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# NEMATODE-INDUCED SYNCYTIUM—A MULTINUCLEATE TRANSFER CELL

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SUMMARY

The formation and structure of a syncytium induced by the potato cyst-nematode (*Heterodera rostochiensis* Woll.) in potato roots is described.

At the permanent feeding site of the nematode larva, usually in the root cortex, the larva pierces a cell with its mouth stylet and injects saliva. Cell wall dissolution occurs to incorporate neighbouring cells into a syncytium. A column of cells is incorporated towards the vascular tissue. Centripetal advance is limited by the lignified xylem, then syncytial spread continues laterally along xylem parenchyma and pericycle cells.

Wall protuberances form on syncytial walls adjacent to conducting elements. This indicates the syncytium is a multinucleate transfer cell, and by ingesting syncytial contents the larva is the nutrient sink. As syncytial expansion occurs, sieve elements are crushed and probably cease to function, hence protuberance development continues only against xylem elements.

Cell alterations on incorporation into the syncytium involve expansion, loss of cell vacuole, nuclear hypertrophy and a proliferation of cytoplasmic organelles free to move through wall gaps into the communal cytoplasm.

'Boundary formations' and microtubules are associated with the growing ends of protuberances, and appear to be involved in their synthesis. Fibrillar material, possibly cellulose microfibrils, occurs between the plasmalemma and the membrane of the 'boundary formation', and the forming protuberance.

To induce the formation of the syncytium, the larva controls the differentiation of unspecialized cells to cells with a specific physiological function. The occurrence of wall protuberances suggests that transfer cells form as a response to solute flow.

## INTRODUCTION

After hatching from eggs in soil, larvae of cyst nematodes (*Heterodera* spp.) and root-knot nematodes (*Meloidogyne* spp.) invade roots of their host plant, usually behind the root tip or where a lateral root emerges (Mankau & Linford, 1960). With their mouth stylet the larvae cut through cell walls and they migrate intracellularly until they settle permanently at a site where they feed (Mountain, 1965; Dropkin, 1969). At this stage the larvae are no more than 0·5 mm long. At the position where they feed the larvae induce the formation of multinucleate syncytia (Dropkin, 1969). *Heterodera* larvae become females if they induce syncytia of sufficient size otherwise they become males (Trudgill, 1967). The females enlarge greatly, rupture the cortex and remain with the head embedded and cemented in the roots (Green, 1970). Males have only modest food requirements and develop to only 1–1·5 mm long.

Investigations with the light microscope indicate that the syncytia induced by *Heterodera* larvae form after saliva is injected into the cells of the pericycle, endodermis

and cortex of susceptible hosts (Cole & Howard, 1958; Mankau & Linford, 1960; Endo, 1964). Dissolution of the cell wall, breakdown of the vacuole, formation of granular cytoplasm and fusion of neighbouring protoplasts produces a syncytium with walls of irregular thickness (Endo, 1964, 1971*a*). Aberrant nuclei and mitochondria have also been reported (Piegat & Wilski, 1963, 1965) and histochemical work has shown hyperactivity of all the enzymes tested except phenolase (Endo & Veech, 1970). Radioautographic techniques indicate increased DNA and RNA synthesis (Endo, 1971*b*), and direct analysis shows increased free amino acid content (Doney, Fife & Whitney, 1970).

An electron-microscope study of syncytia induced by *H. glycines* showed dissolution of cell walls and large areas of cytoplasm with many organelles (Gipson, Kim & Riggs, 1971). Electron microscopy of the giant cells induced by the closely related genus *Meloidogyne* revealed irregular wall ingrowths (Bird, 1961; Huang & Maggenti, 1969*b*; Paulson & Webster, 1970). There were no microtubules at the cytoplasm near the wall ingrowths which might indicate how they were formed (Paulson & Webster, 1970), but tubular and vesicular aggregates were observed and these were called 'boundary formations' (Esau, Cheadle & Gill, 1966; Huang & Maggenti, 1969*b*). The cytoplasm of giant cells induced by *Meloidogyne* spp. is similar to that of the syncytia induced by *Heterodera* spp., but the giant cells probably form only by repeated endomitosis without any dissolution of the cell wall (Huang & Maggenti, 1969*a*).

This paper shows that similar irregular ingrowths of the cell wall occur in syncytia induced by the potato cyst-nematode *H. rostochiensis*, that these ingrowths indicate that multinucleate cells induced by nematodes can be classed as 'transfer cells', and discusses their function and formation.

## MATERIALS AND METHODS

Pentland Crown or King Edward potatoes were grown in steam-sterilized soil (John Innes Compost No. 1) or sand in a greenhouse, and inoculated with 30–50 cysts (dead females containing eggs) of potato cyst-nematode (*H. rostochiensis* Woll.). Infected roots were harvested at intervals and fixed for microscopy. Some material was also collected from plants grown in the field.

### *Light microscopy*

Roots were fixed in 5 % glutaraldehyde adjusted to pH 7·2 with phosphate buffer, dehydrated in cellosolve (ethylene glycol mono-ethyl ether) and embedded in glycol methacrylate. Sections 0·5–2·0 $\mu$m thick were cut on dry glass knives, stained with PAS (periodic acid/Schiff reagent) and toluidine blue (Feder & O'Brien, 1968) or methylene blue and azure II for Araldite sections (Jeon, 1965), and viewed with a Zeiss Ultraphot II.

### *Electron microscopy*

Roots were fixed in glutaraldehyde as above then postfixed in buffered 1 % $OsO_4$ for 1 h, dehydrated in cellosolve and embedded in Araldite. Silver–gold (70–100 nm thick) sections were cut with glass knives, floated on to 15 % ethanol, collected on carbon-celloidin or uncoated 3-mm grids and stained with saturated uranyl acetate in 50 % ethanol followed by alkaline lead stain (Millonig, 1961). The specimens were examined with an AEI EM 6B electron microscope at 60 kV.

RESULTS

*Light microscopy*

The first cells altered by stylet penetration were usually in the root cortex (Figs. 1, 2). A column of cells towards the vascular tissue was then sequentially altered; the cell walls broke down, the vacuoles were lost and the cytoplasmic contents were increased. These changes were accompanied by nuclear and cell enlargement (Fig. 2). The length of the column of cells depended on how far out into the cortex the larval head was situated. Once the alterations in the column of cells reached the vascular tissue, formation of the syncytium spread longitudinally in both directions along vascular parenchyma and pericycle cells adjacent to the xylem (Figs. 3, 4). Cell wall breakdown appeared to occur only as a cell was incorporated into the syncytium. Wall fragments or stubs left after this process were not altered, but they became farther apart as syncytia expanded.

As soon as cells adjacent to the xylem were incorporated, their cytoplasm was filled with vesicles, small vacuoles and plastids, and wall protuberances were formed (Figs. 4–7). These protuberances stained red with PAS reagent and were only resolved in light microscopy in sections of less than 2 $\mu$m thick, otherwise they appeared as a uniformly thickened wall. The protuberances were strictly localized on the syncytial wall adjacent to all conducting xylem vessels for as long as the cells were in contact (Fig. 4) and where the syncytium wall abutted any other cell type no protuberances were found (Figs. 5, 7, 10). The electron-microscope investigations did show that at an early stage some thickening occurred adjacent to sieve tubes. The protuberances penetrated into the cell and the extent of their development appeared to depend on syncytium size and xylem vessel size, i.e. on the nematode's demand for solutes from the syncytium and the quantity being carried by the vessel. In some cells the protuberances developed so that their extent was 10 times the normal wall thickness (Figs. 4, 5, 7). Syncytial walls that were not adjacent to vascular tissue were often thicker than normal cell walls, but lacked protuberances (Figs. 6, 10). The centre of the outer cortical walls usually stained green with toluidine blue, which indicated that lignin or at least some polyphenol was present (O'Brien & Thimann, 1967). The centre of some wall stubs and fragments left inside syncytia also stained green in toluidine blue. Definite evidence of hydrolysis of the wall inside syncytia, using the criterion of O'Brien & Thimann (1967) and O'Brien (1970), is shown in Fig. 8. Here an internal syncytial wall does not stain in toluidine blue or PAS reagent, and is weakly birefringent in polarized light. This indicates that the birefringent section contained only cellulose fibrils – presumably an intermediate stage in complete wall dissolution.

Some cell wall fragments remained to delineate original cell boundaries (Figs. 3, 11). These were more obvious near the nematode in the cortical cells, which were incorporated at an early stage of the formation of the syncytium (Fig. 9). Cell walls next to the nematode head showed no signs of dissolution and were no different from other limiting walls of the syncytium (Fig. 9). Although no nematode stylet was seen penetrating a syncytium, stylets were seen adjacent to the syncytial wall (Fig. 1). The lack of wall protuberances in this region (Fig. 9) indicates that solutes from these cells

do not pass across the walls at this point, so that ingestion of syncytial contents occurs here after the stylet penetrates into the syncytial cytoplasm.

In primary roots and close to the root tip the syncytia formed progressively away from the tip. In secondary roots, syncytium extension occurred equally both towards and away from the tip.

The syncytial cytoplasm when full of small vacuoles, mitochondria and plastids showed signs of oriented flow, especially where small vacuoles were found between wall stubs (Figs. 5, 10). Here they lost their normal, nearly spherical shape and became elongated. Similarly, in later stages, some nuclei became elongated as if oriented by mass flow, and appeared to be caught on wall fragments (Fig. 11). This may have been caused by release of pressure brought about by damage to the syncytia before fixation. Clumping of syncytial nuclei, previously reported by Endo (1964), was not evident, but some accumulations of nuclei were seen where a gap in the wall was small and they may have been trapped there by the flow of cell contents.

An obvious feature when staining with azure II and methylene blue or PAS reagent was the lining up of plastids near the walls and wall fragments, especially in later stages when few small vacuoles were present (Fig. 7).

*Electron microscopy*

Early stages of syncytium formation showed profound alterations in cellular organization. The ground cytoplasm frequently became more dense, and contrast was lost (Fig. 12). As found by Gipson *et al.* (1971), cell wall dissolution was evident (Figs. 12–14). Most wall stumps and fragments had rounded edges, not jagged ones as might be expected from mechanical disruption (Fig. 15). The tonoplast appeared to break down and the vacuoles filled with an amorphous electron-dense substance (Fig. 14), their former position finally becoming invaded by cytoplasm and organelles (Fig. 13). Protoplasts bulged through wall gaps into neighbouring cells (Fig. 14), and eventually merged to form a continuous syncytial protoplast (Fig. 15). The cytoplasm was filled with short elements of smooth endoplasmic reticulum (SER), mitochondria, small vacuoles, vesicles and non-hypertrophied Golgi bodies (Fig. 15). Frequently longitudinally oriented tubules of SER were found (Fig. 16). Nuclei became amoeboid and large with prominent nucleoli and dense chromatin grouped around the nuclear envelope (Figs. 16, 17).

In vascular parenchyma cells, wall protuberances were evident as soon as cell-wall gaps were found. These occurred at first on walls adjacent to sieve elements (Fig. 18), then soon after they were observed adjacent to xylem elements (Fig. 19). After rapid lateral expansion of the syncytium the sieve elements were probably crushed and became non-functional. Whereas progressively more anastomosing protuberances were found on walls adjacent to the xylem, protuberances adjacent to the phloem failed to develop further and were not observed a week after invasion of the roots by the larvae. The protuberances were completely invested with plasmalemma and frequently associated with mitochondria, SER and vesicular aggregates or 'boundary formations' (Figs. 20, 21) (Huang & Maggenti, 1969*b*). The latter first occur where protuberances will be formed and later are mainly associated with the growing ends of

protuberances (Fig. 22). The aggregates are bounded by plasmalemma and consist of vesicles or tubules of varying dimensions between the plasmalemma and the wall. Huang & Maggenti (1969*b*) suggest that they are concerned with protuberance formation; our findings are consistent with this view. The vesicular and tubular elements can sometimes be seen to be continuous with the plasmalemma (Fig. 24) and the membrane of the vesicles is similar in appearance to that of the plasmalemma. The vesicles of the 'boundary formations' are therefore probably sections of tubular infolding of the plasmalemma containing cytoplasm, which are grouped about the wall protuberances. Fibrillar material can be seen within the wall protuberances (Figs. 22–24). These fibrils are in some cases connected with the outer surface of the plasmalemma or the membrane of the vesicles (Figs. 25, 26).

Microtubules were associated with these protuberances only during early stages, presumably when the protuberances were forming, but they were fewer and less localized than microtubules that are involved in secondary thickening of differentiating xylem elements. Their function, however, in directing precursors for wall synthesis to the appropriate site is probably similar (Wooding & Northcote, 1964).

The protuberances gradually extended into the cytoplasm in response to increasing nematode demand for nutrients, and formed an anastomosing network, although still completely surrounded by plasmalemma. Mitochondria and plastids were usually present in the cytoplasm near the protuberances, and mitochondria were sometimes trapped within the network at a later stage (Fig. 27).

## DISCUSSION

This study confirms that dissolution of the cell wall (Piegat & Wilski, 1963) is evident from the initial stages of syncytium induction by *H. rostochiensis*, and results in the formation of the syncytium. This agrees with work on syncytia induced by other species of *Heterodera* (Mankau & Linford, 1960; Endo, 1964; Gipson *et al.* 1971). No mitosis was seen in syncytia (Endo, 1964; Endo & Veech, 1970), although Piegat & Wilski (1963) reported an initial mitotic stimulation in syncytium induction by *H. rostochiensis*. It also confirms that cell-wall protuberances are formed in syncytia induced by *H. rostochiensis* and shows that they are localized against vessels and absent next to the nematode head.

Huang & Maggenti (1969*a*, *b*) did not say where wall protuberances in *Vicia faba* giant cells induced by *M. javanica* are localized. Preliminary observations which we have made show that protuberances also occur adjacent to conducting elements and sometimes on common walls between giant cells when giant cells are induced in *Coleus* by *M. arenaria*.

There are many reports of wall protuberances occurring at specific locations in plants (e.g. Wooding & Northcote, 1965). Such cells with protuberances are called 'transfer cells' (Gunning, Pate & Briarty, 1968; Gunning & Pate, 1969; Pate & Gunning, 1969). The wall ingrowths give the protoplast unusually high surface : volume ratios, and 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. Their function is therefore interpreted as intensive selective transport over short distances. In roots, transfer cell ingrowths have been reported only at root hair bases (Guttenberg, 1968), associated with legume nodules (Pate, Gunning & Briarty, 1969) and parasitic haustoria (Dörr, 1968).

We suggest that syncytia or giant cells induced by nematodes are a multinucleate form of transfer cell, and that the wall protuberances form as a result of nematode demand for nutrients. Nutrients required for syncytium maintenance must be provided by the xylem sap. Presumably this contains adequate organic compounds as well as salts, although the origin of the organic compounds in the xylem sap is unknown. The active metabolism of the syncytium and withdrawal of solutes by the nematode from the syncytium must result in a steep chemical gradient of nutrients across the plasmalemma. At the sites of massive transport of solutes across the plasmalemma adjacent to the xylem, the formation of protuberances may occur as a direct response to the chemical gradient. Thus the nematode has induced the differentiation of unspecialized cells to cells with a specific physiological function. As is typical of transfer cells, mitochondria associated with protuberances are found together with dense cytoplasm and prominent elements of smooth endoplasmic reticulum. The main difference, however, between syncytial transfer cells induced by *H. rostochiensis* and conventional transfer cells is that in the former the nutrient sink is the nematode, whereas in the latter the demand for the nutrient is by actively growing or secreting tissue (Pate, Gunning & Milliken, 1970; Gunning & Pate, 1969). This suggests that the induction stimulus for the formation of transfer cells is the flow of solutes and solvent through the cell rather than that the transfer cells are first formed and that these stimulate the flow. The plasmalemma of normal transfer cells remains intact throughout. The nematode's hollow stylet penetrates into the cytoplasm and removes material, thus creating mass flow in the syncytium, and alignment of cytoplasmic structures. A balance is then maintained between demand and supply of nutrients. As female nematodes grow the syncytium extends, existing wall protuberances grow and new protuberances form adjacent to the xylem. This stable situation lasts for about 30 days until the nematode matures, when it requires less food and the syncytial contents start to degenerate.

The final appearance of ingrowths is characteristic for a given plant, and may even differ for transfer cells located next to xylem or phloem in the same plant (Gunning, Pate & Green, 1970). Few reports of the sequence of protuberance formation exist. Gunning & Pate (1969) report that normal transfer cell ingrowths may form in about a day, accompanied by intensive Golgi activity. When the protuberances are mature microtubules are not particularly obvious and do not follow the irregular profiles, Golgi bodies are not hypertrophied and few 'boundary formations' are evident.

During early stages of protuberance formation, however, we have found that microtubules, 'boundary formation' and smooth endoplasmic reticulum are all present near these sites of wall synthesis.

The fibrils between the plasmalemma and membranes of the 'boundary formation' and the wall could represent cellulose microfibrils, thus the membrane system ('boundary formation') might represent an increased local plasmalemma area neces-

sary for the localized synthesis of protuberance materials. Hemicellulose matrix polysaccharides are probably derived from Golgi vesicles which are directed to the site of deposition by microtubules; the membrane flow from the Golgi vesicles to the plasmalemma would bring about an extension of the cell membrane (Northcote, 1971). Therefore if the situation is similar to that thought to occur at cell plate and cell wall formation (Roberts & Northcote, 1970) the matrix material polysaccharides may be deposited first from the Golgi bodies and appear as a relatively textureless area, followed by an accumulation of cellulose microfibrils at the plasmalemma. This graded sequence may be seen in Fig. 25. Tu & Hiruki (1971*b*) also found microtubules and Golgi-derived vesicles at this stage; hence it appears probably that microtubules play some part in protuberance formation. Tu & Hiruki (1971*a*) also noted fibrous material with the 'boundary formation' associated with wall ingrowths, and similar 'boundary formations' have been seen in non-pathogenic cells, such as the secretory cells of the stigma, associated with wall ingrowths (Rosen, 1971). Both Huang & Maggenti (1969*b*) and Tu & Hiruki (1971*a*) commented that 'boundary formations' preceded and were associated with wall ingrowths. 'Boundary formations' may become especially noticeable when abnormally large ingrowths are synthesized as a result of virus or nematode demands or intense secretory activity.

The reports of increased enzymic activity in giant cells (Endo & Veech, 1969; Veech & Endo, 1970) and syncytia (Endo & Veech, 1970) and the general appearance in the cytoplasm of intense metabolic activity is not surprising as the energetic and synthetic needs are for massive selective transport of nutrients into the cytoplasm and the maintenance of necessary functions despite a constant removal of material by the nematode.

The presence of lignin or polyphenols in the limiting syncytial wall, and in some wall fragments, may play a significant part in limiting cell wall dissolution (O'Brien & Thimann, 1967) and it also renders impermeable the walls not concerned with the flow of nutrients. Evidently the mature lignified xylem is resistant to dissolution of its walls and limits progressive centripetal formation of the syncytia. Initial expansion of the cells crushes adjacent cortical cells that have not been incorporated, and possibly releases polyphenols which on oxidation polymerize and limit dissolution of the outer wall.

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ABBREVIATIONS ON PLATES

| | | | |
|---|---|---|---|
| *bf* | 'boundary formation' | *p* | protuberance |
| *f* | cell wall fragment | *pa* | plastid |
| *fm* | fibrillar material | *pl* | plasmalemma |
| *g* | Golgi body | *pp* | P-protein |
| *m* | mitochondrion | *s* | cell-wall stub |
| *mt* | microtubule | *ser* | smooth endoplasmic reticulum |
| *n* | nematode | *v* | small vacuole |
| *nu* | nucleus | *x* | xylem vessel |
| *o* | outer wall of the syncytium | | |

Figs. 1–11 are light micrographs.

Fig. 1. Nematode stylet (arrow) in a cortical cell beneath the epidermis, adjacent to the first cell to be incorporated into the syncytium. × 1200.

Fig. 2. Syncytial expansion occurs towards the vascular tissue from the initial site where the nematode (*n*) induces its formation. × 400.

Fig. 3. After incorporating cells between the initiation site and vascular tissue, syncytium expansion spreads laterally in pericycle and vascular parenchyma cells adjacent to the xylem. × 570.

Fig. 4. Protuberances (*p*) on the syncytial wall adjacent to a xylem vessel. × 3400.

Fig. 5. Localized syncytial wall protuberances adjacent to xylem vessels and absent next to xylem parenchyma cells. Note also that vacuoles in the syncytial cytoplasm near the protuberances are spherical, but those near wall gaps are elongated. × 2400.

Fig. 6. Syncytial wall protuberances (*p*) occur adjacent to the xylem, but not on outer syncytial walls (*o*). × 2500.

Fig. 7. Localized wall protuberances next to xylem vessels in 2 syncytia. Plastids (*pa*) line internal walls in the syncytia, especially those walls nearer to the protuberances. × 1500.

Fig. 8. Photographed in polarized light. The weakly birefringent wall (arrow) probably contains cellulose alone. × 2400.

Fig. 9. Nematode head (*n*) next to syncytial wall. There are no wall ingrowths here although the syncytial wall is thickened. Cell wall fragments (*f*) delineate original cell boundaries. × 2500.

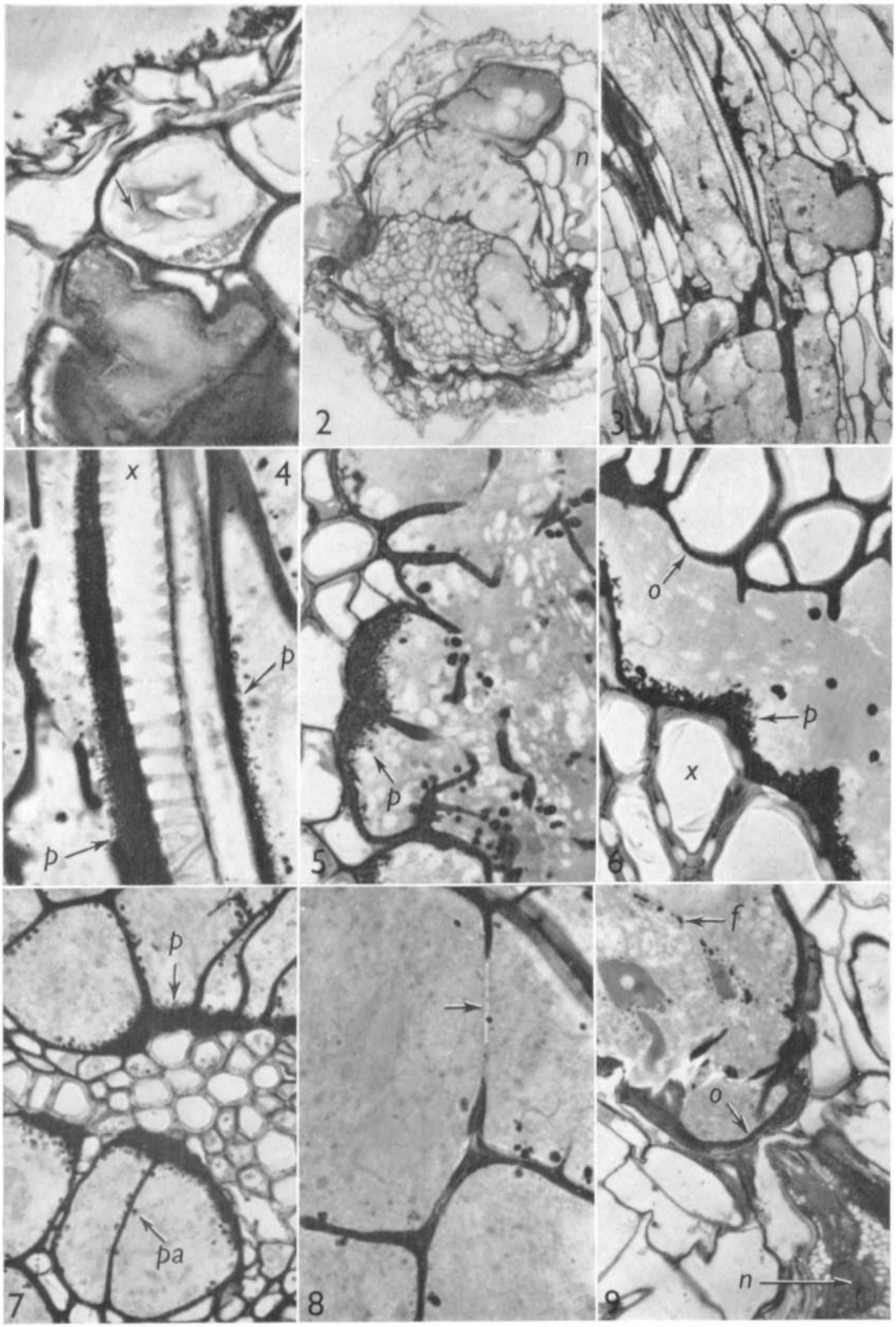

1
2
n
3
4
x
p
p
5
p
6
o
p
x
7
p
pa
8
9
f
o
n

Fig. 10. Cytoplasmic vacuoles between wall stubs are elongated whereas other vacuoles are nearly spherical. Note also the thickened outer syncytial wall which stained for lignin. × 3300.

Fig. 11. Elongated nuclei (*nu*) in syncytial cytoplasm apparently caught on wall fragments. × 750.

Figs. 12–27 are electron micrographs.

Figs. 12–14. These show early stages in formation of a syncytium. Considerable cellular disruption is evident. Cell walls have dissolved in places, and the position formerly occupied by the vacuole is taken by amorphous material (arrow in Fig. 14). Organelles migrate via wall gaps (Fig. 14), occasionally SER rings around organelles (Fig. 13). Fig. 12, × 7000; Fig. 13, × 6800; Fig. 14, × 4700.

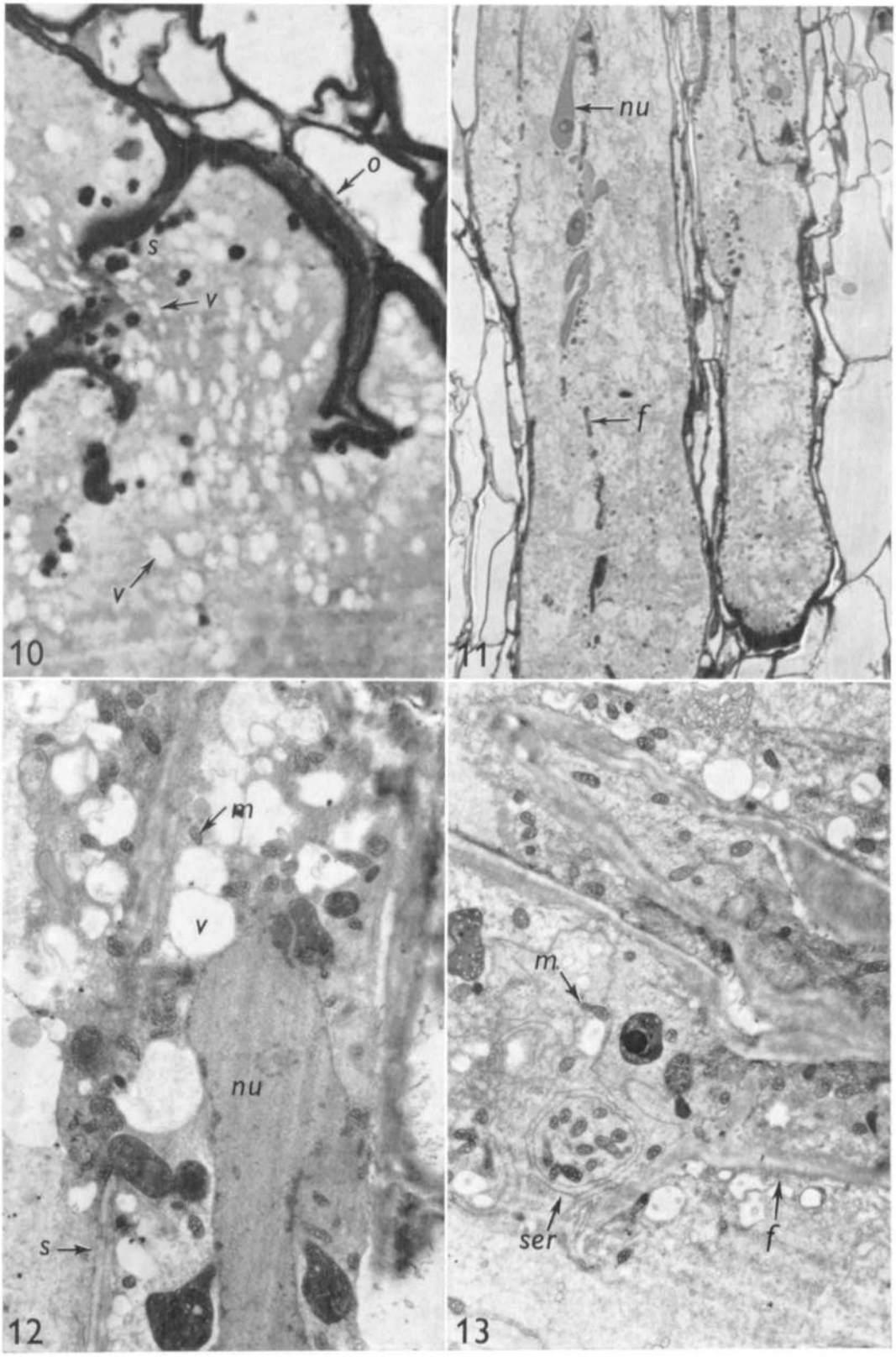
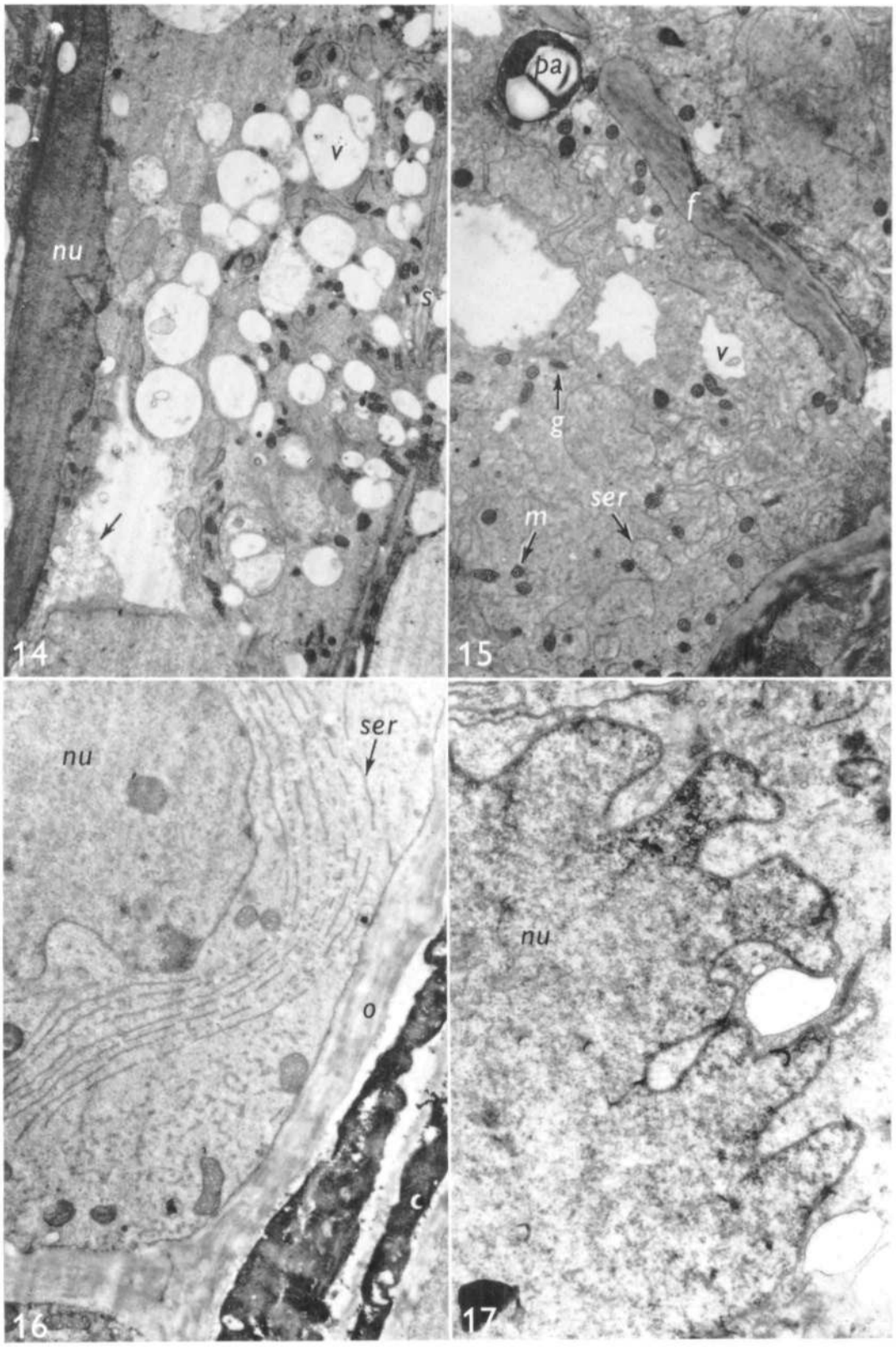

o
s
v
v
10
nu
f
11
m
v
nu
s
12
m
ser
f
13

Fig. 14. See legend on p. 800

Fig. 15. The cytoplasmic organization in the syncytium recovers after wall dissolution; some rounded wall fragments remain. Large areas of cytoplasm are found with SER, mitochondria, small vacuoles, plastids and Golgi bodies. × 6500.

Fig. 16. A nucleus with dense chromatin near a region of the nuclear envelope. Longitudinally oriented SER tubules run between the nucleus and the outer syncytial wall. Cells outside the syncytium have been crushed by syncytial expansion and their contents have degenerated. × 12000.

Fig. 17. Hypertrophied syncytial nucleus with an amoeboid profile. × 18000.


nu
v
s
14
pa
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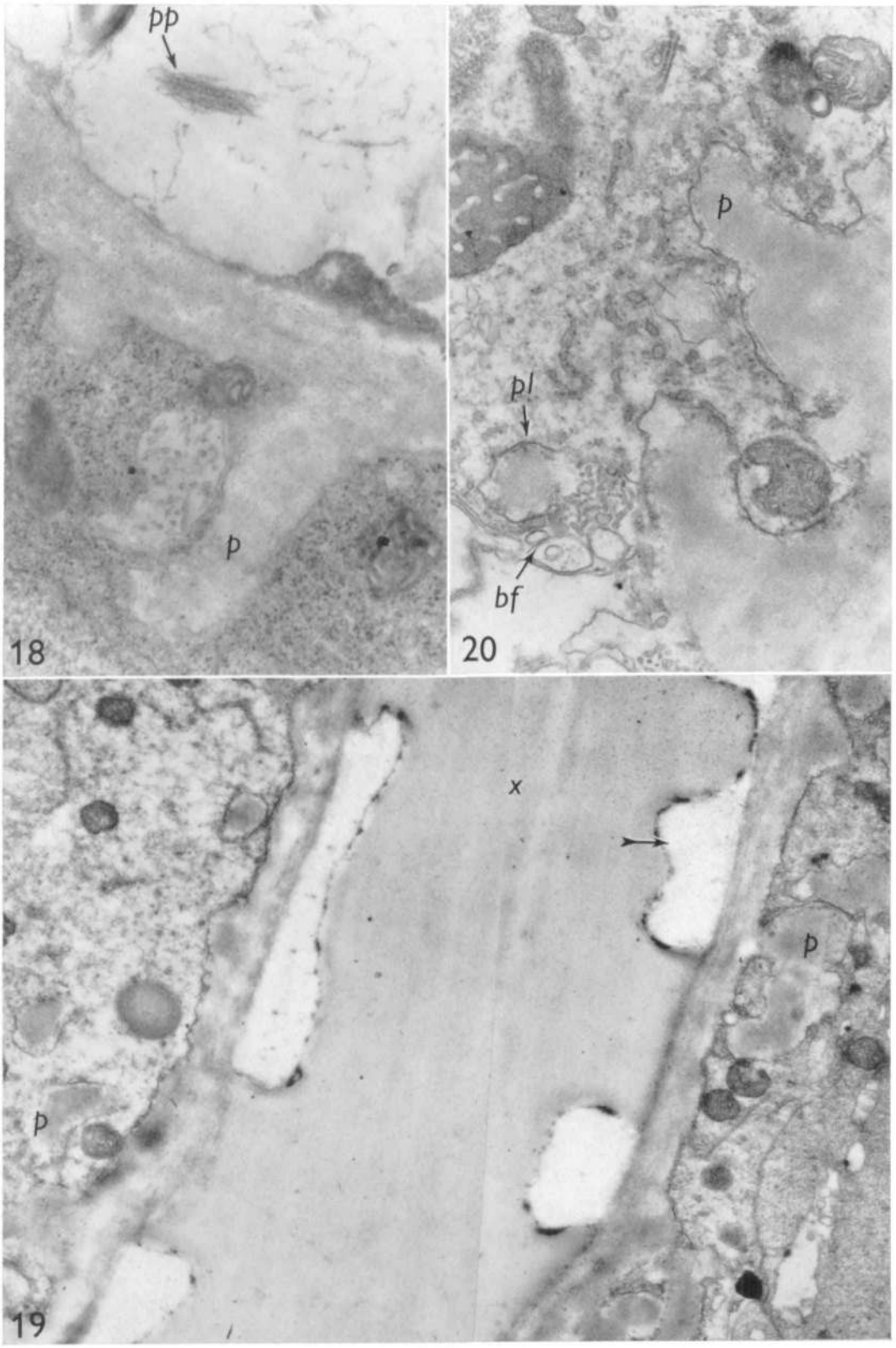
m
ser
15
nu
ser
o
c
16
nu
17

Fig. 18. Wall protuberances found at an early stage of syncytium formation, next to a sieve element containing P-protein (*pp*). × 27 000.

Fig. 19. Protuberances on syncytial walls on both sides of a xylem element. Note the difference in staining between lignified xylem thickenings (arrow) and non-lignified wall material. × 12 000.

Fig. 20. The plasmalemma is continuous over wall protuberances and 'boundary formations' (*bf*). × 26 000.


pp
p
18
p
pl
bf
20
x
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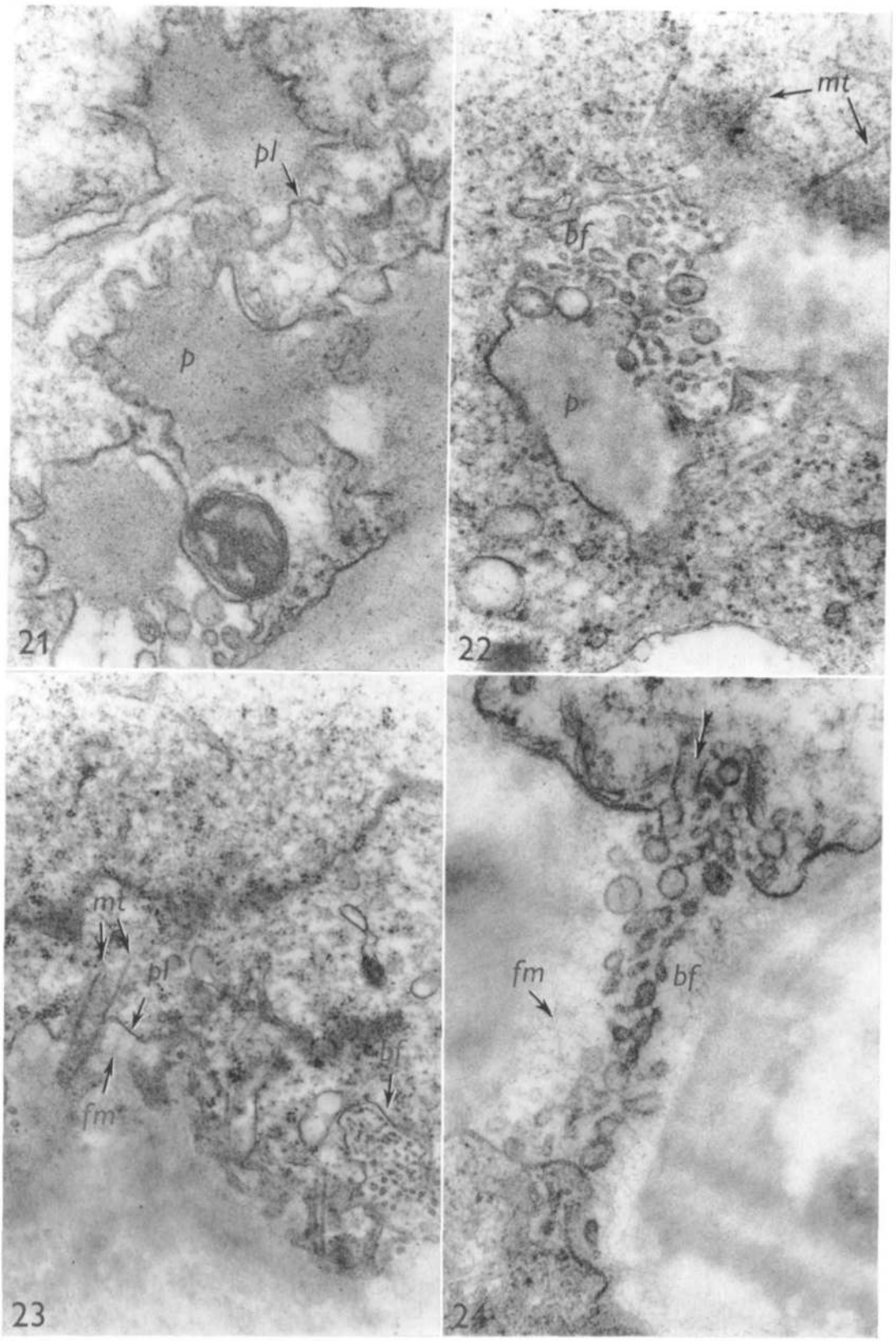
p
19

Fig. 21. Convoluted plasmalemma around wall protuberances. × 37 000.

Fig. 22. 'Boundary formation' at the end of a growing protuberance with microtubules (*mt*) closely associated. × 41 000.

Fig. 23. Fibrillar material (*fm*) occurs under the plasmalemma where rapid wall synthesis is thought to occur. × 27 000.

Fig. 24. The plasmalemma is sometimes continuous with vesicular elements (tailed arrow) of the 'boundary formation'. Fibrils can be seen between elements of the 'boundary formation' and the mature wall. × 56 000.


pl
p
21
mt
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p
22
mt
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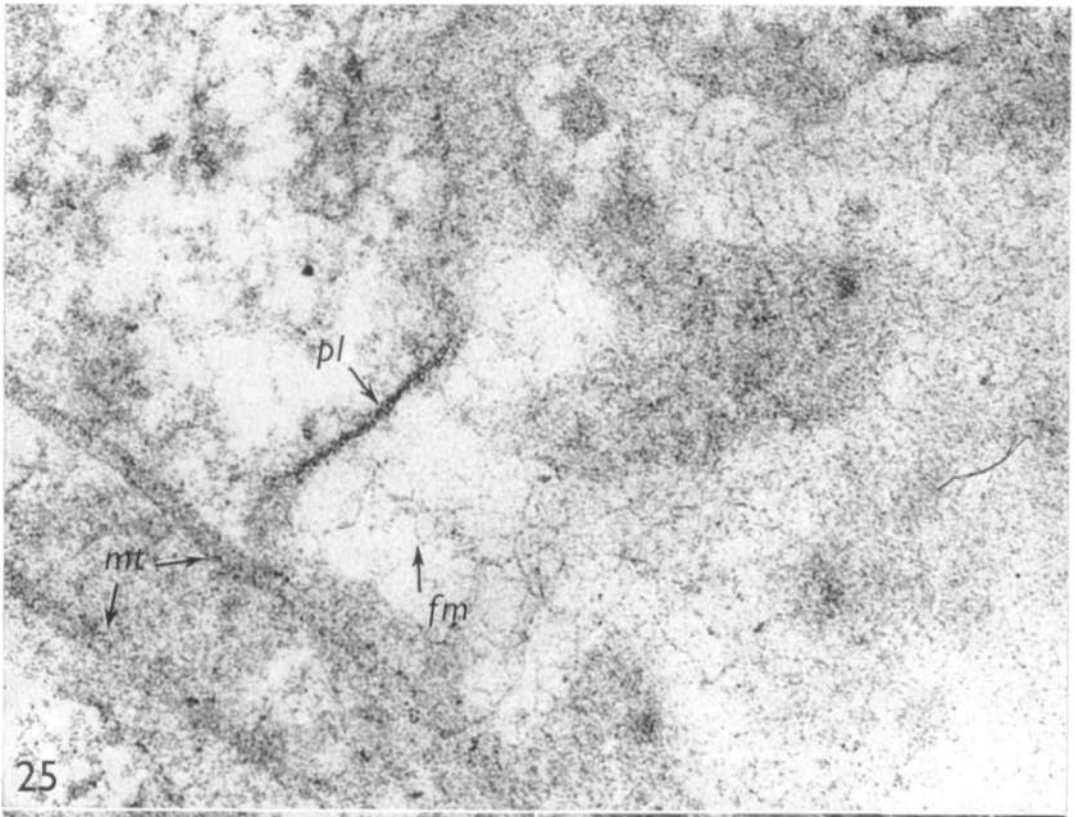
fm
bf
23
fm
bf
24

Fig. 25. An enlargement from Fig. 23 showing wall formation under the plasmalemma. The number of fibrils beneath the plasmalemma increases towards the mature wall. Microtubules are also present. × 120000.

Fig. 26. Fibrillar material, in some cases running from 'boundary formation' vesicular membrane, increasing towards the mature wall. × 133000.

Fig. 27. Extensive anastomosing network of wall protuberances. Plastids abound in the cytoplasm next to them, and mitochondria are sometimes trapped within the network. × 11000.

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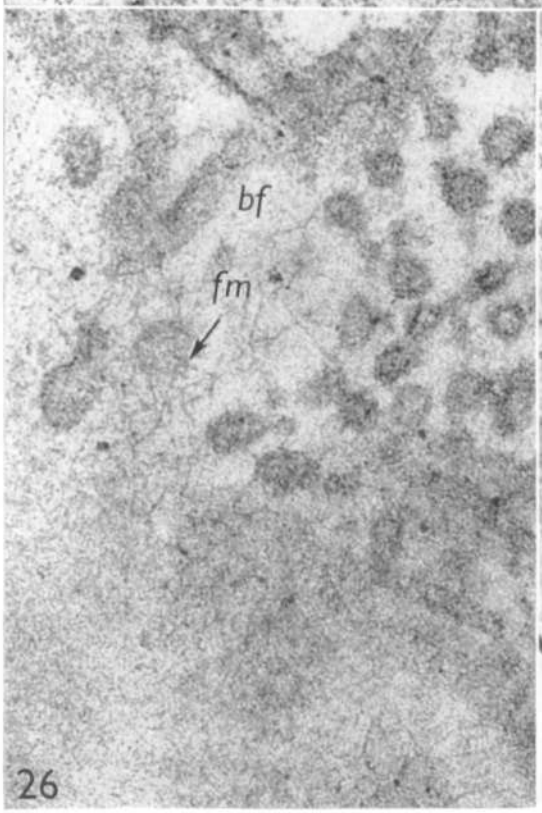


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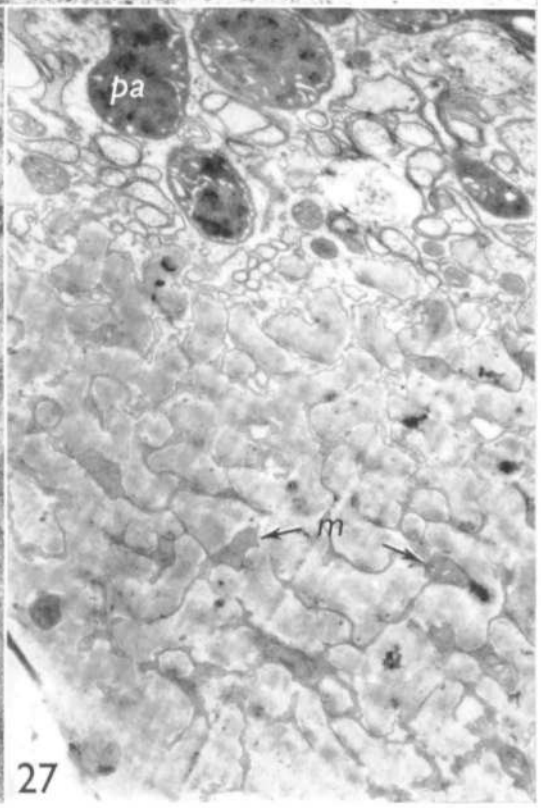


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