of protein (34), was employed to get a comparative estimate of the protein content in these giant cells, using a cytophotometer. Percentage transmission

was converted to extinction and the results obtained are summarized in Fig. 5.

It can be seen from this histogram that the nematode stimulus had a marked effect on protein synthesis. Whether or not this is a direct response to nematode stimuli or merely the culmination of a chain of events triggered off in response to an initial stimulus remains unanswered. For example, it may be argued that the set-back to the plant brought about by heat treatment may have also resulted in a breakdown of protein synthesis and further giant cell development. However, because of the apparently healthy state of the heat-treated gall and the surrounding tissues, it seems that there is some evidence here to support the theory that this synthesis of protein is a direct response to a nematode stimulus. Normal cells in control, and treated and untreated infected plants contained much the same amount of protein, but slight differences between histogram columns *P*, and *N* and *O* were observed. This was probably due to the irregular distribution of cytoplasm in these cells which made it impossible to guarantee uniform thickness of cytoplasm throughout the 10 μ thick section.

From these results it is clear that the dense

FIGURE 1

Low power longitudinal section of a young tomato root after 24 hours' exposure to *M. javanica* larvae. Fixed in Zenker's and stained with gallocyanin and showing several nematodes (*nem*). \times 340.

FIGURE 2

Longitudinal section of a tomato root viewed under oil immersion. Fixed in Zenker's 4 days after infection and stained with gallocyanin. Showing the start of giant cell formation; the cell wall (*cw*) is breaking down (*cwb*), the nuclear envelope (*ne*) is just visible, and the nucleoli (*nu*) are large and well stained. The nematode (*nem*) has been cut in transverse section. \times 1700.

FIGURES 3 *a* and *b*

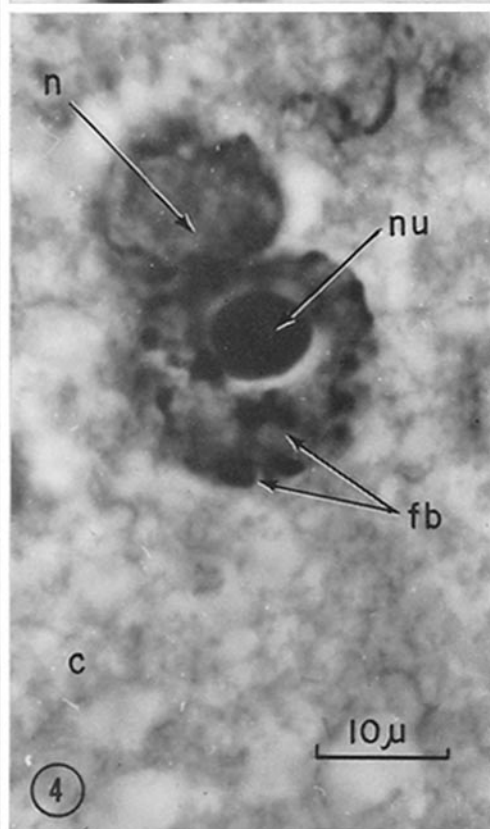
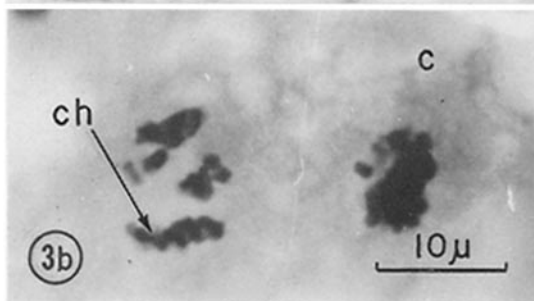
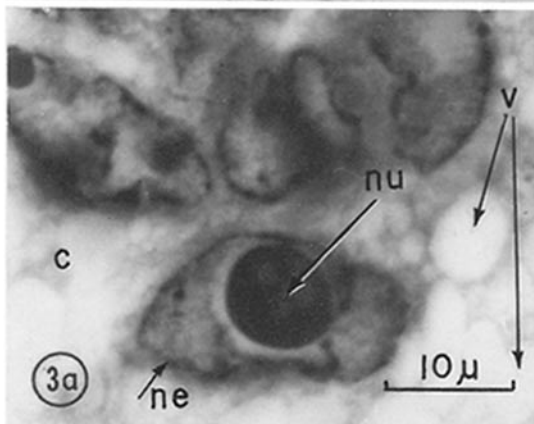
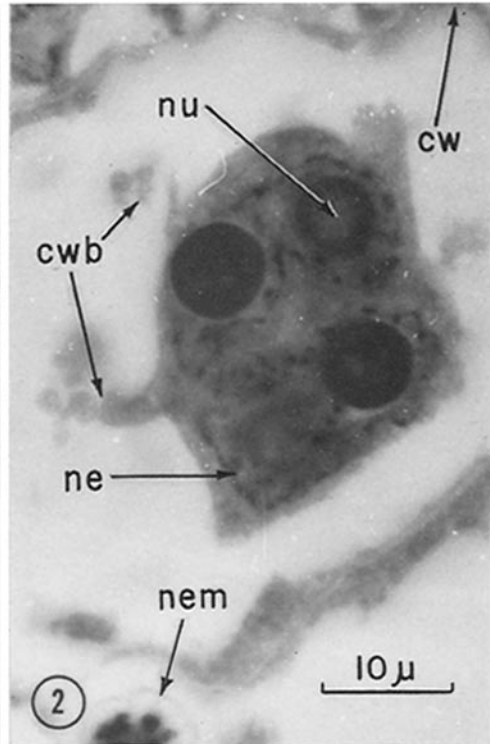
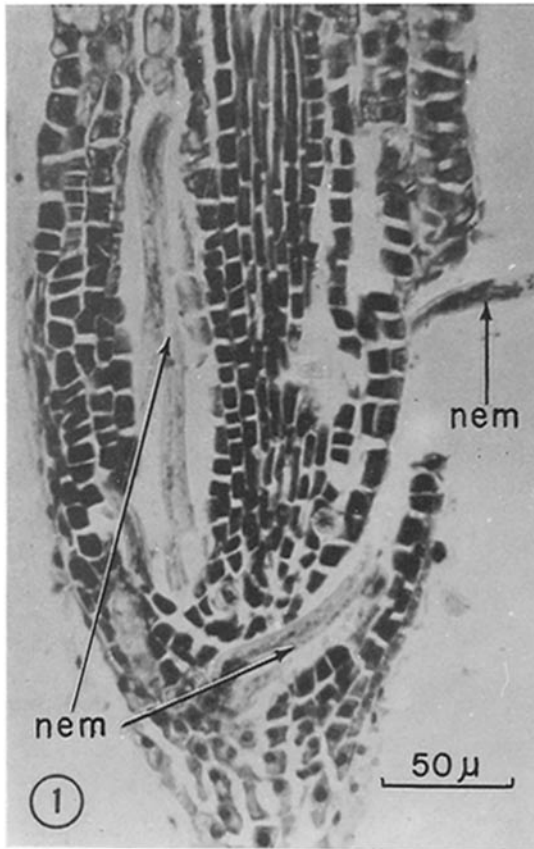
Longitudinal sections of a tomato root viewed under oil immersion. Fixed in Zenker's 13 days after infection and stained with gallocyanin.

Fig. 3 *a*. Large nucleoli (*nu*), vacuoles (*v*), cytoplasm (*c*), and more pronounced nuclear envelopes (*ne*). \times 1700.

Fig. 3 *b*. Chromosomes (*cv*) visible during nuclear division in an adjacent giant cell of the same group. \times 1700.

FIGURE 4

Longitudinal section of a tomato root viewed under oil immersion. Fixed in Zenker's 30 days after infection and stained with gallocyanin. Showing dense cytoplasm (*c*), nucleus (*n*), nucleolus (*nu*), and Feulgen-positive bodies (*fb*). \times 1700.



granular appearance of the cytoplasm in giant cells is closely associated with protein, and that the rate of protein synthesis in these structures is correlated with the demands of the nematode.

3. Histochemistry and Ultrastructure of the Mature Giant Cell Wall, Cytoplasm, Nuclei, and Nucleoli

(A) CELL WALL: The mature giant cell wall is from five to ten times thicker than the cell walls

of the surrounding cells. However, in places it is of dimensions similar to those of the surrounding cell walls (Fig. 9). The giant cell wall has an irregular surface with numerous projections jutting out into the cytoplasm. As has been mentioned, the giant cells associated with one nematode are connected to each other but these connections are usually only detected in serial sections, and most sections show three or four discrete giant cells each with its own intact wall.

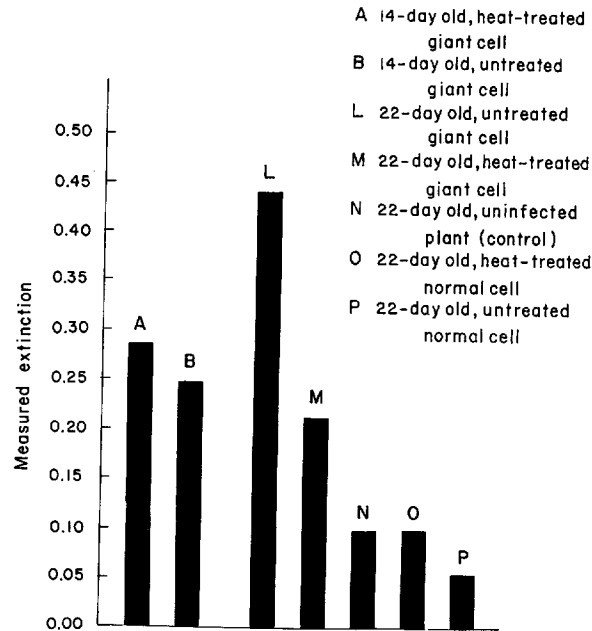


FIGURE 5

Histogram showing the effect that the stimulus from the nematode has on protein synthesis in giant cells. These extinction figures were obtained from 10μ thick sections of the cytoplasm of mercuric-bromophenol-blue-stained giant and normal cells using a cytophotometer with a filter giving maximum transmission at $610 m\mu$.

FIGURE 6

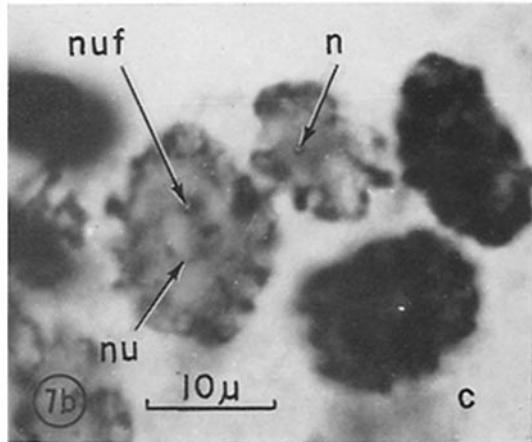
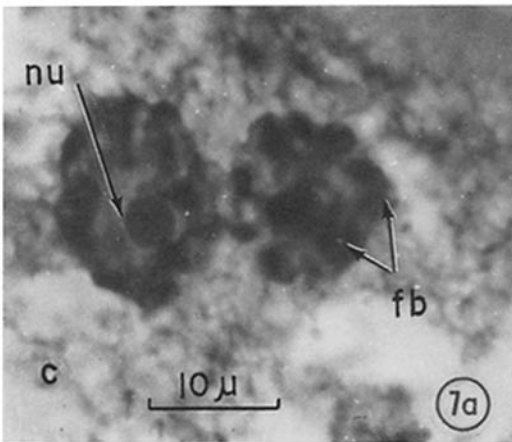
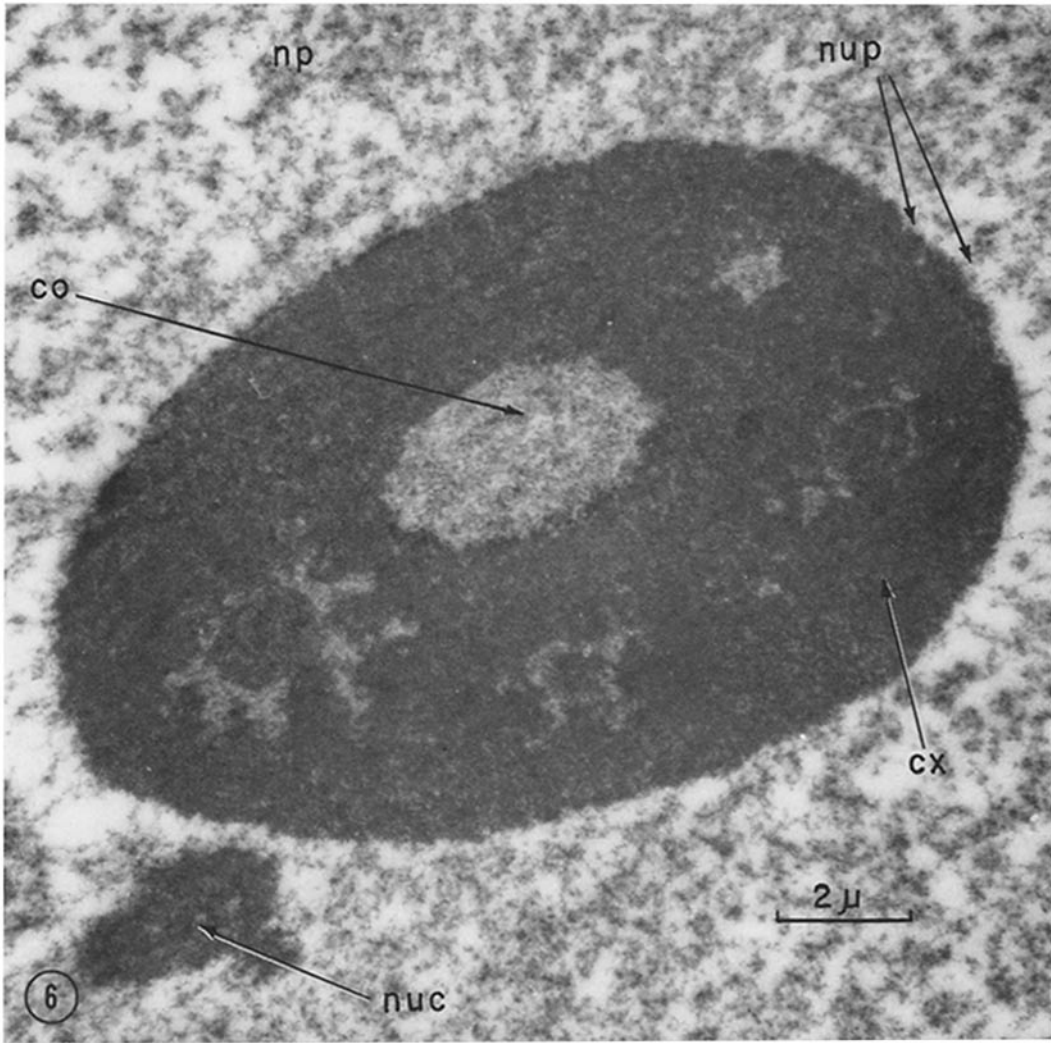
Electron micrograph of a nucleolus in a 23-day-old giant cell fixed in 1 per cent osmium tetroxide for 30 minutes at 5°C and treated with ferric chloride, phosphotungstic acid, and uranyl acetate and embedded in Araldite. Showing part of a nucleolar connection (*nuc*), the core (*co*), and cortex (*cx*) of the nucleolus and the nucleolar processes (*nup*) connecting nucleolus and nucleoplasm (*np*). $\times 1700$.

FIGURES 7 a and b

Longitudinal sections of a tomato root viewed under oil immersion. Fixed in formalcalcium and stained with gallocyanin.

Fig. 7 a. Nucleolus (*nu*), Feulgen-positive bodies (*fb*) and cytoplasm (*c*). $\times 1700$.

Fig. 7 b. This has been treated with ribonuclease and shows the nuclei (*n*), nucleolar Feulgen-positive granules (*nuf*), and the unstained RNA-containing nucleolus (*nu*) and cytoplasm (*c*). $\times 1700$.



Information on the composition of the giant cell wall was obtained using the cytochemical technique described by Jensen (25). Formol-calcium-fixed roots containing mature giant cells were sectioned serially and then subjected to different extraction procedures as described (25). They were then tested for carbohydrates using the P.A.S. technique. Control slides which were not subjected to any extraction showed giant cell walls staining a uniform deep red colour. These techniques showed that all normal polysaccharide components of cell wall, *viz.*, pectic substances, hemicellulose, insoluble non-cellulosic polysaccharides, and cellulose appear to be present in the giant cell wall. Tests for lignin gave negative results and it seems that this substance is absent from giant cell walls of the tomato. These results are in accord with those of Dropkin and Nelson (19) who obtained positive results for cellulose and pectin and negative results for lignin, suberin, and starch in the giant cell walls of soybean.

(B) CYTOPLASM: The cytoplasm of the mature giant cell is dense and granular and contains much protein. It also contains RNA as it gives a positive stain with gallocyanin but not if the section is pretreated with ribonuclease (7), trichloroacetic acid (44), or perchloric acid (21). The cytoplasm also contains traces of carbohydrate and fat, giving faint positive reactions to the P.A.S. and Sudan black B reagents. Electron micrographs (Figs. 9 to 12) show that the cytoplasm contains many mitochondria, proplastids, Golgi bodies, and a dense endoplasmic reticulum. It

closely resembles the cytoplasm of meristematic tissue during interphase and is similarly associated with active metabolic activities such as protein synthesis.

(C) NUCLEI AND NUCLEOLI: A typical giant cell nucleus is large and irregular in shape (Figs. 4, 7 *a* and *b*, 8, 11 to 13) and contains a large nucleolus (Figs. 2, 3 *a*, 4, 6, 8, 12, and 13), and a number of Feulgen-positive bodies usually scattered irregularly along the nuclear membrane and which, at times, are connected to the nucleolus (Figs. 4, 6, 12, and 13). The nucleoplasm is granular and bounded by a double-membraned nuclear envelope which is often disrupted in the region of the Feulgen bodies (Fig. 11). In a few sections these structures appear to be in the process of extrusion into the cytoplasm. These Feulgen bodies are similar in internal structure to adjacent bodies in the cytoplasm, which differs in that they are surrounded by a membrane and which have been termed proplastids (Fig. 11). Some sections examined in these experiments showed a close association between mitochondria and the nuclear envelope. This phenomenon has been described in different tissues by many workers and is thought to be at least one of the ways in which mitochondria are formed (24).

Giant cell nucleoli (Figs. 6, 8, and 12) resemble normal nucleoli in that they lack a surrounding membrane and contain RNA (Fig. 7 *a*) which can be removed by ribonuclease (Fig. 7 *b*). The nucleoli also contain DNA (Fig. 7 *b*) in the form of a few small Feulgen-positive granules which

FIGURE 8

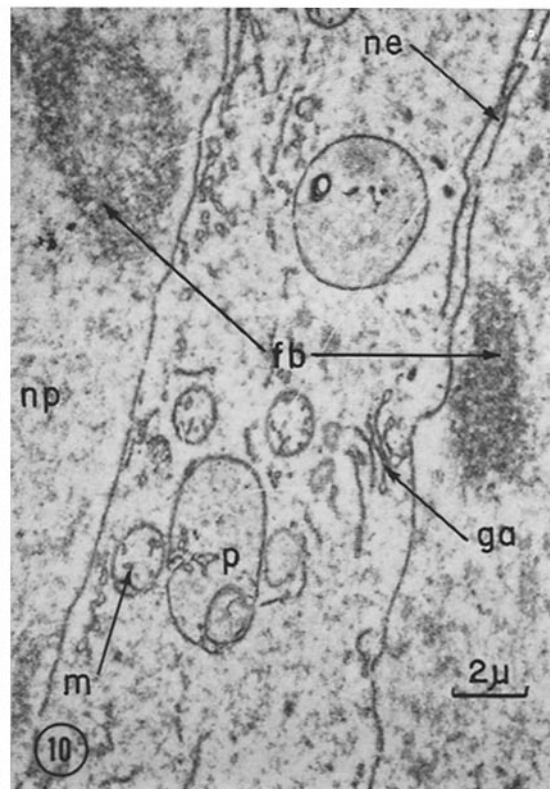
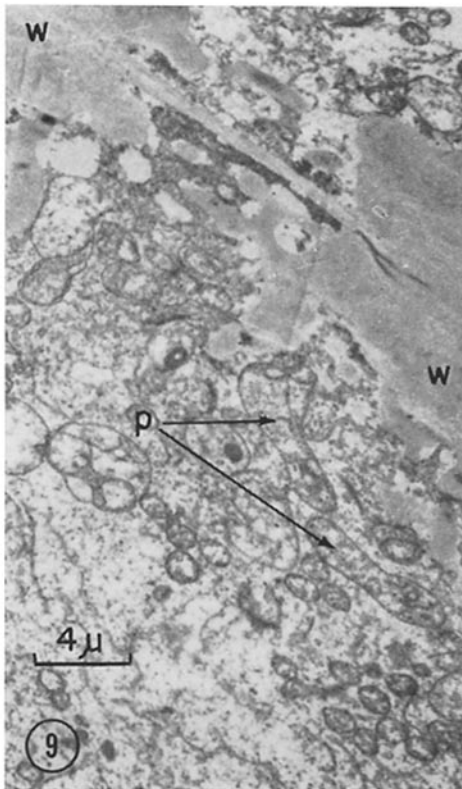
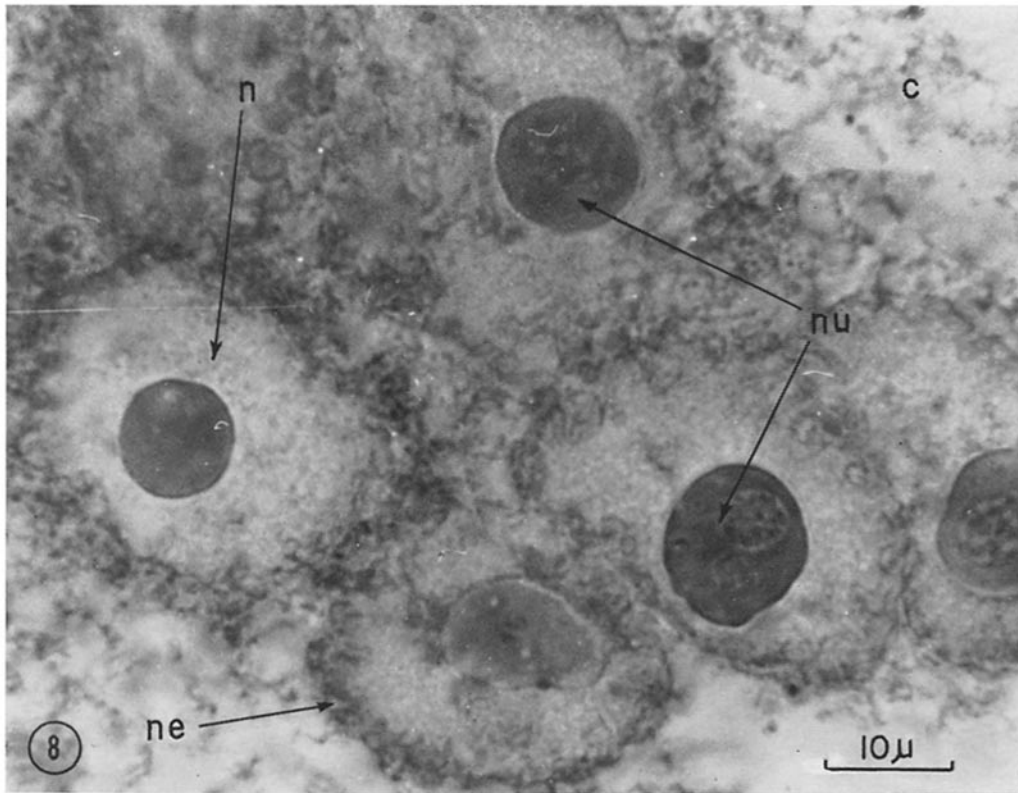
Longitudinal section of a tomato root viewed under oil immersion. Fixed in formol-calcium and stained with sudan black B. Showing large fat-containing nucleoli (*nu*), dense cytoplasm (*c*), and large nuclei (*n*), with irregular nuclear envelopes (*ne*). $\times 3000$.

FIGURE 9

Electron micrograph of part of a giant cell wall in a 23-day-old giant cell fixed in 1 per cent osmium tetroxide for 30 minutes at 5°C and treated with ferric chloride, phosphotungstic acid, and uranyl acetate and embedded in Araldite. Showing dense cytoplasm with proplastids (*p*) and part of the giant cell wall (*w*). $\times 5000$.

FIGURE 10

Electron micrograph of part of a 20-day-old giant cell fixed in 2 per cent potassium permanganate for 30 minutes at 5°C and embedded in methacrylate. Showing two nuclei with nucleoplasm (*np*), nuclear envelopes (*ne*), and Feulgen-positive bodies (*fb*) and between them some cytoplasm with a Golgi apparatus (*ga*), proplastids (*p*), and mitochondria (*m*). $\times 25,000$.



appear at times to be connected to the Feulgen bodies in the nucleoplasm; there is also a concentration of DNA around the nucleolus which can be seen in ribonuclease-treated specimens as a Feulgen-positive halo. The nucleoli also contain fat as they stain strongly with sudan black B and osmium tetroxide (Figs. 6, 8, and 12). Under the electron microscope (Figs. 6 and 12) the nucleoli of the giant cells are seen to consist of a dense outer cortex surrounding a much lighter central core. Nucleoli with this type of structure have been described in plant roots (28). In addition to the connections between the Feulgen bodies and the nucleolus there are numerous fine processes radiating from the cortical region of the nucleolus and connecting with the granular nucleoplasm (Fig. 6).

The structure of the mature giant cell and its various components at different magnifications together with an adult female *Meloidogyne* and its egg sac are displayed, drawn to scale, in the three-dimensional diagrams (Fig. 13).

DISCUSSION

Extreme hypertrophy or galling and giant cell formation in response to the genus *Meloidogyne* appear to be two quite different phenomena. The former reaction, which can occur within a few hours of infection, may be produced merely by surface feeding without actual entry of the larvae into the root (32). On the other hand, giant cell development is established over a much longer period of time, usually in the vascular tissues, and results in complex structural alterations within the root.

Most workers have not been able to detect mitosis occurring within the giant cells and have come to the conclusion that it is limited to the first few days of development, after which nuclear division takes place amitotically or cell wall breakdown at the ends of the giant cells gives rise to more nuclei. However, Nemeč (39) detected mitosis in young giant cells and maintained that this occurred several times during the life of the giant cell and that such phenomena, which took place rapidly, could pass unnoticed in the course of a month or two. Nemeč's work has only recently been substantiated by Krusberg and Nielsen (27) who showed that mitosis occurred in the giant cells of a vine cutting.

The observations described in this paper have given further support to Nemeč's theory of nuclear multiplication by mitosis in giant cells, as opposed to the theory of amitotic budding of nuclei (47). Nemeč's suggestion that the dense and granular giant cell cytoplasm is associated with intense metabolism is undoubtedly true, and both this paper and that of Owens and Novotny (40) provide evidence to support this. Nemeč went even further and described two types of mitochondria, a long filamentous type and a thicker round-ended type. However, it seems unlikely that, with the limited techniques at his disposal, he was able to detect these structures. Recently Dropkin and Nelson (19) have discarded these suggestions on the basis of light microscope observations. During the present investigations numerous similarly sized mitochondria were observed.

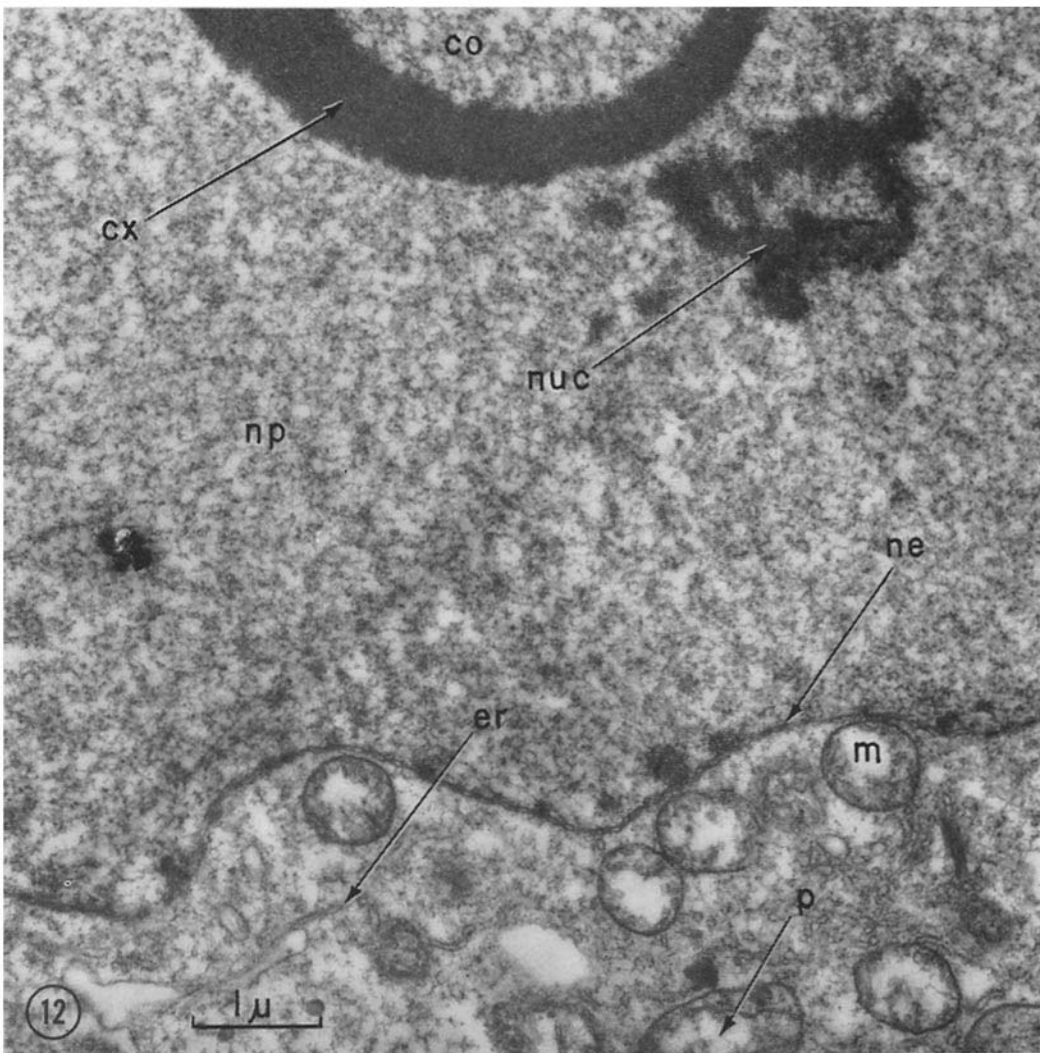
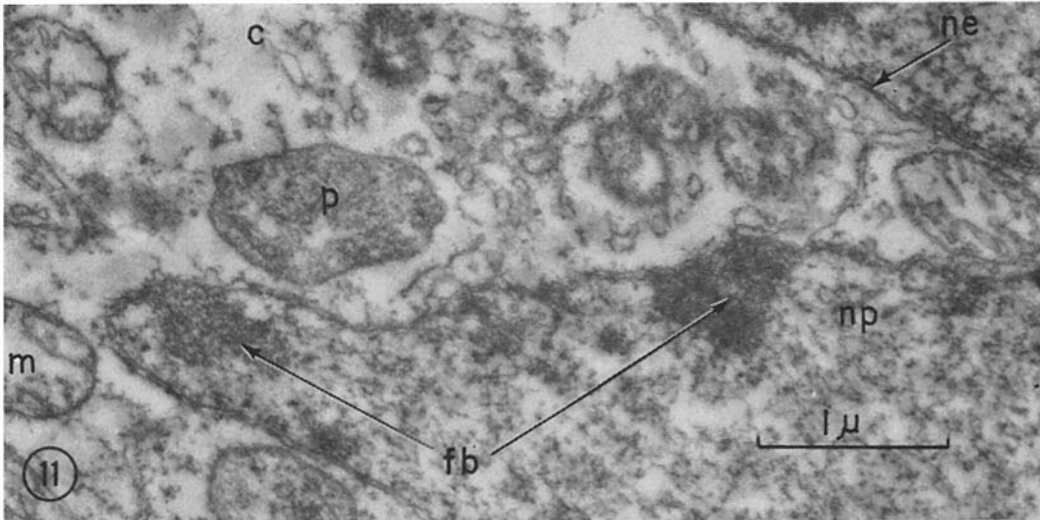
The size and irregular shape of the giant cell nucleus, the large nucleolus and the presence of chromatin bodies distributed irregularly along

FIGURE 11

Electron micrograph of part of a nucleus in a 30-day-old giant cell fixed in 1 per cent osmium tetroxide for 30 minutes at 5°C and treated with ferric chloride, phosphotungstic acid, and uranyl acetate and embedded in Araldite. Showing Feulgen-positive bodies (*fb*) in the nucleoplasm (*np*) adjacent to the nuclear envelope (*ne*) and proplastids (*p*) and mitochondria (*m*) in the cytoplasm (*c*). $\times 17,000$.

FIGURE 12

Electron micrograph of part of a nucleus in a 30-day-old giant cell fixed with 1 per cent osmium tetroxide for 30 minutes at 5°C and treated with ferric chloride, phosphotungstic acid and uranyl acetate and embedded in Araldite. Showing part of a nucleolus, with its core (*co*) and cortex (*cx*) and a nucleolar connection (*nuc*) lying in the nucleoplasm (*np*). Nuclear envelope (*ne*). The dense cytoplasm includes endoplasmic reticulum (*er*), proplastids (*p*), and mitochondria (*m*).



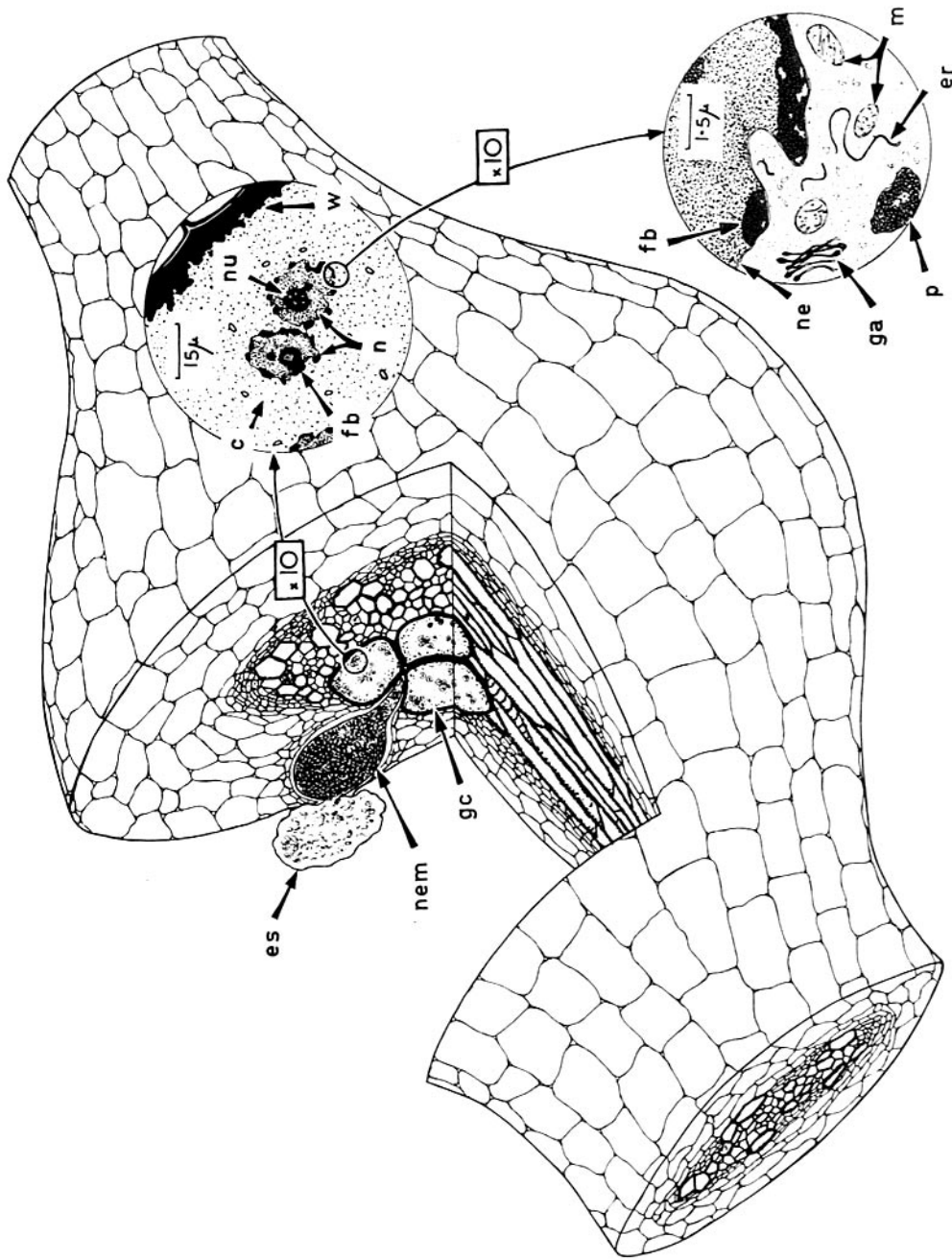


FIGURE 13

Semi-diagrammatic scale drawing of a single infection in a young tomato root, showing the adult female nematode (*nem*) with its egg sac (*es*) protruding out of the gall. Three giant cells (*gc*) are depicted with thickened cell walls (*w*) and large irregular nuclei (*n*) surrounded by nuclear envelopes (*ne*) and containing Feulgen-

positive bodies (*fb*) which in one case show the described connection with the nucleolus (*nu*). The cytoplasm (*c*) is dense and contains mitochondria (*m*), proplastids (*p*), endoplasmic reticulum (*er*), and Golgi apparatus (*ga*).

the nuclear membrane have been commented on by many workers (12, 17, 19, 26, 27, 37, 39, 47, 48). Nemeč reported the movement of chromatin bodies from the nucleus to the cytoplasm. He did not think that this was associated with the formation of mitochondria because he could not find chromatin bodies in the cytoplasm next to the nucleus. He assumed that these bodies must be broken down on their entry into the cytoplasm. Since these interesting observations, very little has been added to our knowledge of the giant cell nucleus. Some electron micrographs show a disruption of the nuclear envelope in the region of these Feulgen-positive bodies and their structure in osmium-fixed sections is similar to that of similarly sized bodies in the cytoplasm, referred to as proplastids.

However, in permanganate-stained sections there appears to be little similarity in structure, other than a dimensional one, between nuclear Feulgen-positive bodies and the proplastids in the cytoplasm. The reason for this dissimilarity between osmium- and permanganate-treated sections could be due to the fact that permanganate staining results in the removal of RNA and histones from tissues (11). It may be that a phenomenon similar to that originally described by Nemeč (39) is, in fact, occurring, *viz.*, that these Feulgen-positive bodies are moving from the nucleus to the cytoplasm and that this movement is associated with a depolymerization of the DNA to give rise to a Feulgen-negative substance which is located in the cytoplasm and which is eluted by treatment with permanganate.

Similar types of reactions have been reported to occur in the tissues of various invertebrates (1, 4, 7, 8, 23, 38, 45). However, it must be stressed that only a few sections in these experiments have shown what appears to be Feulgen-positive bodies being extruded into the cytoplasm and these may be due to sectioning artifacts. It has been stated by Bernhard (4) on the topic of transfer of nuclear material into the cytoplasm that "morphological investigations of this essentially dynamic phenomenon are in many cases of only restricted value." Nevertheless, in these experiments many sections viewed under the electron microscope have shown connections between the nucleolus and the Feulgen-positive bodies on the nuclear envelope. The nuclear envelope is often irregular

in these regions in a manner which suggests transport of material from the nucleus into the cytoplasm. Whilst there is as yet no experimental evidence that DNA makes RNA (10), experiments with tritiated cytidine (22) and radioactive leucine and uridine (53) lend support to the hypothesis that RNA synthesis begins in the chromatin portion of the nucleus and that RNA carries genetic information from the nucleus into the cytoplasm where proteins are made.

Electron microscope observations of the nucleolus in these experiments have not revealed structures such as nucleolonema which have been described in vertebrate tissues (52). Giant cell nucleoli differ slightly in appearance from the nucleoli which have been described for plant root material and this is probably because they are associated with a prolonged and less disturbed period of protein synthesis than are the nucleoli of the meristematic tissues which have been described (51). These differences are probably the result of the physiological conditions prevailing in the cytoplasm (9), which, in turn, is probably influenced by the stimulus from the nematode. It is generally agreed that this stimulus is contained in the salivary secretions which Linford (30) has observed exuding from the stylet of *Meloidogyne*.

Numerous problems remain unsolved. These include the precise nature and origin of the stimulus and the effect that this stimulus has on the host plant. Does it merely trigger off a series of cell reactions which lead to the formation of the giant cell without further stimulus from the nematode, or is a continuous stimulus from the nematode essentially for the phenomena of cell wall breakdown, mitosis, and protein synthesis which together lead to the formation of the giant cell? Observations from many sources indicate that a constant stimulus is required but further evidence must be forthcoming before this hypothesis can be firmly established.

I wish to thank Dr. N. T. Flentje for encouragement; Dr. S. G. Tomlin and Professor R. K. Morton for allowing me to use the Philips and Siemens electron microscopes; Dr. P. G. Martin for the use of the Lison histophotometer; and Messrs. P. P. Thomson and B. J. Jeffery for technical assistance.

Received for publication, June 30, 1961.

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