JOURNAL OF BACTERIOLOGY, Nov. 1970, p. 989-1009
Vol. 104, No. 2

Printed in U.S.A.

Protoplasmic Organization of Hyphal Tips Among Fungi: Vesicles and Spitzenkörper

STANLEY N. GROVE AND CHARLES E. BRACKER
Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana 47907

Received for publication 10 August 1970

Hyphal tips of fungi representing *Oömycetes*, *Zygomycetes*, *Ascomycetes*, *Basidiomycetes*, and *Deuteromycetes* were examined by light and electron microscopy and compared with respect to their protoplasmic organization. In all fungi studied, there is a zone at the hyphal apex which is rich in cytoplasmic vesicles but nearly devoid of other cell components. Some vesicle profiles are continuous with the plasma membrane at the apices of these tip-growing cells. The subapical zones of hyphae contain an endomembrane system which includes smooth-surfaced cisternae associated with small clusters of vesicles. The findings are consistent with the hypothesis that vesicles produced by the endomembrane system in the subapical region become concentrated in the apex where they are incorporated at the expanding surface. Septate fungi (*Ascomycetes*, *Basidiomycetes*, and *Deuteromycetes*) have an apical body (Spitzenkörper) which is associated with growing hyphal tips. In electron micrographs of these fungi, an additional specialized region within the accumulation of apical vesicles is shown for the first time. This region corresponds on the bases of distribution among fungi, location in hyphae, size, shape and boundary characteristics to the Spitzenkörper seen by light microscopy. This structure is not universally associated with tip growth, whereas apical vesicles are widespread among tip-growing systems.

Hyphal tips of fungi are of interest cytologically because growth is restricted to the rounded portion of the apex, and cell components involved directly in cell expansion are likely to be concentrated in this region. Differences between growing and nongrowing regions of hyphae are reflected by internal organization as seen with light and electron microscopy (3, 5–8, 10–12, 21). In particular, a small densely staining or refractive spheroid body called the "Spitzenkörper" has been observed by light microscopy in living and fixed hyphal tips of various fungi (5–8, 12). Brunswik (5) concluded that the Spitzenkörper was a special structure connected with apical growth of hyphae. Girbardt (6, 7) noted that in some *Deuteromycetes* and *Basidiomycetes* the Spitzenkörper disappeared when hyphal extension ceased and reappeared before tip growth resumed. He also observed that the position of the Spitzenkörper in the apex was correlated with the subsequent direction of hyphal elongation. McClure, Park, and Robinson (12) detected the Spitzenkörper with phase-contrast microscopy in hyphae of septate fungi (i.e., *Ascomycetes*, *Basidiomycetes*, and *Deuteromycetes*) but not in phycomycetous species (i.e., *Oömycetes*, *Zygomycetes*).

Recent electron microscopic studies (3, 8, 10–12) show that in several fungi the hyphal apex is filled with cytoplasmic vesicles, to the exclusion of nearly all other organelles. In contrast, the subapical portions of hyphae are rich in other protoplasmic components. In *Oömycetes*, for example, dictyosomes which produce the vesicles of the tip region are located subapically (10). The apical vesicles are interpreted as being secretory vesicles involved in wall elaboration and plasma membrane increase at the growing apex (3, 10, 12) or as vehicles for secretion of enzymes (8). Based on electron micrographs of *Aspergillus niger*, McClure et al. (12) suggested that these apical accumulations of vesicles are the ultrastructural equivalent of the Spitzenkörper. Girbardt (8) likewise correlated the clusters of apical vesicles with the Spitzenkörper and recognized at least two types of apical organization based principally on the morphology of the Spitzenkörper. However, the observation that hyphae of fungi such as *Pythium ultimum*, an oömycete, have no detectable Spitzenkörper (10, 12) but do have an accumulation of apical vesicles (10) is sufficient reason to question "whether the Spitzenkörper is in fact the light microscopic equivalent of the collection of apical vesicles" (10).

In this report, we illustrate the ultrastructural

organization of hyphal tips in several taxonomically diverse fungi and show evidence of subapical organization that supports a hypothesis for hyphal tip growth involving vesicular additions at the apex. Electron micrographs of fixed hyphae are correlated with light microscopy of growing hyphae to interpret the correspondence between cell components in living and fixed cells. Special emphasis is placed on cytoplasmic vesicles and on the Spitzenkörper with respect to the distribution and morphology of these components in diverse groups of fungi. We show that concentrations of vesicles are of general occurrence at sites of hyphal growth. Additionally, the septate fungi possess a specialized region within the apical zone which is distinct from the cluster of apical vesicles. This specialized region corresponds to the spherical Spitzenkörper seen by light microscopy.

MATERIALS AND METHODS

The following fungi were studied: *Oömycetes—P. aphanidermatum* (Edson) Fitzpatrick; *Zygomycetes—Gilbertella persicaria* (Eddy) Hesseltine; *Ascomycetes—Ascodesmis nigricans* van Tieghem, *Neurospora crassa* Shear and Dodge; *Basidiomycetes—Armillaria mellea* (Vahlex Fries) Quélet, *Rhizoctonia solani* Kuehn [imperfect stage of *Thanatephorus cucumeris* (Frank) Donk]; *Deuteromycetes—Aspergillus niger* van Tieghem, *Fusarium oxysporum* Schlectendahl f. sp. *lycopersici*, *Verticillium albo-atrum* Reinke and Berth.

For light microscopy, sterile microscope slide cover glasses (24 by 50 mm) were prepared with a thin layer of either Difco potato dextrose agar (PD Agar), or Difco potato dextrose broth containing 14 to 16% Difco gelatin (PD Gelatin). These were point-inoculated with hyphae or spores and incubated at room temperature (ca. 24 C) on moist filter papers in petri dishes until the hyphae were at least 3 to 4 mm long. For observation, excess medium and mycelium were removed from each microscope cover-glass culture with a razor blade, and a cover glass (22 by 40 mm) was gently lowered to the surface of the medium so that numerous air pockets were trapped between the cover glass and the medium. Air under the cover glass permitted the cultures to remain aerobic and was favorable for hyphal growth. The hyphae were observed and photographed as they grew through regions of the preparation which were free from air pockets. A Zeiss WL microscope equipped with phase-contrast and Nomarski interference-contrast optics was used.

For electron microscopy, cultures were incubated at 24 C on PD Agar overlaid with permeable cellophane (cellulose xanthate, DuPont). To insure that hyphal tip growth was not interrupted before fixation, the fixative was flooded directly onto cultures in petri dishes. Small portions of mycelium from the edge of the hyphal mats were then transferred to vials and fixed for 30 min at room temperature (ca. 24 C) with 2% glutaraldehyde or with 1% formaldehyde (prepared from paraformaldehyde) plus 2% glutaraldehyde in 0.1 M sodium cacodylate (*p*H 7.2). This was followed by postfixation with 1% OsO_4 in 0.1 M sodium cacodylate (*p*H 7.2) for 2 to 4 hr at room temperature. Hyphae were subsequently washed, and soaked in 0.5% aqueous uranyl acetate for 2 to 4 hr, dehydrated in a graded series of increasing ethanol concentrations followed by anhydrous acetone, and embedded in Araldite 6005 (CIBA, USA; reference 18). The embedded hyphae were oriented in the microtome parallel to the diamond knife so that serial longitudinal sections through hyphal apices could be obtained. The thin sections were mounted on collodion-coated grids, stained for 10 min with lead citrate (17), and examined in a Philips electron microscope (model 200) at 60 kv. A 54,864-line-per-inch diffraction grating replica (Ladd Research Industries, USA) was used as the magnification standard.

RESULTS

In the hyphal tips of all the fungi examined, there is a zone at the apex which is rich in cytoplasmic vesicles but nearly devoid of other cell components (Fig. 1, 6, 16, 22, 28–30, 33, 38–40). Ribosomes are scarce in the apical cytoplasm (Fig. 1, 2, 16, 22, 23, 28–30, 33, 38–40) as compared to the subapical zones (Fig. 5, 16, 21, 42). The wall at the apex of most hyphae usually has a more uneven profile than the lateral wall (Fig. 1, 38, 40) and may have bumps along the inner margin (Fig. 2, 7, 39, 40) which are about the same size as the apical vesicles. Profiles showing continuity between vesicle membranes and plasma membrane are seen in hyphal apices (Fig. 2, 8, 38–40) but not in subapical regions of the same hyphae.

The subapical zone contains representatives of all protoplasmic components including mitochondria, nuclei, ribosomes, and an endomembrane system consisting of rough-surfaced endoplasmic reticulum, smooth-surfaced cisternae, and cytoplasmic vesicles (Fig. 5, 16, 21, 28, 30, 38, 42). In *Oömycetes*, dictyosomes of the Golgi apparatus are the endomembrane components which produce vesicles (Fig. 5; references 9, 10). The non-oömycetous fungi we studied do not have dictyosomes consisting of stacks of cisternae. Instead, each has a characteristic endomembrane system in the subapical zone which includes some form of smooth-surfaced cisternae or tubules which are associated with elaborations of vesicles (Fig. 16, 21, 42–44). These cisternae and tubules, like dictyosomes, occur in cytoplasmic zones of exclusion which contain few or no ribosomes and represent single-cisterna Golgi apparatus. The subapical vesicles have the same appearance as those concentrated in hyphal tips.

In the oomycete and zygomycete species, apical vesicles are of two general types based on their

KEY TO LABELING OF FIGURES: *CV, coated vesicle; D, dictyosome; ER, endoplasmic reticulum; G, Golgi cisterna; M, mitochondrion; MT, microtubule; N, nucleus; PM, plasma membrane; R, ribosome; S, Spitzenkörper; T, tubule; V, cytoplasmic vesicle; W, hyphal wall.*

FIG. 1. *Hyphal tip of Pythium aphanidermatum taken from one of a series of longitudinal sections through the hypha. The apical region of the cell has few ribosomes (R), and is occupied mainly by vesicles (V) of two general types. The small vesicles have condensed, densely staining matrix. The larger vesicles have dispersed, lighter-staining matrix, resembling the hyphal wall (W). Some vesicles are intermediate between the two predominant forms. Vesicles are distributed more or less uniformly throughout the apical zone, with no evidence of specialized regions within the cluster of vesicles. A few mitochondria (M) from the subapical zone intrude into the apical region. Glutaraldehyde fixation. Scale line = 1 μm.*

FIG. 2. *Enlargement of part of the apical region from Fig. 1 showing large vesicles with lightly staining contents and small vesicles with denser contents. The plasma membrane (PM) is convoluted beside the tip wall (W), and a fusion profile showing continuity between plasma membrane and vesicle membrane is evident at the arrow where material with the same stainability as the contents of small vesicles is confluent with the tip wall. Ribosomes (R) are scarce in the ground cytoplasm. Glutaraldehyde fixation. Scale line = 0.1 μm.*

FIG. 3. *Phase-contrast micrograph of a growing hyphal tip of P. aphanidermatum. When observed with the microscope, the light area in the curved apex contains numerous rapidly moving particles that are not resolved in the micrograph. This region corresponds to the zone of vesicles shown in Fig. 1. The elongated dark structures in the subapical region are mitochondria (M). During growth the mitochondria move slowly into and out of the apical zone of particles. Grown on PD Gelatin. Scale line = 1 μm.*

FIG. 4. *Growing hyphal tip of P. aphanidermatum observed by Nomarski interference-contrast optics. A zone in the apex (indicated by brackets) contains very small particles that are not resolved in the micrograph and corresponds to the light zone in Fig. 3. Mitochondria (M) are in the subapical region. Grown on PD Agar. Scale line = 1 μm.*

(FIG. 1-4 on page 992)

FIG. 5. *Longitudinal section showing the subapical region of the same hypha as that in Fig. 1. This region is 20 to 30 μm behind the hyphal apex. The part of the micrograph toward the top of the page is closest to the hyphal apex. Paranuclear stacks of smooth-surfaced cisternae are dictyosomes (D) of the Golgi apparatus. The dictyosomes, like the apical vesicles (Fig. 1), are in cytoplasm that contains few ribosomes. Vesicles associated with dictyosomes in this subapical region appear the same as those concentrated in the apical zone. Endoplasmic reticulum (ER), ribosomes (R), and mitochondria (M) are found in the subapical region. Glutaraldehyde fixation. Scale line = 1 μm.*

(FIG. 5 on page 993)

FIG. 6. *Hyphal tip of Gilbertella persicaria taken from one of a series of longitudinal sections through the hypha. As with P. aphanidermatum the apical zone is populated mainly by large and small vesicles that are distributed throughout the tip region. Large vesicles are concentrated at the apex. Vesicles intermediate in size and staining characteristics between the large and small ones are also present. Ribosomes (R) and vesicles are mixed throughout the cytoplasm in the posterior portion of the apical zone, but the ribosomes are scarce near the apex where the large vesicles are concentrated. Formaldehyde-glutaraldehyde fixation. Scale line = 1 μm.*

FIG. 7. *An apical region in G. persicaria showing the closely packed large vesicles (V) lining the apical wall (W) in a crescent-shaped cluster. The contents of the large vesicles and the inner layer of the wall have similar staining characteristics. The smaller vesicles have very dense contents but are nearly excluded from the cluster in the apex. Formaldehyde-glutaraldehyde fixation. Scale line = 1 μm.*

FIG. 8. *Enlargement of part of a hyphal apex of G. persicaria showing several large vesicles (V) near the wall (W) and two profiles (arrows) that suggest fusion of vesicles with plasma membrane. The vesicle contents and the inner layer of the wall have similar staining characteristics. Formaldehyde-glutaraldehyde fixation. Scale line = 1 μm.*

(FIG. 6-8 on page 994)

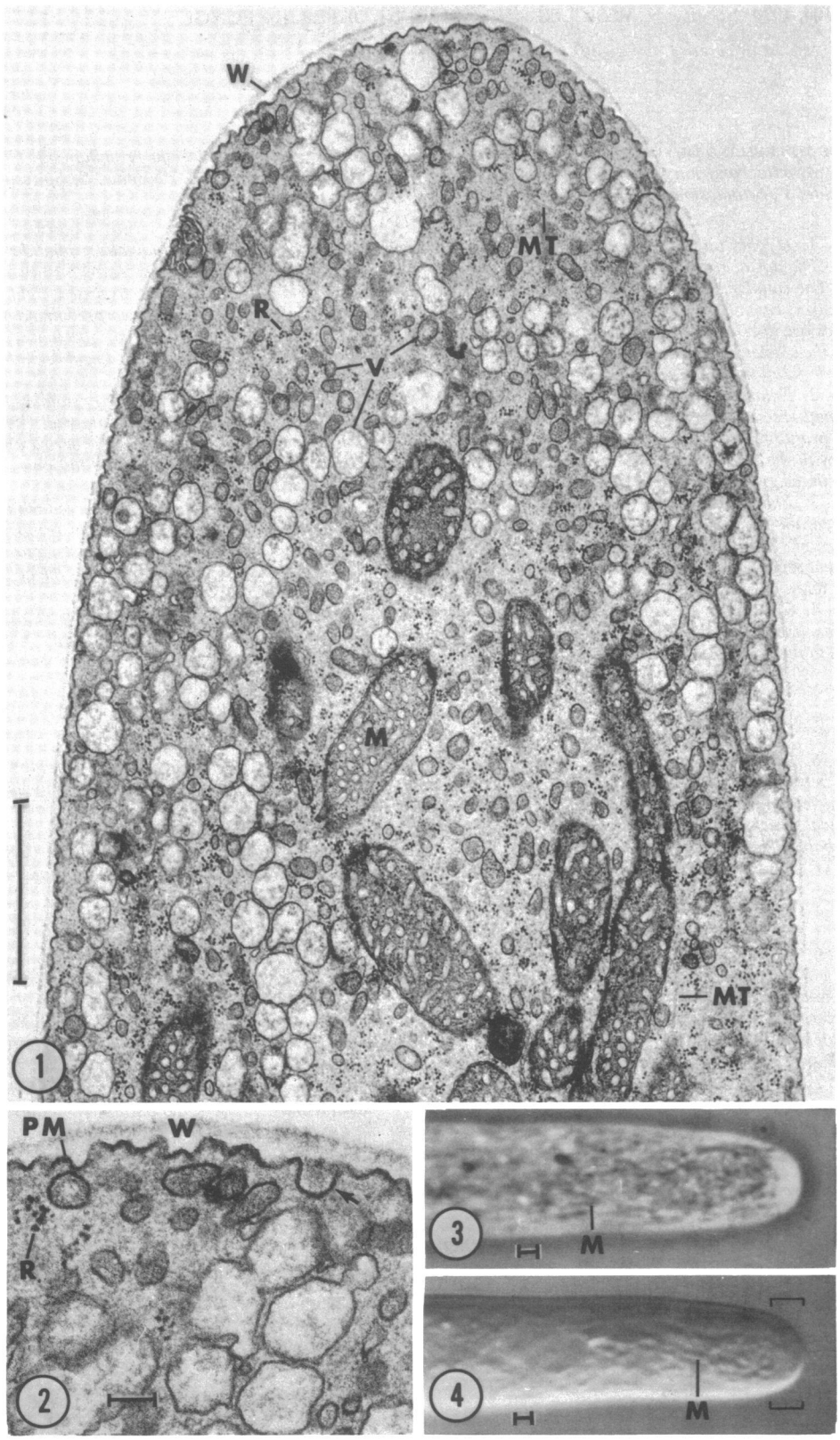

FIG. 1–4

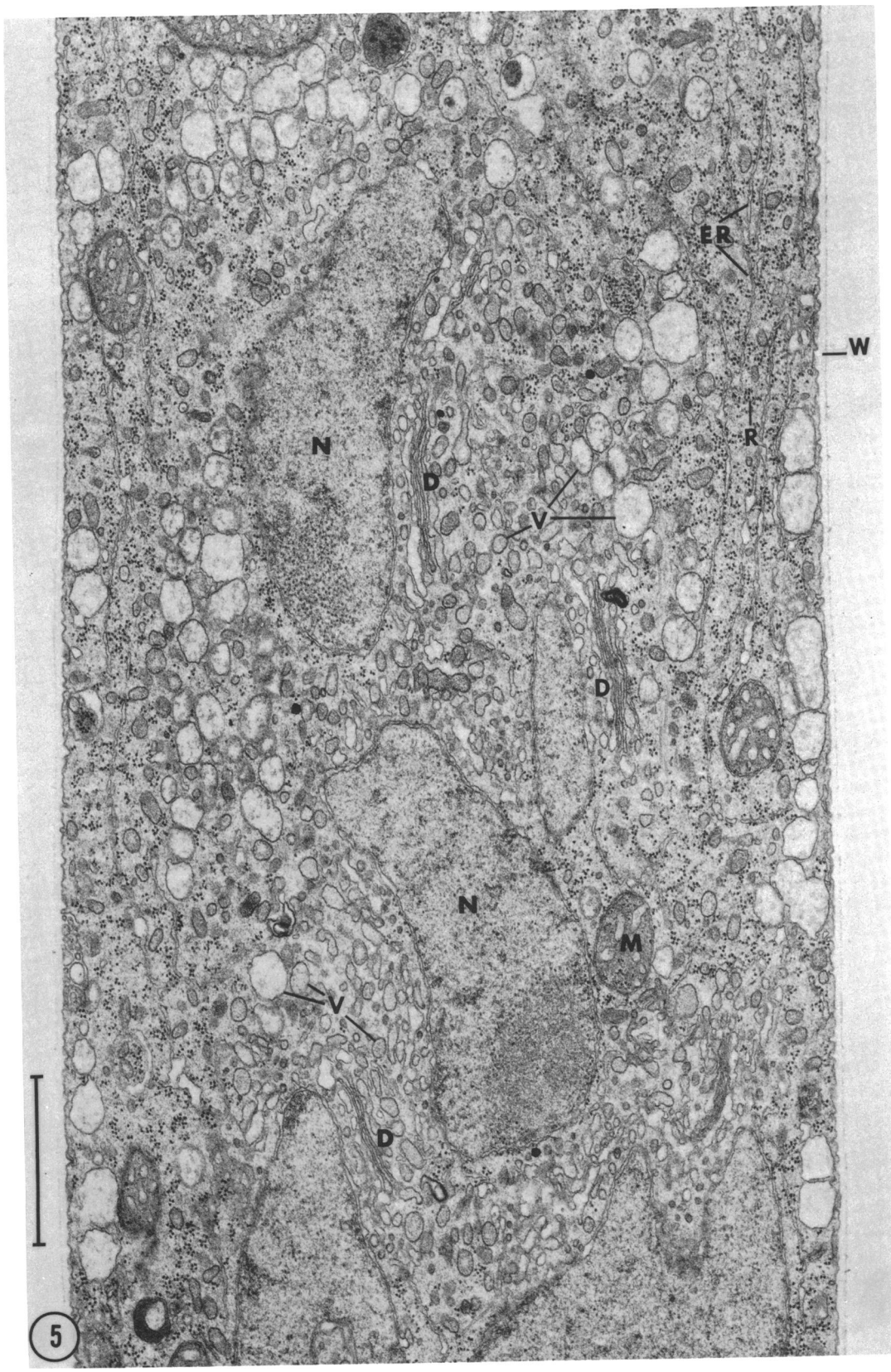

FIG. 5

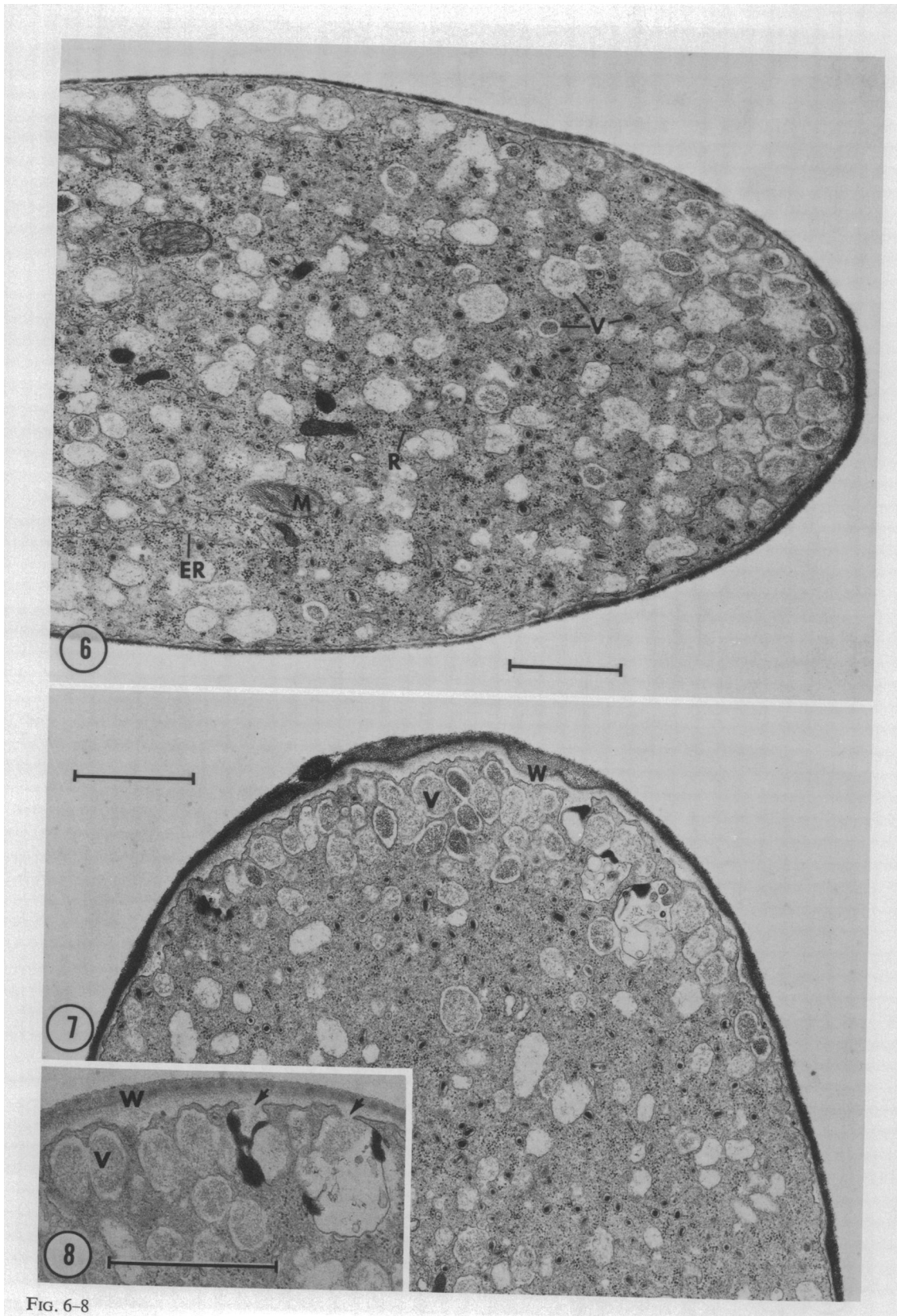

FIG. 6–8

size and stainability (Fig. 1, 2, 6–9), but a few vesicles are intermediate between the two main forms. In *P. aphanidermatum*, the vesicles are distributed rather uniformly throughout the apical zone (Fig. 1). However, in *G. persicaria* a band of closely packed large vesicles is typically associated with the apical plasma membrane (Fig. 7, 8). There is an incursion of ribosomes up to this band of apical vesicles, but few ribosomes are found between the vesicles at the apex (Fig. 6–8). In *P. aphanidermatum* on the other hand, ribosomes are scarce in the entire apical zone (Fig. 1, 2). Posterior to the apical band of large vesicles in *G. persicaria*, the large and small vesicles are uniformly distributed in the cytoplasm of the hyphal tip in a region which may extend basipetally for a distance equivalent to more than one hyphal diameter (Fig. 6). Unlike the organization of hyphal tips in septate fungi, shown subsequently, we have not detected specialized regions amidst the accumulations of apical vesicles in *P. aphanidermatum* and *G. persicaria*, although many hyphal apices have been serially sectioned. Additionally, in thin sections, we did not observe an "apical corpuscle" such as that described by Bartnicki-Garcia et al. (1) for *Mucor rouxii*, another zygomycete.

In living hyphae of *P. aphanidermatum* and *G. persicaria*, observed by light microscopy, the apices contain a zone of rapidly moving particles (Fig. 3, 4, 10–14) that correspond to the vesicles seen in electron micrographs (Fig. 15). In the large, rapidly growing hyphae of *G. persicaria*, the vesicles are easily resolved (Fig. 10–14), and their motion can be observed. These vesicles exhibit a swarming motion. Those in the subapical region migrate along the lateral wall from the subapical region toward the apex and appear to adhere to the inner surface of the wall at the hyphal apex. This results in a crescent-shaped band lining the apical wall (Fig. 10–12) and corresponds to the pattern of large apical vesicles observed in electron micrographs (Fig. 7). Individual vesicles at the apex disappear from view as the hypha elongates, but they are constantly replaced by other vesicles from the subapical zone. Some of the unattached vesicles may return to the subapical region via a route near the center of the hypha. When growth ceases, the zone of vesicles remains for a few minutes, but the band of large vesicles at the apex disappears. In general, if growth does not resume after several minutes, vacuolation of the cytoplasm occurs and the zone of vesicles is dispersed. In growing hyphae of *P. aphanidermatum* a similar zone of vesicles is observed, although vesicles are not as well resolved with light microscopy as those in *G. persicaria*. There may be an initial build up of vesicles in the apex as growth slows or stops, as in *P. ultimum* (10), but if growth does not resume, the zone of vesicles is dispersed and vacuolation of the cytoplasm occurs. No crescent-shaped band lining the apical wall has been seen in the *Pythium* species.

In contrast to the apical organization of the foregoing fungi, a specialized region within the cluster of apical vesicles occurs in hyphae of the ascomycete, basidiomycete, and deuteromycete species (i.e., septate fungi) we have examined (Fig. 16, 22, 23, 28–30, 33, 34, 38–40). In electron micrographs, it usually measures less than half of the diameter of the hypha and is located in our specimens near the central axis of the hypha; thus, it is found in near median longitudinal sections. It may occur immediately adjacent to the apical wall, or it may be separated from the wall by a short distance. This region lacks a distinct boundary or limiting membrane and is characterized by having a different consistency from the surrounding cytoplasm. It consists of either a vesicle-free area (Fig. 28–30, 38–40), which in some sections has a densely-staining core (Fig. 28, 29, 39), or an aggregate of very small vesicles (Fig. 16, 22, 23, 33, 34) or tubules (Fig. 30, 34). It is usually surrounded by the large apical vesicles (Fig. 16, 22, 23, 28–30, 33, 38–40). In some specimens, a cluster of ribosomes is associated with this specialized zone (Fig. 16, 22, 23, 30, 34) in contrast to the general scarcity of ribosomes in apical zones of hyphae.

In living hyphae of septate fungi observed by light microscopy, the apices also contain a zone of rapidly moving particles, corresponding to the apical vesicles seen in electron micrographs. In addition, we have consistently observed a phase-contrast-dark region within the apical zone of vesicles. It is roughly spherical, but may also appear flattened or irregular, has no distinct boundary, and has a diameter which is usually less than half of the diameter of the hypha (Fig. 17–19, 24–26, 31, 35–37, 41). This region corresponds to the Spitzenkörper described in previous studies of living hyphae (6–8, 12). It is specifically associated with the expanding surface at the hyphal apex and disappears when hyphal growth stops only to reappear when growth resumes. It is not attached to the inner surface of the apical wall, because it moves about slightly in the apex, and we frequently observe space between the wall and the Spitzenkörper (Fig. 17, 18, 24, 25). It is not the collective equivalent of the apical vesicles. In large, rapidly growing hyphae of septate fungi (e.g., *A. nigricans*, *N. crassa*, *R. solani*), in which apical vesicles can be resolved by light microscopy, the zone of vesicles often occupies all of the

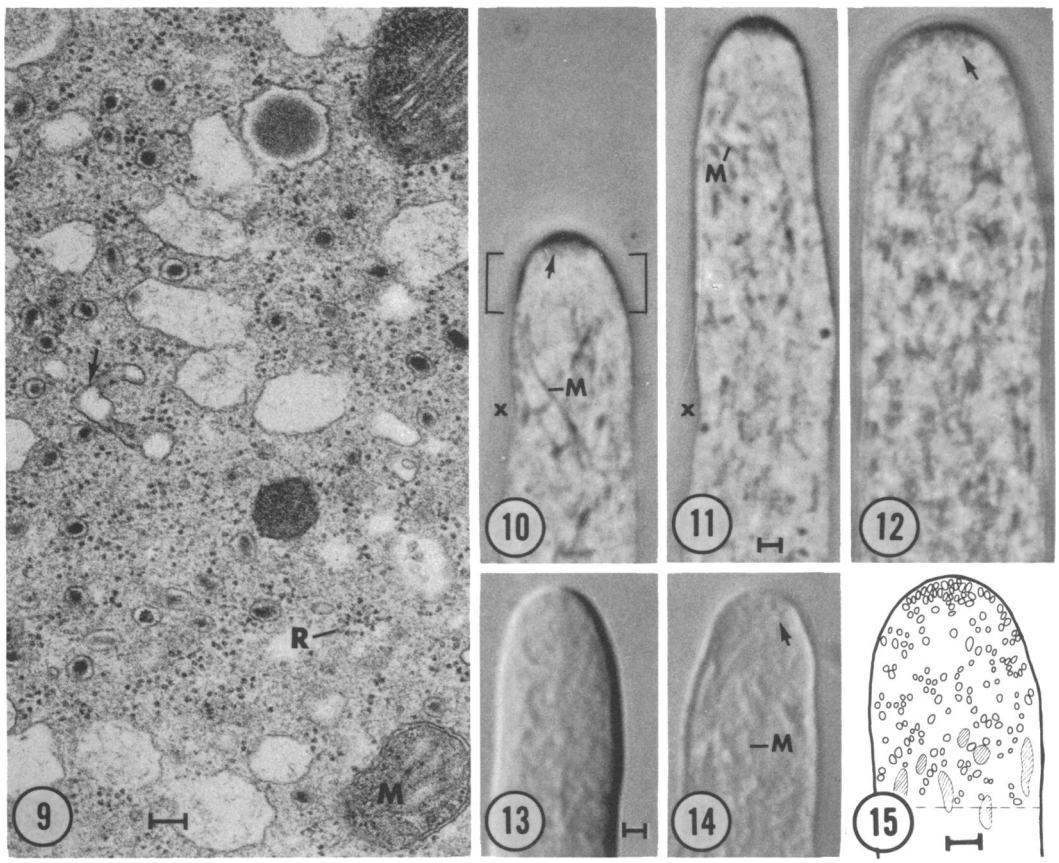

FIG. 9. *Enlargement of part of the posterior portion of the apical zone in a hypha of G. persicaria. Note the large and small vesicles with densely staining contents, large vesicles with less dense contents, ribosomes (R), and the smooth-surfaced cisterna (arrow). Formaldehyde-glutaraldehyde fixation. Scale line = 0.1 μm.*

FIG. 10–12. *Growing hyphae of G. persicaria as observed by phase-contrast optics. A crescent-shaped band of dark particles is adjacent to the apical wall. The particles (arrows) are apical vesicles and can be seen in the zone under the curved apex. They migrate from the subapical region along the hyphal wall to the apical region where many adhere to the wall and gradually disappear as the hyphae elongate. The clear region (brackets) behind the apex corresponds to the region containing vesicles and ribosomes in Fig. 6 and 7. Mitochondria (M) are in the subapical region and are represented by the filamentous dark bodies, best seen in Fig. 11. Figure 11 is of the same tip as Fig. 10 but taken about 45 sec later. Bends (×) in the wall serve as markers to show the amount of elongation. Grown on PD Gelatin. Scale line = 1 μm.*

FIG. 13 and 14. *Growing hyphae of G. persicaria as observed by Nomarski interference-contrast optics. The hypha in Fig. 14 was grown on PD Gelatin, whereas that in Fig. 13 was grown on PD Agar. The different refractive indices impart different images to the hyphae. The particulate nature of the region adjacent to the apical wall is best seen in Fig. 14. The particles (arrow) correspond to apical vesicles. Mitochondria (M) are in the subapical region. Scale line = 1 μm.*

FIG. 15. *A tracing of the apical region from an electron micrograph reduced to a lower magnification. The hyphal outline, apical vesicles, and the mitochondria from the electron micrograph have been traced. The row of vesicles at the apex corresponds to the band of particles seen in the light micrographs. Scale line = 1 μm.*

curved apical region (Fig. 25, 35–37), but the Spitzenkörper takes up less than half of this area (Fig. 17–19, 24–26, 31, 32, 35–37, 41). The particles corresponding to the apical vesicles remain for a few minutes when hyphal growth ceases, even though the Spitzenkörper disappears. If growth does not resume after several minutes, vacuolation occurs and the zone of vesicles also disappears.

A graphic demonstration of the correspondence between the images of hyphal tips obtained by light and electron microscopy is seen when trac-

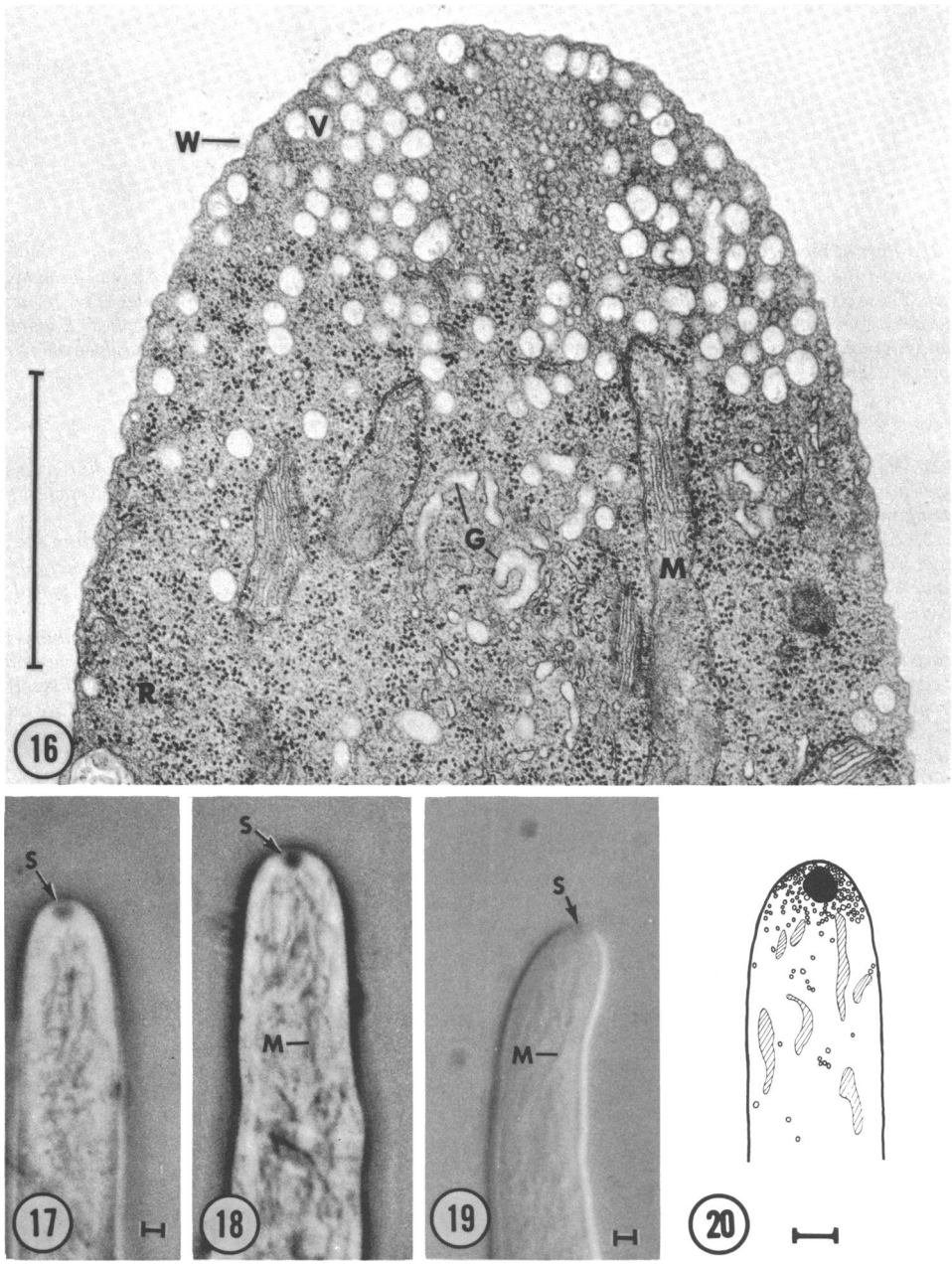

FIG. 16. *Median longitudinal section of a hyphal tip of Aspergillus niger. Amidst the cluster of apical nesicles (V) there is a zone consisting of an aggregate of smaller vesicles and clusters of ribosomes. The larger vesicles do not intrude into this area. Note the abrupt decline in the concentration of ribosomes (R) in the apical zone as compared to the subapical portion in which apical vesicles are not concentrated. Smooth-surfaced Golgi cisternae (G) and a few vesicles occur immediately basipetal to the apical zone. Both the hyphal wall and the apical vesicles are electron transparent with this method of preparation. Formaldehyde-glutaraldehyde fixation. Scale line = 1 μm.*

FIG. 17 and 18. *Phase-contrast micrographs of growing hyphae of A. niger showing the dark Spitzenkörper (S) within the light apical areas. Numerous minute rapidly moving particles can be seen in these light areas when observed through the microscope, but they are not resolved in the micrographs. The Spitzenkörper is not touching the apical wall. Mitochondria (M) are represented by the dark filamentous structures in the subapical region. Grown on PD Gelatin medium. Scale line = 1 μm.*

FIG. 19. *Nomarski interference contrast micrograph of a growing hyphal tip of A. niger. The Spitzenkörper (S) appears as a bump in the hyphal apex. Mitochondria (M) are in the subapical region. Grown on PD Agar. Scale line = 1 μm.*

FIG. 20. *Tracing taken from an electron micrograph of an A. niger hyphal tip. The outlines of the hypha, large apical vesicles, and mitochondria have been traced. The specialized region containing microvesicles but no large vesicles is represented by the inked-in spot and agrees with the relative size, shape, and location of the Spitzenkörper in light micrographs (Fig. 17, 18). Scale line = 1 μm.*

FIG. 21. *Part of the subapical region about 20 μm behind the apex in a hypha of A. niger. The top of the micrograph is nearest the apex. Several clusters of inflated smooth-membrane cisternae (G) are scattered throughout the cytoplasm. These cisternae occur in association with vesicles that appear the same as apical vesicles (Fig. 16) and are surrounded by zones of exclusion that contain few ribosomes compared to the rest of the protoplast. Endoplasmic reticulum (ER) and mitochondria (M) are common in the subapical region. Formaldehyde-glutaraldehyde fixation. Scale line = 1 μm.*

(FIG. 21 on page 999)

FIG. 22. *Hyphal tip of Neurospora crassa showing vesicles (V) in the apical zone. A specialized area (arrows) within the cluster of large apical vesicles contains minute vesicles and only a few of the larger apical vesicles. Formaldehyde-glutaraldehyde fixation. Scale line = 1 μm.*

FIG. 23. *Enlargement of part of a hyphal apex in N. crassa showing details of the specialized area in the apex. The apical wall (W) is at the right. This specialized region contains small vesicles (arrows) and clusters of ribosomes, and is surrounded by the larger apical vesicles. Except for this part of the apex, the rest of the apical zone is relatively free from ribosomes. Formaldehyde-glutaraldehyde fixation. Scale line = 0.1 μm.*

FIG. 24 and 25. *Phase-contrast micrographs of growing hyphal tips of N. crassa showing the Spitzenkörper (S). A light area is visible between the Spitzenkörper and the apical wall in Fig. 24. Particles corresponding to apical vesicles can be observed in the light areas by direct observation through the microscope but are not well resolved in the micrograph. Mitochondria (M) may be excluded from the apical region as in Fig. 25, or they may move into the apical region and appear to contact the Spitzenkörper as in Fig. 24 (see also Fig. 22). Grown on PD Gelatin. Scale line = 1 μm.*

FIG. 26. *Growing hypha of N. crassa as seen by Nomarski interference-contrast optics. The Spitzenkörper (S) is near the center of the apical region and appears as a bump. Mitochondria (M) from the subapical region appear to contact the Spitzenkörper. Grown on PD Agar. Scale line = 1 μm.*

FIG. 27. *A tracing taken from an electron micrograph of N. crassa, showing the distribution of the apical vesicles relative to the Spitzenkörper. The hyphal profile, large apical vesicles and mitochondria are outlined. The specialized region containing microvesicles and ribosomes is inked in. The location and size of this region correspond closely to the Spitzenkörper seen in the light micrographs (Fig. 24–26). Scale line = 1 μm.*

(FIG. 22–27 on page 1000)

FIG. 28. *A hyphal tip of Verticillium albo-atrum showing the apical zone with a concentration of vesicles (V) and few ribosomes. The apical zone gradually merges with the subapical zone that contains mitochondria (M), endoplasmic reticulum (ER), ribosomes (R), and Golgi cisternae (G). A vesicle-free region at the apex amidst the vesicles has a densely staining core and corresponds to the Spitzenkörper. Glutaraldehyde fixation. Scale line = 1 μm.*

FIG. 29. *Enlargement of part of the apical zone from Fig. 28 to illustrate details of the apical vesicles and the Spitzenkörper region at the apex. Scale line = 1 μm.*

FIG. 30. *Hyphal tip of Fusarium oxysporum showing the shallow apical zone and part of the subapical zone. The subapical zone is denoted by the presence of mitochondria (M). The clear region (arrows) in the center of the cluster of apical vesicles stains the same as ground cytoplasm. In the posterior part of this region is a cluster of ribosomes (R) and minute membranous tubules (T). Glutaraldehyde fixation. Scale line = 1 μm.*

FIG. 31. *Phase-contrast micrograph of V. albo-atrum showing the Spitzenkörper (S) in the apex a of growing hypha. Mitochondria (M) are in the subapical region. Grown on PD Gelatin. Scale line = 1 μm.*

FIG. 32. *Growing hyphal tip of F. oxysporum as seen by phase-contrast optics. The Spitzenkörper (S) occupies part of the apical region. Mitochondria (M) and other dark bodies are in the subapical region. Grown on PD Gelatin. Scale line = 1 μm.*

(FIG. 28–32 on page 1001)

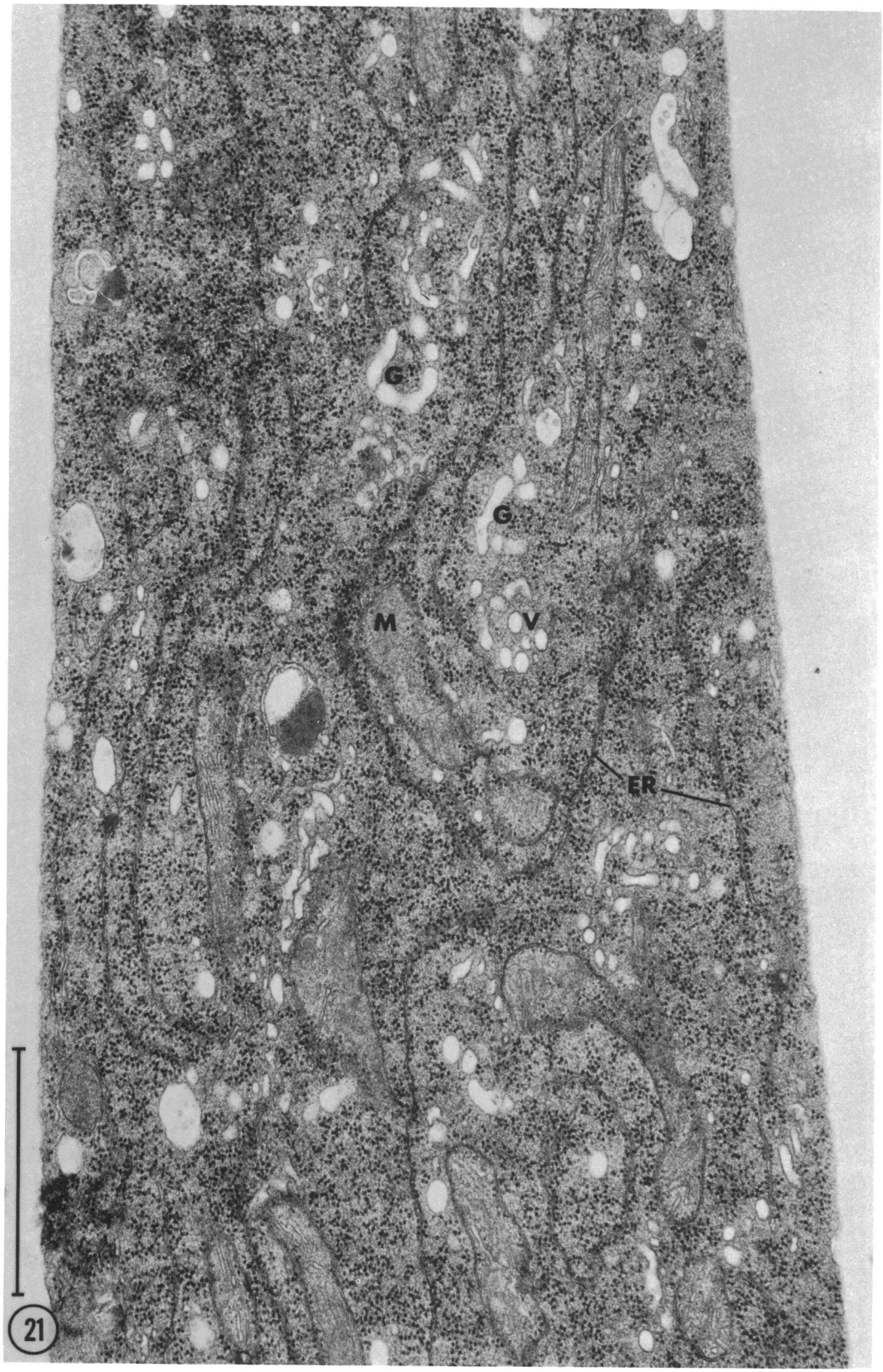

FIG. 21

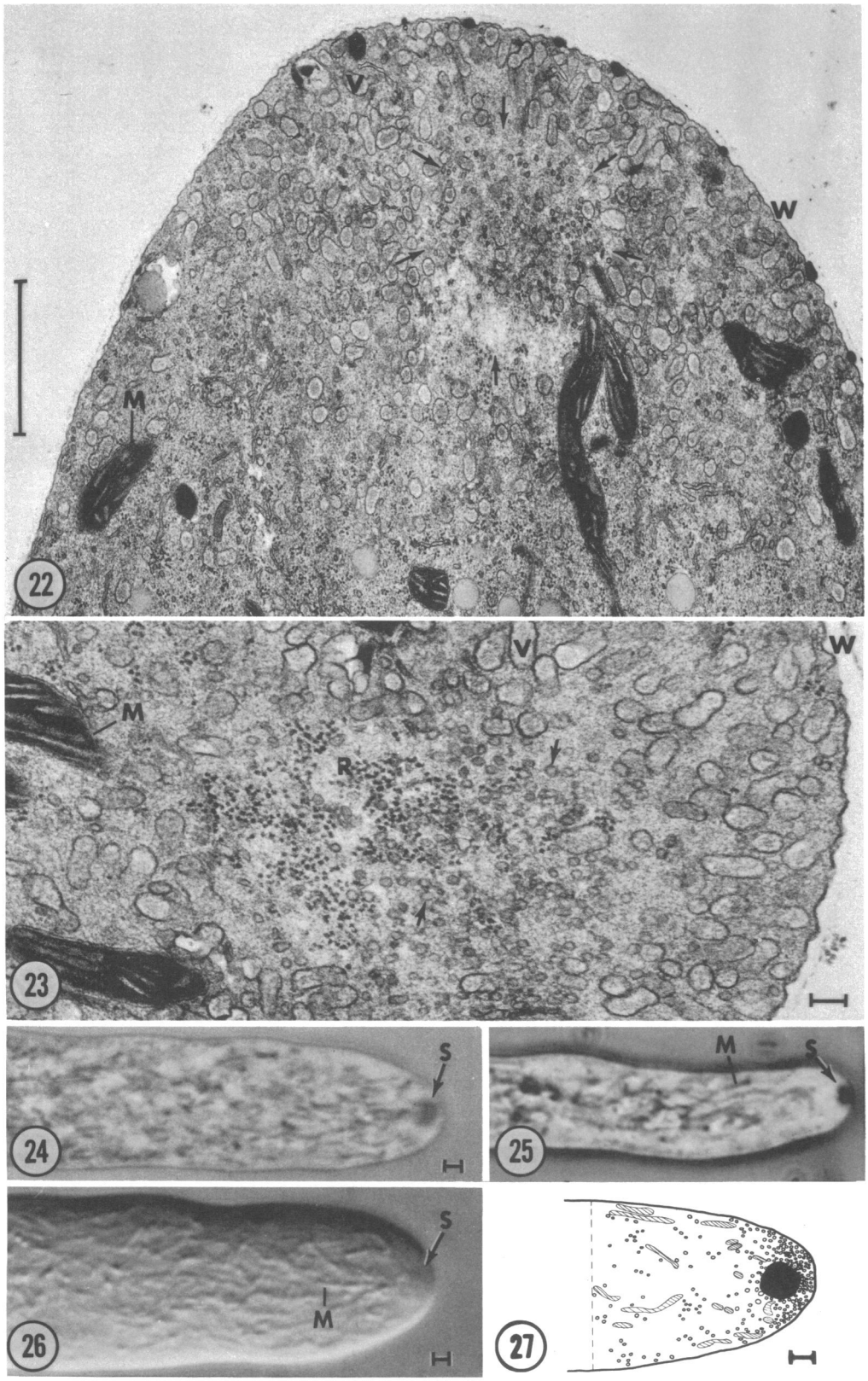

FIG. 22–27

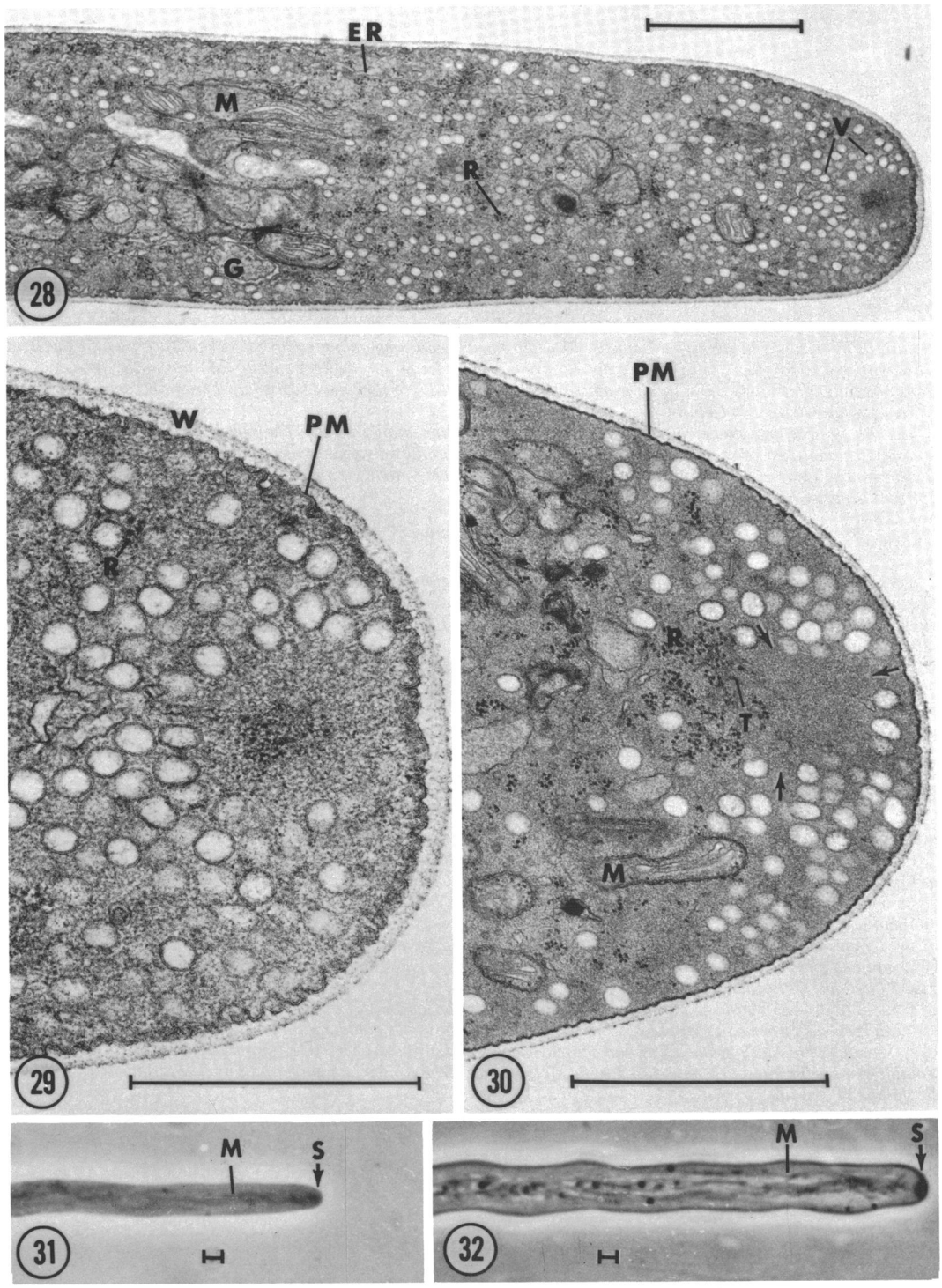

FIG. 28–32

FIG. 33. *Part of a hyphal tip of Ascodesmis nigricans in longitudinal section. The specialized region or Spitzenkörper of the apical zone (arrows) appears as a diffusely mottled dark-staining area within the mass of apical vesicles. Glutaraldehyde fixation. Scale line = 1 μm.*

FIG. 34. *Enlargement of part of the same hyphal tip as in Fig. 33 to show details of the dark-staining region in the apex. It consists of a concentrated grouping of small tubules (T) and small vesicles (arrows) intermingled with a cluster of ribosomes (R). Larger apical vesicles (V) surround the specialized region. The apical wall (W) is at the top. Scale line = 0.1 μm.*

FIG. 35. *Phase-contrast micrograph of A. nigricans showing the dark Spitzenkörper (S) within the light zone in the apex of a growing hypha. Moving particles corresponding to apical vesicles are observed in the light area but are not resolved in the micrograph. Mitochondria (M) occupy the subapical region. Grown on PD Gelatin medium. Scale line = 1 μm.*

FIG. 36 and 37. *Growing hyphae of Rhizoctonia solani as seen by phase-contrast optics. The dark Spitzenkörper (S) are in light zones in the hyphal apices. Moving particles corresponding to apical vesicles are seen in the light areas with the microscope, but they are not resolved in the micrograph. Mitochondria (M) are in the subapical regions and may alternately move into the apical zone as in Fig. 37, or they may be excluded from the apical region as in Fig. 36. Grown on PD Gelatin. Scale line = 1 μm.*

FIG. 38. *Apical and subapical zones in a hyphal tip of Rhizoctonia solani. The apical zone is nearly devoid of ribosomes in contrast to the subapical zone. Vesicle fusion profiles give the wall at the apex a lumpy irregular appearance. The clear zone (arrows) among the cluster of apical vesicles is the specialized region interpreted as the Spitzenkörper. Glutaraldehyde fixation. Scale line = 1 μm.*

(FIG. 33–38 on page 1003)

FIG. 39. *Median longitudinal section through the apical zone of a hypha of Armillaria mellea. The convoluted plasma membrane at the apex of this hypha is closely associated with vesicle profiles. The vesicle-free region among the vesicles contains a small densely-staining core (arrow). Microtubules (MT) extend into the apical zone from the subapical zone. Glutaraldehyde fixation. Scale line = 1 μm.*

FIG. 40. *A hyphal tip of Armillaria mellea showing the apical cluster of vesicles (V) and the central region that is free from vesicles (arrows). This is a different hypha from the one shown in Fig. 39 and the section is slightly off median. The irregular profile of the plasma membrane (PM) and the wall in the hyphal apex suggests vesicular fusion with the plasma membrane. Glutaraldehyde fixation. Scale line = 1 μm.*

FIG. 41. *Phase-contrast micrograph of A. mellea showing a Spitzenkörper (S) in the apex of a growing hyphal tip. Mitochondria (M) are visible in the subapical region. Grown on PD Gelatin. Scale line = 1 μm.*

(FIG. 39–41 on page 1004)

FIG. 42. *Longitudinal section through part of the subapical zone about 30 μm behind the apex of the hypha shown in Fig. 39. The top of this micrograph is nearest the apex. Clusters of vesicles (V) which appear similar to hyphal tip vesicles are scattered throughout the subapical zone. The clusters of vesicles are associated with Golgi cisternae or tubules (G) in zones of cytoplasm which contain few ribosomes compared to the surrounding protoplasm. Some of the tubules are branched or fenestrated, much the same as the periphery of a dictyosome cisterna. Mitochondria (M) and endoplasmic reticulum (ER) occur throughout this region. Compare the concentration of ribosomes with Fig. 39 and 40. Glutaraldehyde fixation. Scale line = 1 μm.*

FIG. 43 and 44. *Smooth-surfaced, fenestrated or tubular cisternae (G) in the subapical region of A. mellea. Each cisterna resides in a zone of cytoplasm which contains few ribosomes compared to the surrounding protoplasm. Vesicles (V) similar to those in the apex, and coated vesicles and protuberances (CV) are associated with the cisternae. Similar coated profiles are associated with dictyosomes in Pythium aphanidermatum. Glutaraldehyde fixation. Scale line = 0.1 μm.*

(FIG. 42–44 on page 1005)

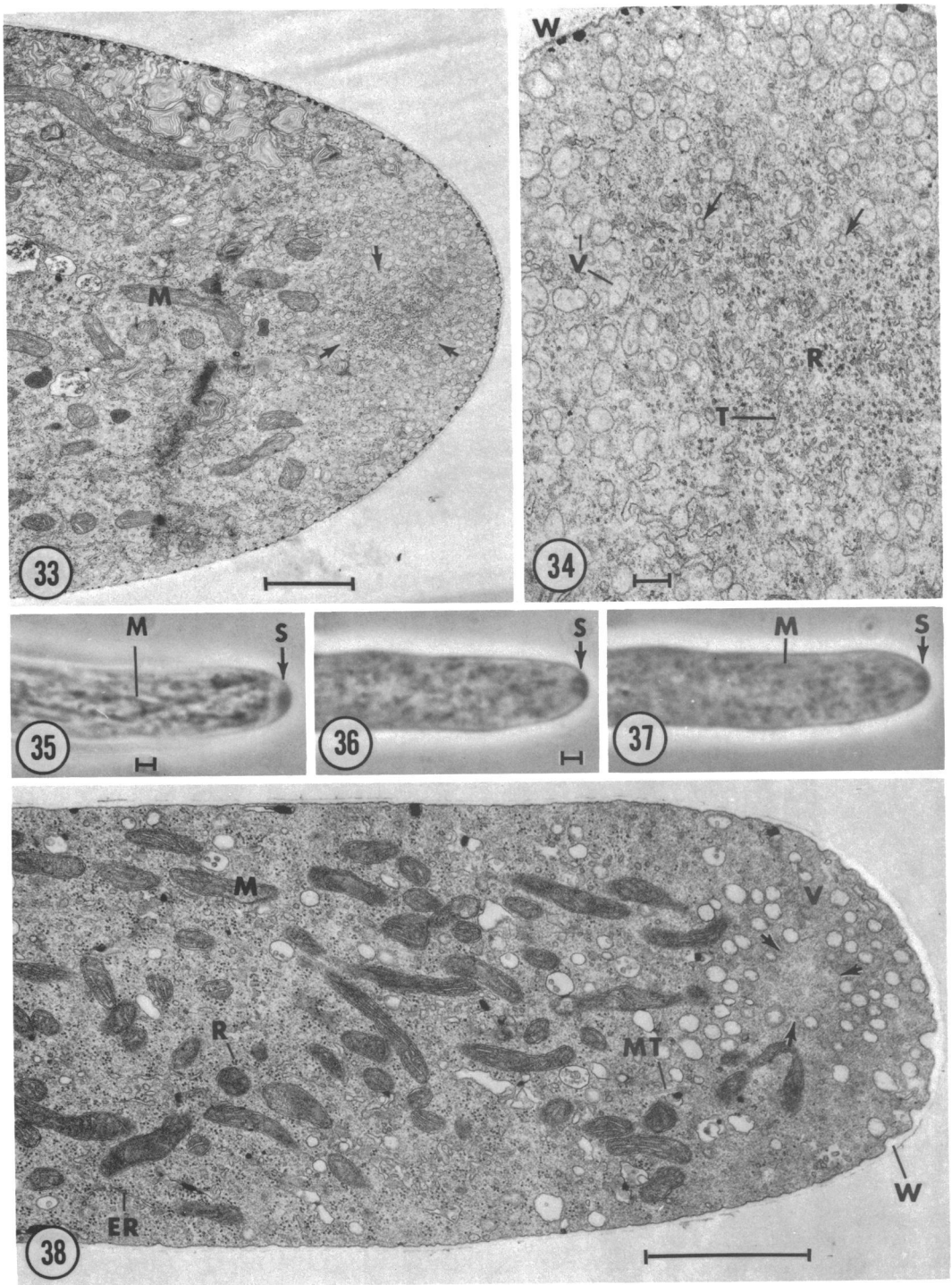

FIG. 33–38

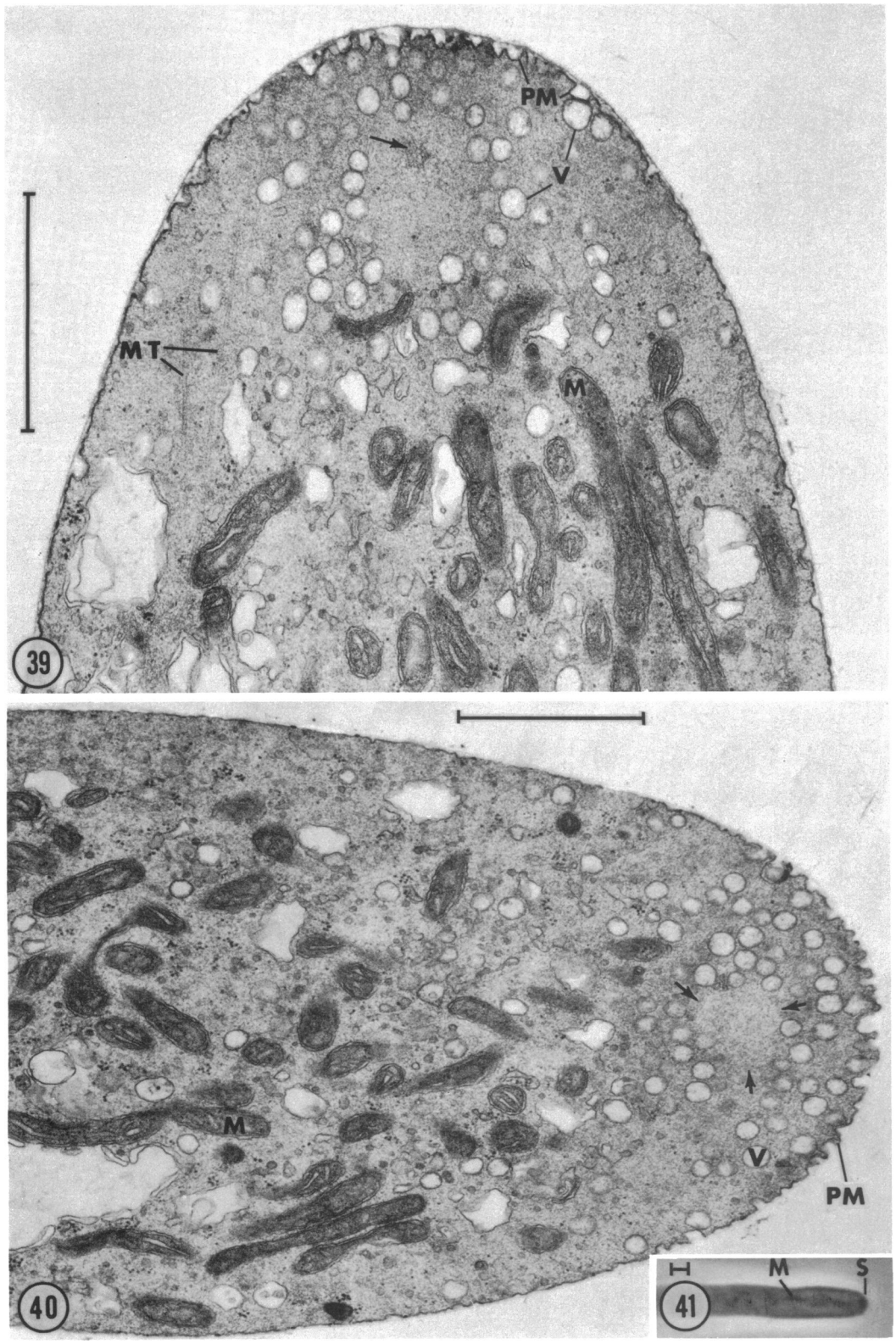

FIG. 39–41

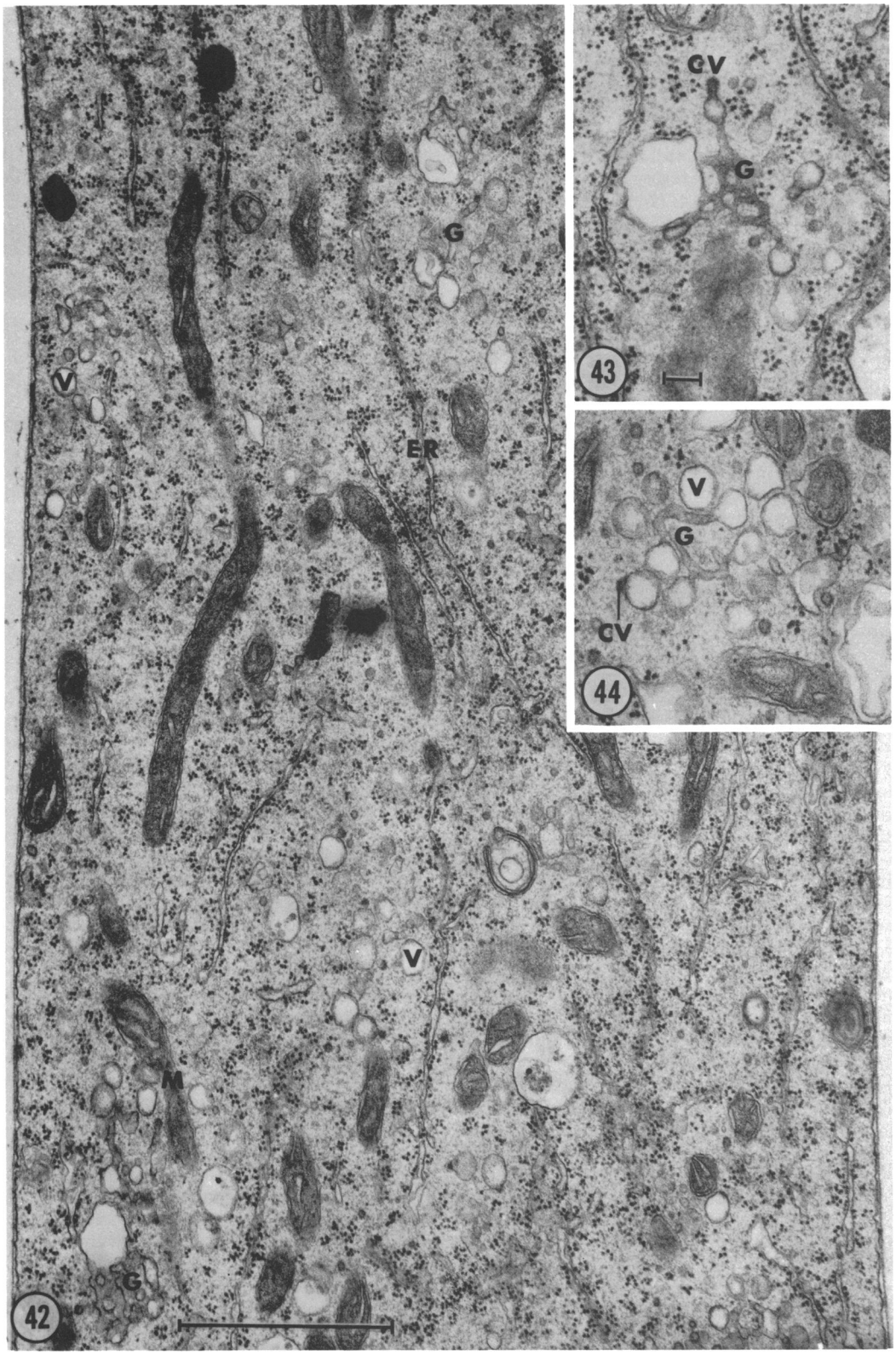

FIG. 42–44

ings of electron micrographs of hyphal tips are reduced in size and compared with light micrographs of living hyphae. In a tracing of an electron micrograph of *G. persicaria* (Fig. 15), vesicles are aligned along the apical wall in the position of the crescent-shaped dense region in phase-contrast micrographs (Fig. 10–14). In *A. niger*, a tracing (Fig. 20) of an electron micrograph shows that the relative size and shape of the Spitzenkörper and of the zone of apical vesicles is similar to that in the light micrographs (Fig. 17–19). The Spitzenkörper (Fig. 20) is equivalent only to the zone of microvesicles in Fig. 16 and not to the total accumulation of apical vesicles. A tracing of an electron micrograph of *N. crassa* (Fig. 27) also shows a regional distribution in the apex similar to that observed by light microscopy (Fig. 24–26). Thus, the relative size, shape, and location of the Spitzenkörper in hyphal tips prepared for electron microscopy agrees with observations of this region in living, growing hyphal tips.

DISCUSSION

Our observations and those of others (3, 8, 10–12) point out a remarkable similarity in the basic pattern of hyphal tip organization among a spectrum of taxonomically diverse fungi. These comparisons reveal an apical zone of vesicles in fungal hyphae as in other tip-growing cells such as pollen tubes (19), root hairs (2), budding yeast cells (14), and algal rhizoids (20). This type of organization is consistent with the hypothesis that vesicles produced in the subapical region become concentrated in the apex, in a zone with few or no ribosomes, where they are incorporated at the expanding surface. Several lines of evidence support this hypothesis. Budding profiles and associations between vesicles and smooth-membraned cisternae (dictyosomes in *Oömycetes*) in ribosome-free areas of the subapical region suggest that the vesicles are derived endogenously from the endomembrane system (*see also* 8–10). This interpretation is supported by evidence that components of the endomembrane system, as exemplified by the Golgi apparatus, are involved in transforming membranes from endoplasmic reticulum-like to a plasma membrane-like appearance, concomitant with the production of secretory vesicles (9, 10). Fusion profiles showing continuity between vesicle membranes and plasma membrane (*see also* 10, 12) indicate that the vesicle membrane is contributed to the plasma membrane at the apex, and that the vesicle contents are released into the wall region in a process comparable to exocytosis or vesicle-mediated secretion (10, 13). Girbardt (8) has provided evidence against the alternative possibility of endocytosis by growing hyphae in a medium containing 0.01% K_2TeO_3 which stains the plasma membrane, including lomasome-like invaginations, but not the vesicles. If the vesicles were derived from the plasma membrane by endocytosis, their membrane would be expected to retain the stain and appear similar to the plasma membrane. Additionally, the hyphal apex is a region in which there is an ongoing net increase in cell surface—not a decrease, as would be suggested by the utilization of plasma membrane to form endocytotic vesicles at that site. Thus, endocytosis would run counter to the required increase of plasma membrane in the hyphal apex. In light microscopic studies of fungi with large rapidly growing hyphae, we observe the migration of vesicles in the peripheral cytoplasm toward the apex from the subapical regions. The apparent deposition of the apical vesicles at the apex and their disappearance from view can be seen in hyphae of *G. persicaria*. These observations support the scheme for hyphal tip growth mentioned above and discussed in detail by Grove et al. (10).

The content of the vesicles in hyphal apices is not yet established. Girbardt (8) suggests that the apical vesicles produced at the peripheries of Golgi cisternae probably function in the transport of exoenzymes which are extruded at the apex. A similar proposal for the transport of an enzyme was made by Moor (14) for budding yeast cells. Grove et al. (10) suggest that the apical vesicles of *P. ultimum* also contain wall precursors (i.e., polysaccharides) which may contribute to forming the large quantities of new hyphal wall required at the apex, W. J. VanDerWoude (M.S. Thesis, Purdue Univ., Lafayette, Ind., 1969) has isolated secretory vesicles from pollen tube tips and characterized the contents as including polysaccharides by using biochemical methods correlated with cytochemical localization of polysaccharides in the intact cell. Preliminary ultracytochemistry by us, using the silver hexamine method (16), indicates that the apical vesicles in *P. aphanidermatum* give a positive reaction for polysaccharides similar to the reaction of the hyphal wall. Equivalent results with species of *Saprolegnia* have been obtained by I. B. Heath (Ph.D. Thesis, Univ. London, London, England, 1969). These observations are consistent with our previous suggestion (10) that at least part of the apical vesicle contents consist of carbohydrates, and they are also consistent with literature on plant secretion which demonstrates that secretory vesicles contain polysaccharides (4, 13, 15, 16; see discussion in 10). Thus, although the specific composition and roles of apical vesicles in tip growth are not yet certain, they may contribute new plasma membrane, ma-

terial for extracellular coatings (wall precursors), materials to maintain the apical wall in an extensible condition (10, 14), or eoxenzymes (8).

In the fungi we studied there is good correlation between hyphal tip organization in life, as seen by light microscopy, and after fixation, as seen by electron microscopy. Since the individual vesicles are seen in living hyphae of some fungi, the relative positions of these and other cell components can be determined in living and fixed states. We obtained both preservation of apical organization and staining of cellular membranes with the same fixation procedure rather than by using two separate fixations (8). It was not necessary to employ $KMnO_4$ as a fixative to distinguish membranes clearly (see reference 8 for opposite view). Additionally, small vesicles, comparable to the microvesicles described by Girbardt (8) in $KMnO_4$-fixed hyphae, were preserved by aldehyde-OsO_4 fixation procedures. We have not experienced the difficulty of confusing microvesicles with ribosomes (8), since the microvesicles are bounded by a distinct membrane and are of a size and stainability clearly different from ribosomes.

Interpretation of the apical body or Spitzenkörper in hyphal tips is now a matter of some controversy, especially with regard to the light microscopic identity of the Spitzenkörper and a corresponding evaluation of what actually constitutes the Spitzenkörper at the ultrastructural level. Light microscopic studies leave no doubt that the Spitzenkörper is related to apical growth in higher fungi and that its position in the apex is associated with the direction of tip extension (6, 7, and this study). From the image of the Spitzenkörper as seen by light microscopy, one would expect, as stated by Girbardt (8), that it would be an organelle with a special ultrastructure. However, on the basis of electron micrographs of glutaraldehyde-OsO_4 fixed hyphal tips of *A. niger*, McClure et al. (12) first suggested that the Spitzenkörper corresponds to the cluster of cytoplasmic vesicles in the hyphal apex. Recently, Girbardt (8) concluded that the Spitzenkörper seen by light microscopy "represents an area of ground cytoplasm of different size and shape, containing apical vesicles, microvesicles and ribosomes." Throughout that report and in a three-dimensional model of the apical region of *Polystictus versicolor* (8), it is evident that Girbardt considers the Spitzenkörper to consist chiefly of apical vesicles which are closely aggregated into a group in the hyphal apex. Girbardt (8) cautions that it is not known whether the different size and shape of the vesicle accumulation is of functional significance or only represents a summation of single vesicles. When the aggregate of apical vesicles became dispersed, either due to cessation of growth or to unsatisfactory fixation, the Spitzenkörper then disappeared (8). The microvesicles were not shown in hyphae fixed with glutaraldehyde-OsO_4 (which preserves the Spitzenkörper), and the published micrographs do not show ribosomes in the apical region (8). The principal correlation by Girbardt (8) and McClure et al. (12) is between the Spitzenkörper which occurs in the hyphal apex and the cluster of apical vesicles which occurs in the same region.

A further complication in defining the Spitzenkörper is the apparent variation of apical organization among major groups of fungi. Two types of apical organization in hyphal tips were identified by Girbardt (8). Type I was found in *Phycomycetes*, had a longer portion of the hyphal tip devoted to vesicles in electron micrographs, and had an indistinct dark crescent-shaped band near the apical wall in living hyphae when seen with phase-contrast microscopy. *Ascomycetes*, *Basidiomycetes*, and *Deuteromycetes* had type II apical structure, which was characterized in electron micrographs by a sharper division between the apical accumulation of vesicles and the subapical region. This type also had a stainable, phase-contrast-dense, spherical Spitzenkörper which was interpreted as corresponding to the apical cluster of vesicles. A vesicle-free region in the apex was observed occasionally, but its vital existence was questioned because of the possibility that it was induced during fixation by dispersion of a compact cluster of vesicles. However, Girbardt suggests that it is a matter of definition whether type I hyphal tips have a Spitzenkörper or whether this designation should be limited to the spherical body of type II hyphal tips.

We think it is unlikely that the apical mass of vesicles in toto constitutes the spheroid Spitzenkörper seen by light microscopy. Instead, there is a positive correlation between the Spitzenkörper and the smaller specialized zone that occurs within the cluster of apical vesicles and which sometimes contains microvesicles, tubules, and a few ribosomes. The distribution of this zone among fungi agrees with the occurrence of spherical Spitzenkörper which have been observed only among the *Ascomycetes*, *Basidiomycetes*, and *Deuteromycetes* (5–8, 12). If, on the other hand, the apical vesicles did constitute the Spitzenkörper, then in the *Oömycetes*, which contain a distinct zone of apical vesicles (10–12), we would expect to find a Spitzenkörper. But no Spitzenkörper were seen by McClure et al. (12) or by us (see also reference 10) with phase-contrast microscopy of living hyphae.

The shape, size, and position of the specialized regions shown here are consistent with our light

microscopic observations of Spitzenkörper and with those of others (5–8, 12). The lack of a distinct boundary or limiting membrane in our study agrees with the observation reported by McClure et al. (12) that the Spitzenkörper have vague outlines and can not be sharply focused with the light microscope. If the Spitzenkörper were the collective equivalent of hyphal tip vesicles, we would expect the entire apical zone to be Spitzenkörper, but photographs and descriptions consistently portray a smaller structure within the apical zone (5–8, 12). This is especially evident when the tracings of electron micrographs are compared with light micrographs of living hyphae in which the specialized region corresponds closely to the Spitzenkörper, whereas the zone of apical vesicles corresponds to the lighter area in the apex that surrounds the Spitzenkörper. Sometimes a space is observed between the Spitzenkörper and the apical wall in living hyphae which corresponds to the region between the specialized zone and the apical wall in electron micrographs, and which is occupied by apical vesicles adjacent to the plasma membrane. The specialized zone which we term Spitzenkörper is usually completely surrounded by apical vesicles. The suggestion that a vesicle-free zone within the accumulation of apical vesicles is a preparation artifact (8) does not seem valid because the specialized zones in some fungi are tightly packed with microvesicles, whereas others contain tubular profiles and ribosomes with no apparent dilution of the ground cytoplasm, and still others have no discernible fine structure. It seems improbable that dispersion of a cluster of vesicles would occur in one instance, whereas in another the vesicles remain closely packed. It is unlikely that microvesicles, tubules, ribosomes, or densely staining areas would be the result of a disruption of the Spitzenkörper, because in the septate fungi shown here the apical vesicles are quite closely packed together and there is still a specialized zone. However, the possibility remains that some, but not all, of the apical vesicles may be included in the specialized region in association with the microvesicles, tubules, or ribosomes and may thus contribute minimally to the light microscopic image that is termed Spitzenkörper.

For the *Phycomycetes* shown here, which have type I apical organization (8), we favor excluding the crescent-shaped band of apical vesicles from the definition of Spitzenkörper. Several observations support this suggestion. In *G. persicaria* the band is composed entirely of large apical vesicles, and ribosomes and microvesicles are apparently excluded. In *P. aphanidermatum* and in *P. ultimum* (10), we did not observe a distinct band or other type of phase-contrast-dark region in the apical zone. This may be a matter of variation within the type I organizational pattern, or it may represent a third type, since Girbardt's (8) characterization of *Phycomycetes* does not include *Oömycetes*, although several *Oömycetes* have been cited as examples of fungi with no Spitzenkörper (10, 12). However, in the fungi with type II apical organization, considerable variation also exists, especially in the character of the specialized region. Therefore, we suggest that pending further investigation of this variation, type I and II be retained to designate the different major kinds of apical organization and that the specialized region in type II be considered as the ultrastructural equivalent of the phase-dense, spherical Spitzenkörper. A diagram comparing the forms of apical organization observed in this study is shown in Fig. 45.

Thus, the regions which we describe as the ultrastructural equivalents of the Spitzenkörper are not uniform in their characteristics. Some are comprised of aggregates of microvesicles, whereas others appear as regions devoid of vesicles. In spite of the fact that these regions are distinct from the rest of the vesicle mass, we do not mean to imply that the Spitzenkörper is functionally dissociated from the apical vesicles or unrelated to the process of tip growth. On the contrary, the Spitzenkörper seems to be a regional differentia-

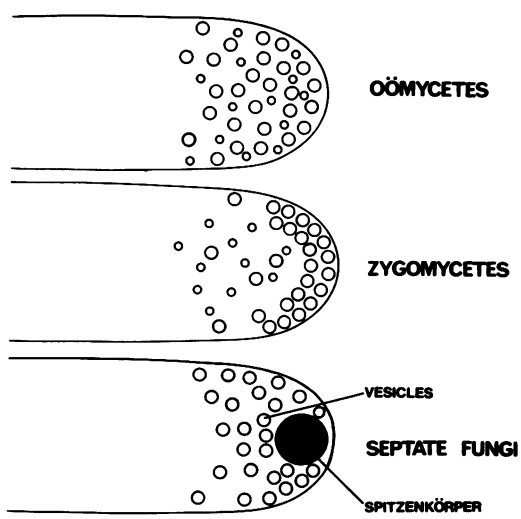

FIG. 45. *Diagrammatic comparisons of the principal forms of apical organization in hyphae, based on representatives from major taxonomic groups.*

tion of the apical zone, intimately associated with the apial vesicles and with hyphal tip growth in some fungi, but its occurrence in tip-growing systems is less general than that of the vesicles.

ACKNOWLEDGMENTS

This study was supported by grants GB-3044 and GB-6751 from the National Science Foundation and by a National Science Foundation traineeship granted to S. N. Grove; it was Journal Paper no. 4101 of the Purdue University Agricultural Experiment Station.

LITERATURE CITED

1. Bartnicki-Garcia, S., N. Nelson, and E. Cota-Robles. 1968. A novel apical corpuscle in hyphae of *Mucor rouxii*. J. Bacteriol. 95:2399–2402.
2. Bonnett, H. T., Jr., and E. H. Newcomb. 1966. Coated vesicles and other cytoplasmic components of growing root hairs of radish. Protoplasma 62:59–75.
3. Brenner, D. M., and G. C. Carroll. 1968. Fine-structural correlates of growth in hyphae of *Ascodesmis sphaerospora*. J. Bacteriol. 95:658–671.
4. Brown, R. M., Jr., W. W. Franke, H. Kleinig, H. Falk, and P. Sitte. 1970. Scale formation in chrysophycean algae I. Cellulosic and noncellulosic wall components made by the Golgi apparatus. J. Cell Biol. 45:246–271.
5. Brunswik, H. 1924. Untersuchungen über die Geschlecht und Kernverhaltnisse bei den Hymenomyceten Gattung *Coprinus*. Bot. Ann. K. Goebel. Heft. 5.
6. Girbardt, M. 1955. Lebendbeobachtungen an *Polystictus versicolor* (L). Flora 142:540–563.
7. Girbardt, M. 1957. Der Spitzenkörper von *Polystictus versi color* (L). Planta 50:47–59.
8. Girbardt, M. 1969. Die Ultrastruktur der Apikalregion von Pilzhyphen. Protoplasma 67:413–441.
9. Grove, S. N., C. E. Bracker, and D. J. Morré. 1968. Cytomembrane differentiation in the endoplasmic reticulum-Golgi apparatus-vesicle complex. Science 161:171–173.
10. Grove, S. N., C. E. Bracker, and D. J. Morré. 1970. An ultrastructural basis for hyphal tip growth in *Pythium ultimum*. Amer. J. Bot. 57:245–266.
11. Hemmes, D. E., and H. R. Hohl. 1969. Ultrastructural changes in directly germinating sporangia of *Phytophthora parasitica*. Amer. J. Bot. 56:300–313.
12. McClure, W. K., D. Park, and P. M. Robinson. 1968. Apical organization in the somatic hyphae of fungi. J. Gen. Microbiol. 50:177–182.
13. Mollenhauer, H. H., and D. J. Morré. 1966. Golgi apparatus and plant secretion. Annu. Rev. Plant Physiol. 17:27–46.
14. Moor, H. 1967. Endoplasmic reticulum as the initiator of bud formation in yeast (*S. cerevisiae*). Arch. Mikrobiol. 57:135–146.
15. Northcote, D. H. 1968. Structure and function of plant cell membranes. Brit. Med. Bull. 24:107–112.
16. Pickett-Heaps, J. D. 1968. Further ultrastructural observations on polysaccharide localization in plant cells. J. Cell Sci. 3:55–64.
17. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17:208–212.
18. Richardson, K. C., L. Jarett, and E. H. Finke. 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Technol. 35:313–323.
19. Rosen, W. G. 1968. Ultrastructure and physiology of pollen. Annu. Rev. Plant Physiol. 19:435–462.
20. Sievers, A. 1967. Elektronenmikroskopische Untersuchungen zur geotropischen Reaktion. II. Die polare Organisation des normal washcenden Rhizoids von *Chara foetida*. Protoplasma 64:225–253.
21. Zalokar, M. 1959. Growth and differentiation of *Neurospora* hyphae. Amer. J. Bot. 46:602–610.