INFECTION AND IMMUNITY, Nov. 1982, p. 706–715
0019-9567/82/110706-10$02.00/0

Vol. 38, No. 2

Ultrastructure of the Invasion of Human Hair In Vitro by the Keratinophilic Fungus *Microsporum gypseum*

TOSHIO KANBE AND KENJI TANAKA*

Institute of Medical Mycology, Nagoya University School of Medicine, Showa-ku, Nagoya 466, Japan

Received 24 May 1982/Accepted 14 July 1982

The pattern of invasion of human hair in vitro by the dermatophyte *Microsporum gypseum* was studied by transmission and scanning electron microscopy. Mycelia that invaded the hair cortex through the edge of cuticles showed a flattened "frond" growth in contrast to the filamentous form seen on ordinary laboratory media. The frond cells were characterized by the presence of vesicles formed by invaginations of plasmalemma, and lomasomes were prominent in the region adjacent to the hard keratinized tissue of the hair cortex being degraded as well. The initial perforating organ, which originated from the frond mycelium, appeared as an enlarged spherical cell which integrated with the laterally branched hyphae, as revealed by analysis of a three-dimensional model reconstructed from a series of sections. The fully developed perforating organ consisted of a column of wide and short cells which penetrated perpendicularly through the hair cortex. Through the medulla the filamentous hyphae had grown profusely in a longitudinal direction. Our studies confirm earlier light microscope observations and provide new ultrastructural details on the development of the eroding frond and the perforating organ.

The invasion of hair by dermatophytes has been investigated with light and electron microscopy under natural and experimental conditions. Light micrographs showed that naturally infected hair obtained from biopsy specimens was penetrated and channeled by hyphae or arthrospore chains or both (3). Studies by scanning electron microscopy of scalp hair from subjects infected with *Trichophyton violaceum* (12) and of guinea pig skin infected experimentally with *Trichophyton mentagrophytes* (8) showed in detail the hyphal morphology during in vivo infection. These observations indicated that hyphae of the keratinophilic dermatophytes grew in a filamentous form during in vivo infection of hairs following the same pattern as on ordinary laboratory media. Growth of filamentous fungi is highly polarized at their hyphal tips, where a large number of vesicles, thought to carry wall subunits as well as lytic and synthetic enzymes for the synthesis of cell wall at the tip, are seen by electron microscopy (6).

The keratinophilic dermatophytes, however, show a peculiar morphology when growing in vitro on keratinized tissue (2); they produce flattened fronds of hyphae or eroding mycelium on the surface layers of the material, and a perforating organ develops from these fronds to penetrate the keratinized tissue (4, 10). The development of this saprophytic morphology, although different from the parasitic one seen in natural disease, (2, 10), was extensively studied by light microscopy (4, 5, 10), but there have been few electron microscopic studies. Baxter and Mann (1) reported electron microscopic observations on both the eroding mycelium and the perforating organ of the invasion of human hair in vitro by keratinophilic dermatophytes, but their results were not satisfactory to elucidate the ultrastructure.

The purpose of this paper is to describe the ultrastructural details of the peculiar morphology of the frond formation and of the perforating organs in experimental infection of human hair by the dermatophyte *Microsporum gypseum*.

MATERIALS AND METHODS

Organism. *M. gypseum* strain NUMm-1, a clinical isolate from this University Hospital, was used.

Preparation of conidia and infection to human hair. *M. gypseum* was cultured on YPG (yeast extract, 0.2%; peptone, 1%; glucose, 2%) agar slants for 4 to 5 weeks. Distilled water (10 ml) was added to the cultures, and conidia were suspended by pipetting. The suspension was centrifuged at 3,000 rpm for 10 min and washed three times with distilled water. Spores were kept in the refrigerator. Human hair from a 1.5-year-old boy was washed and sterilized with chloroform-ethanol (1:1) at room temperature for 2 h and stored in a desiccator until used. The hair was infected with the fungus by dipping it into the suspension of conidia and placing it onto a slide glass, which was then cultured in a moist atmosphere in a petri dish at 25°C.

Scanning electron microscopy. The specimens were fixed in 2% OsO_4 vapor at room temperature for 12 h and dried in vacuo. They were mounted on cover slips with silver paste and coated with gold in a vacuum evaporator. Specimens were examined in a Hitachi S-450 scanning electron microscope operated at 20 kV.

Transmission electron microscopy. Samples were fixed in 3.5% glutaraldehyde–2% paraformaldehyde in 0.2 M cacodylate buffer (pH 7.0) (with or without 0.1% ruthenium red) for 4 h at room temperature. After being washed in 0.2 M cacodylate buffer, they were postfixed in 2% OsO_4 in the same buffer (with or without 0.1% ruthenium red) for 4 h at room temperature and soaked in a 0.5% uranyl acetate aqueous solution for 12 h at 4°C. Specimens were subsequently dehydrated through a graded series of acetone before being embedded in Spurr resin. Sections were obtained on a Reichert-Jung OmU4 Ultracut with a diamond knife and picked up on Formvar-coated single-slot grids. They were examined in a JEOL 100 CX electron microscope with an accelerating voltage at 80 or 100 kV.

Reconstruction of the three-dimensional model was carried out by the method of Pellegrini (9). Models were made by tracing the outlines of plasma membrane profiles from micrographs of ultrathin serial sections onto paperboards which were the thickness of the ultrathin sections (0.1 μm) multiplied by the magnification of the negatives.

RESULTS AND DISCUSSION

English (4) described the stages by which detached hairs are attacked by keratinophilic fungi as follows: (i) cuticle lifting, (ii) cortical erosion, (iii) production of penetrating organs, and (iv) colonization of the medulla. We could also recognize these stages, but they overlapped one another during the process of hair infection with *M. gypseum*. Germinated hyphae were found on the hair infected with conidia after 2 days in a moist atmosphere. Most of the hyphae were growing in a filamentous form along the edge of the cuticular scales (Fig. 1A) and resembled the hyphae seen on ordinary laboratory media. In addition, some of the hyphae seemed to wedge beneath the free edge of cuticular cells, which were observed to be slightly lifted (Fig. 1B). This observation is in accord with those described previously for light (5, 10) and scanning electron (8) microscopy of the infections of hair of the keratinophilic fungi. The fronded growth of the invaded hyphae extended in a palm-like form beneath the cuticular scales. Where parts of the cuticles were peeled off, fronded mycelium was exposed and visible by scanning electron microscopy (Fig. 1C). When fronded or eroding mycelium was sectioned tangentially to the hair shaft (Fig. 1D), the hypha consisted of cells of a wide variety of shapes and heterogenous cytoplasmic contents. In contrast to the apical growth of a filamentous hypha cultured on ordinary laboratory media, characterized by the vesicles accumulated at apices, it could not be determined where the most active region of the fronded growth was. We could not, therefore, define a region as the growing tip of the filamentous hypha, even though an extensive examination of a whole series of sections was done. However, a gradient of vacuolar development was observed (Fig. 1D from upper left to lower right), which might suggest a directed growth of fronded mycelium; cells filled with cytoplasmic organelles in lower half were actively growing. In the process of the invasion of hair the hyphae might grow in a flattened fronded form adhering to the surface of the hair cortex, and a portion of frond mycelium would then become the initial perforating organ, which grows perpendicularly to penetrate into the cortex (5). This very early phase of the invasion into the hair cortex is shown in Fig. 2A. The flattened mycelial cell was lifting the cuticular scales and bulged a part of the bottom portion into the hair cortex where the cell wall was irregular and not distinct. The cell contained a nucleus, mitochondria, and endoplasmic reticula and was full of ribosomes. At this stage of development membrane vesicles, demonstrated by the analysis of serial sections, were formed by invaginations of the plasmalemma (Fig. 2A). Figure 2A also shows both the cuticle lifting and the erosion of the cortex, which seemed to occur simultaneously. The early stage of the perforating organ formation is shown in Fig. 2B. Most of the enlarged, rather spherical cell with a large vacuole was embedded in the hair cortex, and both sides of the cell were flanked by many branched hyphae which had pointed tips exfoliating the layers of hair cortex in parallel with the longitudinal axis of the hair shaft. A large spherical cell in which cytoplasmic structures can be seen and the branched parts with pointed tips whose cytoplasm was too dense to discern the internal structures are shown separately in Fig. 2B, but they were eventually determined to be a single cell when the three-dimensional model was reconstructed from the serial sections. No complete septum was demonstrated in the serial section analysis.

An intracellular view of the initial perforating organ at a more advanced stage is shown in Fig. 3A. The cell contained a large number of nuclei, mitochondria, and rough endoplasmic reticulum. Incomplete septa formed by the inward growth of the plasmalemma and the cell wall gave an irregular profile of the cell. Dense melanin granules remained between the cell walls of branched hyphae. Most of the hyphal cells in Fig. 3A were actually integrated into a single large cell, as demonstrated by the three-dimensional reconstruction of 108 consecutive sections (Fig. 3B). The branched portion of the

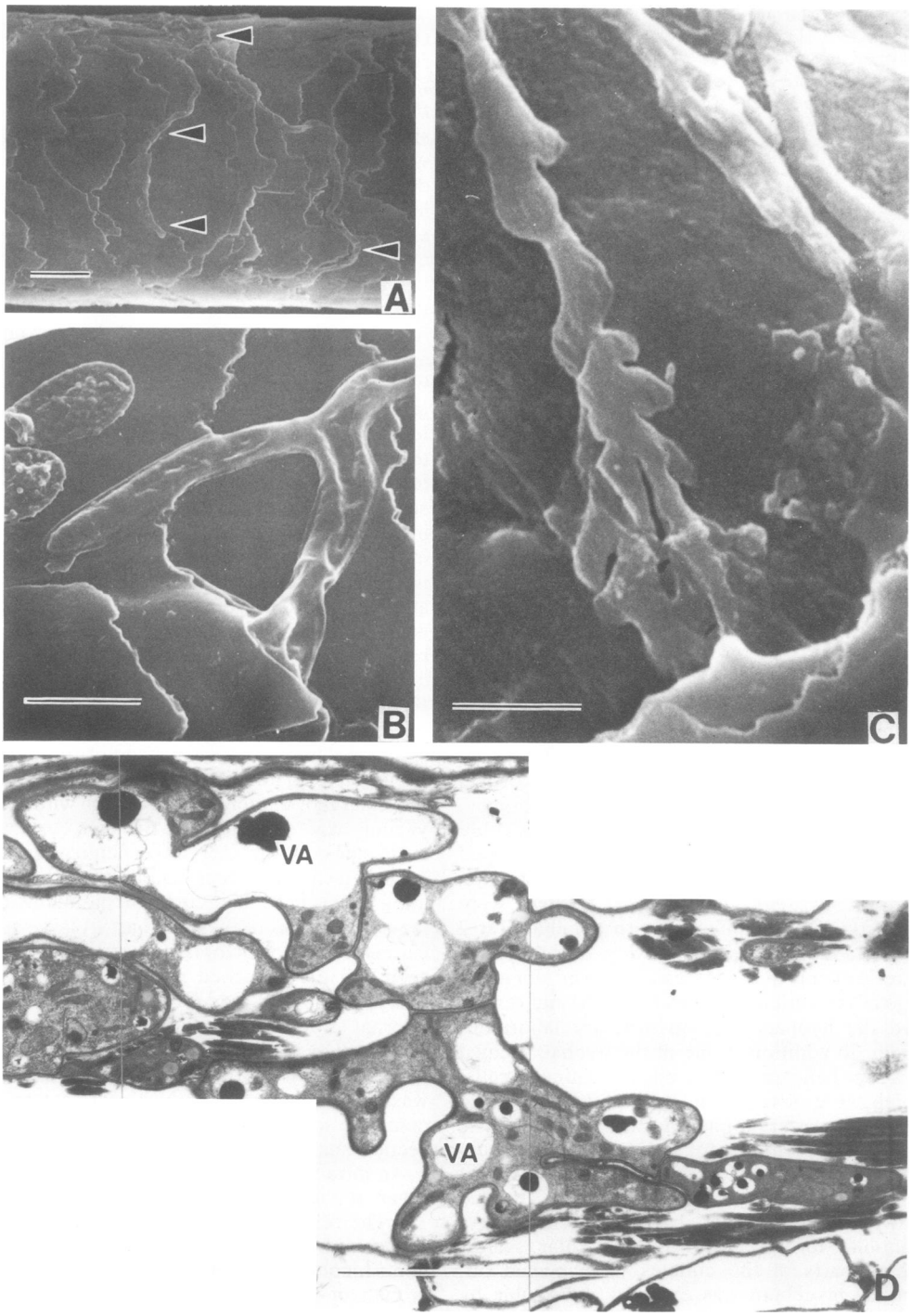

FIG. 1. (A) Scanning electron micrograph of germinated hyphae (indicated by arrows) on the hair surface after 48 h of cultivation. Bar, 10 μm. (B) Scanning electron micrograph of germinated hypha invading beneath the edge of the cuticle cell of the hair which is somewhat raised. Bar, 10 μm. (C) Scanning electron micrograph showing the unusual morphology of fronded hyphae on the hair cortex, where the cuticular cells were peeled off. Bar, 10 μm. (D) Ultrathin section of fronded hyphae, consisting of cells of various forms. Vacuoles in the cytoplasm are more developed in the upper left than in the lower right. Specimen is stained with ruthenium red. Bar, 10 μm. Abbreviation: VA, vacuole.

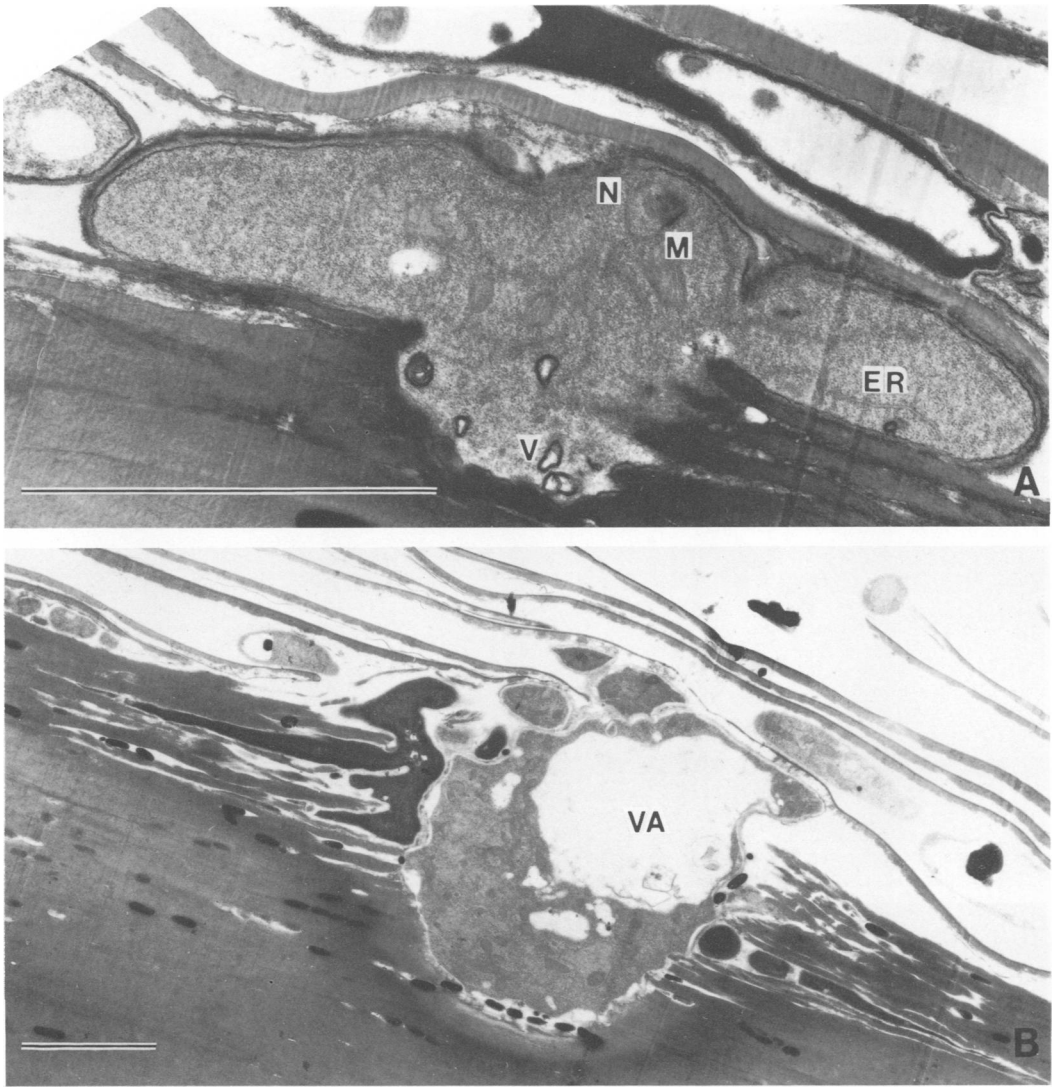

FIG. 2. (A) Ultrathin section of the very early stage of invasion into the hair cortex. The specimen is stained wth ruthenium red. A part of the fronded hypha is pushing the cuticle scales apart and is bulging to invade into the hair cortex. Note the presence of vesicles that are formed by invagination of plasmalemma. Bar, 5 μm. Abbreviations: N, nucleus; M, mitochondria; ER, endoplasmic reticulum; V, vesicle. (B) Ultrathin section of the initial of the perforating organ. The massive cell adhered on the hair grow in spherical form. Dark-stained lateral branches with pointed tips that advance in parallel to the longitudinal axis of the hair cortex. Bar, 5 μm. Abbreviation: VA, vacuole.

upper part of the perforating organ which extended laterally may correspond to the "handles" of English (5). No particular region of the cell appeared to be the most active biosynthetically or with respect to growth. The synthetic activities of the cell were indicated by the presence of numerous cellular organelles (Fig. 2B, 3A, and 4C), and growth seemed to be taking place by deterioration of surrounding hard keratinized tissues, rather than by growth in a single direction. The structure of the cell surface in contact with the degrading hair cortex was irregular and variable (Fig. 4A and B). Cell wall structure was not distinctive, and aggregates of membrane structures or lomasomes were seen in the space between the irregular profiles of the plasmalemma and the keratin fibrils of the degrading hair cortex. Lomasomes are considered to be formed as a result of unbalanced synthesis of the membranes and of the cell wall, and no

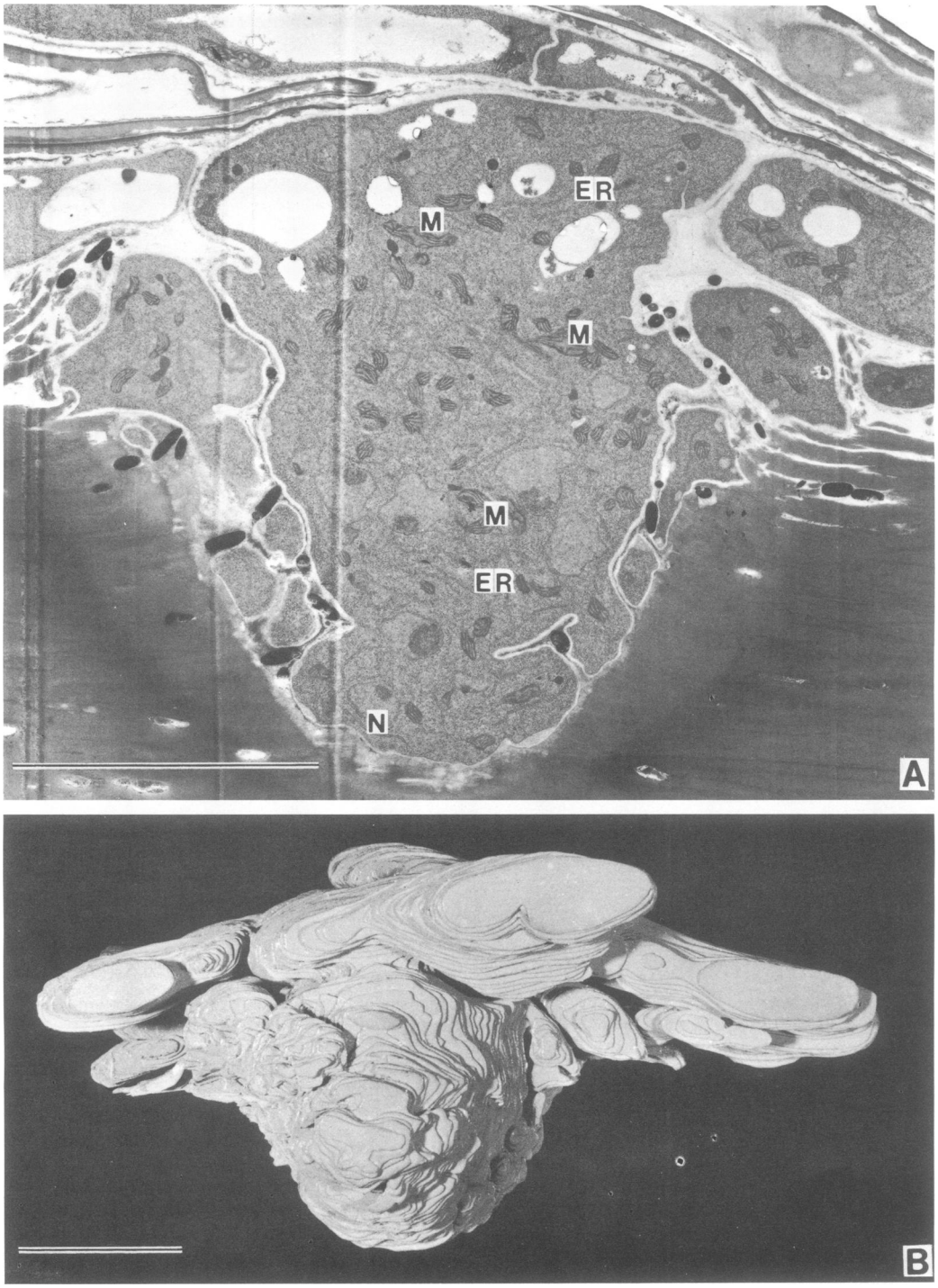

FIG. 3. (A) Ultrathin section of a perforating cell after 4 days of cultivation. Septum formation is started at several points. A central perforating organ initial is associated with laterally extending hyphal parts that correspond to handles. Bar, 5 μm. Abbreviations: M, mitochondria; ER, endoplasmic reticulum; N, nucleus. (B) Three-dimensional model of the perforating cell, reconstructed from 108 ultrathin serial sections of the same cell shown in A. The association of handle parts with the main part of the cell is distinct. Bar, 5 μm.

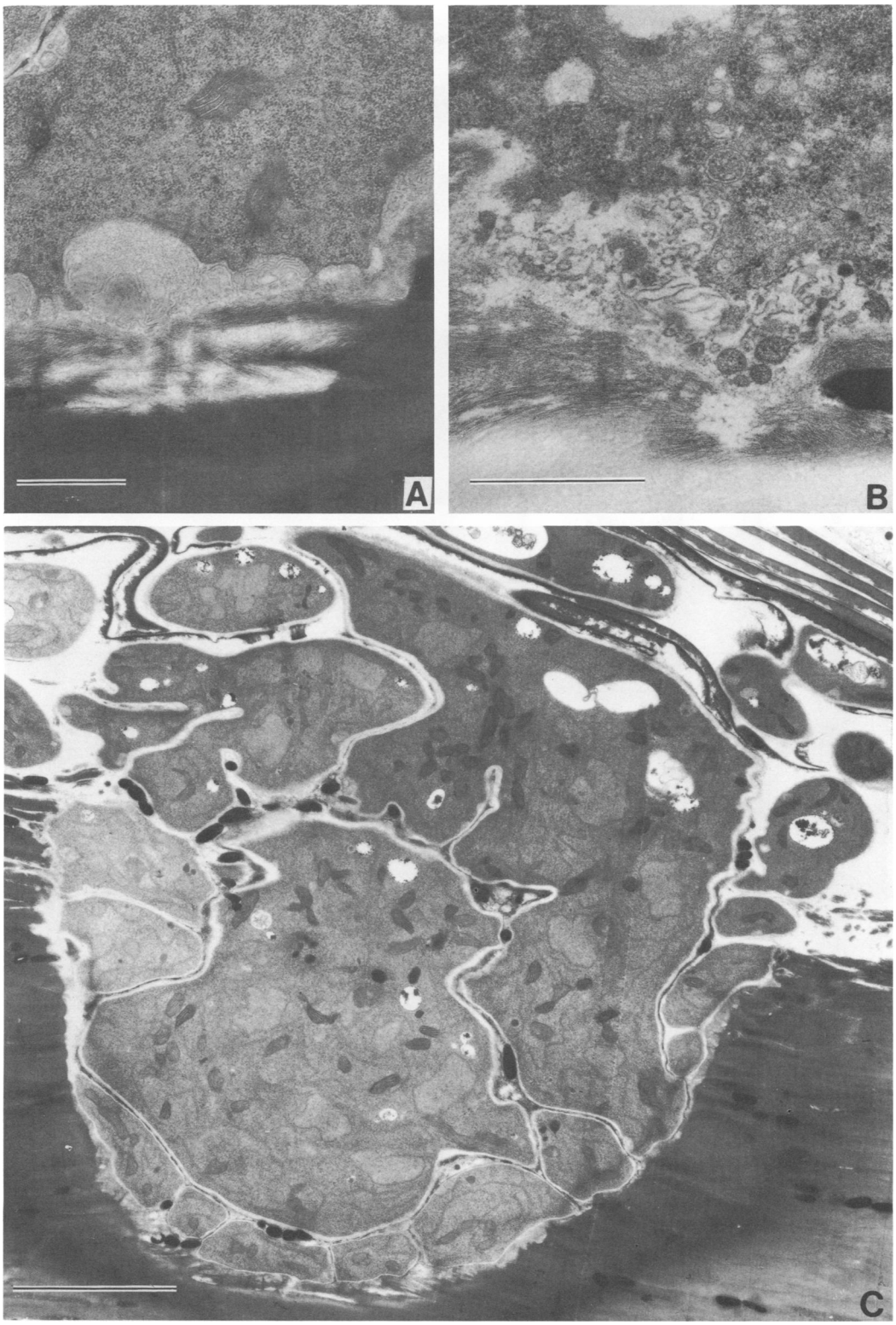

FIG. 4. (A) Thin section showing lomasome-like structure frequently observed in the space between the irregular plasmalemma and the keratin fibrils revealed by the deterioration of the hair cortex. Bar, 1 μm. (B) Thin section showing another type of interspace structure between the cell and the hair cortex. The cell wall is not apparent. Membrane fragments and vesicles are seen in the interspace, and vesicles and multivesicles are in the cytoplasm. Bar, 1 μm. (C) Oblique thin section of a perforating organ after 4 days of cultivation. Septum formation is almost complete, and the perforating organ is composed of cells of irregular form. Melanin granules are seen in the space between the cells. Bar, 5 μm.

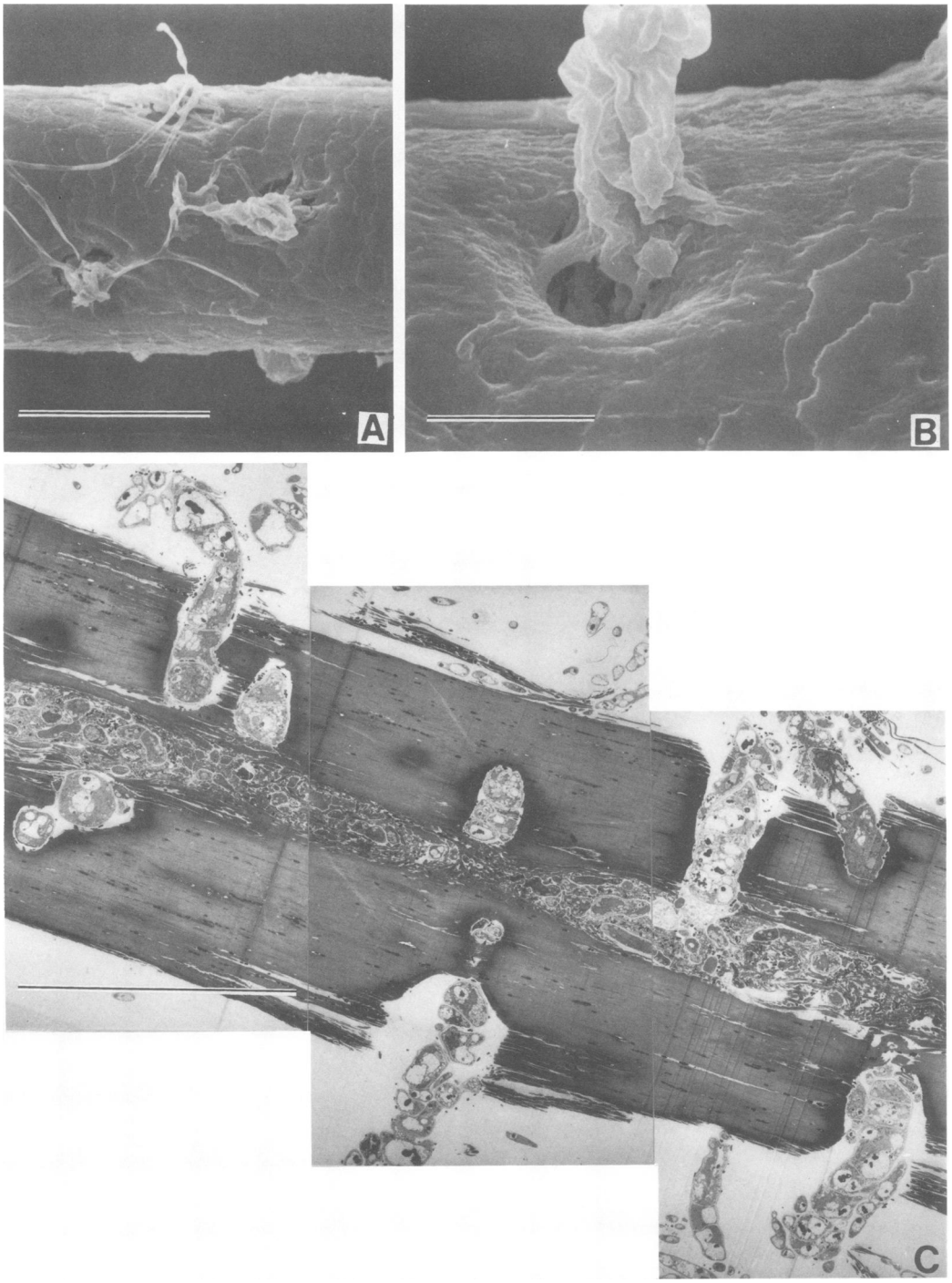

FIG. 5. (A) Scanning electron micrograph of an infected hair after 6 days of cultivation. Three perforating organs connected with filamentous hyphae are shown. Bar, 50 μm. (B) Scanning electron micrograph of a perforating organ of aggregated hyphae, which stands in the perforated pit where part of the hair cuticles and the cortex are dissolved. Bar, 10 μm. (C) Longitudinal section of a hair perforated by fungi after 7 days of cultivation. Many perforating organs penetrate deep into the hair shaft, and the filamentous hyphae fill the medulla. Bar, 50 μm.

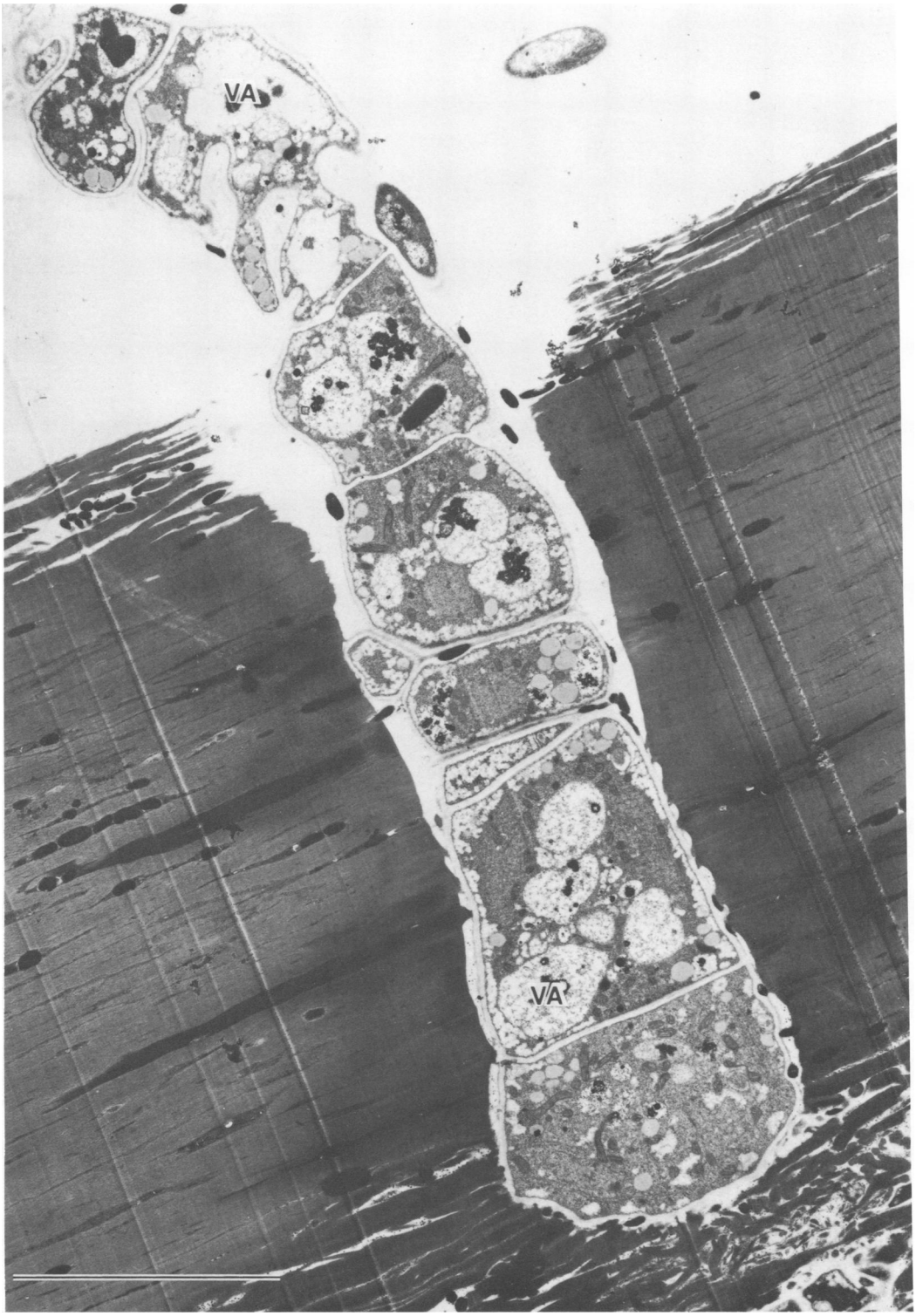

FIG. 6. Near-median section of a perforating organ consisting of a stack of short and wide cells. Vacuoles are more developed in cells close to the outer surface than those close to the medulla. The clear space between the cells and the hair cortex may be formed by keratinase digestion. In addition to the ordinary organelles, lipid and glycogen granules and various vacuoles are seen. Bar, 10 μm.

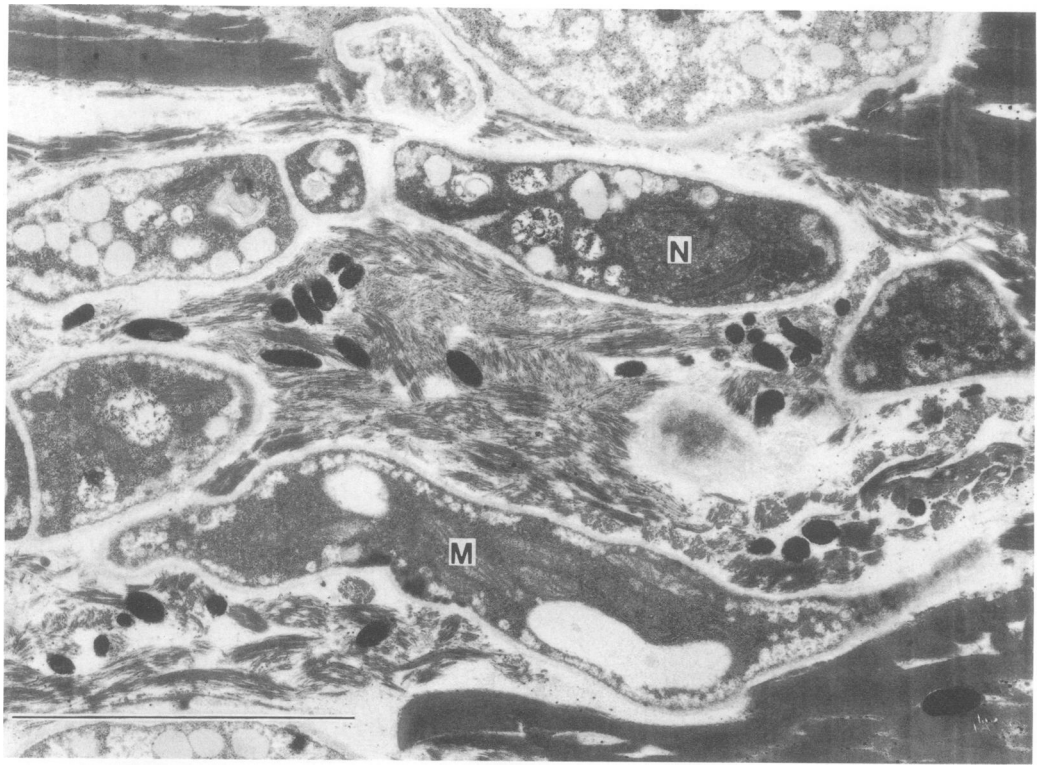

FIG. 7. Higher magnification of a part of the hair medulla with profuse growth of filamentous hyphae. Dark melanin granules are shown in the keratin fibrils which are oriented in different directions. Bar, 5 μm. Abbreviations: N, nucleus; M, mitochondria.

function was attributed to them (7). As the hair cortex was dissociated into keratin fibrils by keratinase (11, 14), however, the secretion of the enzyme appeared to occur at the site where lomasomes were predominate. Various kinds of vesicles were also observed in the peripheral cytoplasm (Fig. 4B). A more advanced stage of perforating organ development is shown in Fig. 4C. A large central cell was surrounded by smaller cells, and the profiles of all cells were irregular.

Part of an infected hair cultured for 6 days was observed by scanning electron microscopy. The hyphal conglomerations of the perforating organs were seen on the hair surface being connected by strands of hyphae (Fig. 5A). Hair cuticles seemed to be digested away around the deep holes of the hair cortex into which hyphae of the perforating organ had penetrated (Fig. 5B). The perforating organs or boring hyphae seemed to penetrate deeply into the hair medulla, a situation which is shown clearly in a longitudinal median section of a hair shaft which had been infected for 7 days (Fig. 5C). The perforating organ, once completed, consisted of a column of short and wide cells arranged perpendicularly from the hair surface to the medulla (Fig. 6) as had been suggested by light microscopy (5). Cells near the surface were more vacuolated than those close to the medulla, and the hair tissues around the perforating organ were digested, leaving a clear space where the dense melanin granules that were not attacked by keratinase remained. In contrast to the boring hypha which consisted of wide and short cells, the hyphal cells in the medulla seemed to be long and filamentous and grew in the direction parallel to the hair axis. A thin section of part of the medulla invaded by hyphae is shown in Fig. 7. Hyphal ultrastructure is not different from that of the perforating organs, and melanin granules can be seen between the hyphal cells in the digested remains of the keratin fibrils.

Werner et al. (13) studied the ultrastructure of hyphal cells in a few species of dermatophyte fungi grown on Sabouraud dextrose agar, but ultrastructural differentiation of the growing filamentous hypha in a longitudinal direction (6) was not described. Fine structure of the mycelium growing in the hair was studied only by Baxter and Mann (1). However, probably because of their use of a 1-week-old preparation in which the infection was too advanced to follow the developmental stages and of their poor fixa-

tion of the material, their results were not satisfactory. In the present studies, we used ruthenium red staining which enhanced cell wall structure and attempted to understand the structure three dimensionally by a model reconstructed from the serial sections. Light microscopy of the developmental morphology of the perforating organ and eroding mycelium carried out by English (4, 5) and others (10) seemed to be quite accurate, as their observations and ours are in complete agreement, although our studies have included the ultrastructural details.

Finally, it must be emphasized that the structures we described here were seen only in the detached hair infected in vitro and were different from that seen in natural diseases. This point still presents us with a major problem for further investigation.

ACKNOWLEDGMENT

We thank T. Hana-ichi for excellent technical assistance in taking scanning electron micrographs.

LITERATURE CITED

1. **Baxter, M., and P. R. Mann.** 1969. Electron microscopic studies of the invasion of human hair *in vitro* by three keratinophilic fungi. Sabouraudia **7**:33–37.
2. **Deacon, J. W.** 1980. Fungi as animal parasites, p. 168–175. *In* Introduction to modern mycology. Blackwell Scientific Publications, London.
3. **Emmons, C. W., C. H. Binford, J. P. Utz, and K. J. Kwon-Chung.** 1977. Medical mycology, 3rd ed. Lea & Febiger, Philadelphia.
4. **English, M. P.** 1963. The saprophytic growth of keratinophilic fungi on keratin. Sabouraudia **2**:115–130.
5. **English, M. P.** 1968. The developmental morphology of the perforating organs and eroding mycelium of dermatophytes. Sabouraudia **6**:218–229.
6. **Grove, S. N.** 1978. The cytology of hyphal tip growth, p. 28–50. *In* J. E. Smith and D. R. Berry (ed.), The filamentous fungi. Edward Arnold Ltd., London.
7. **Heath, I. B., and A. D. Greenwood.** 1970. The structure and formation of lomasomes. J. Gen. Microbiol. **62**:129–137.
8. **Hutton, R. D., S. Kerbs, and K. Yee.** 1978. Scanning electron microscopy of experimental *Trichophyton mentagrophytes* infection in the guinea pig skin. Infect. Immun. **21**:247–253.
9. **Pellegrini, M.** 1980. Three-dimensional reconstruction of organelles in *Euglena gracilis* Z. I. Qualitative and quantitative changes of chloroplasts and mitochondrial reticulum in synchronous photoautotrophic culture. J. Cell Sci. **43**:137–166.
10. **Raubitschek, F., and R. Evron.** 1963. Experimental invasion of hair by dermatophytes. Arch. Dermatol. **88**:837–845.
11. **Takiuchi, I., and D. Higuchi.** 1977. Isolation, purification and biochemical properties of keratinase elaborated from *Microsporum gypseum* Jpn. J. Dermatol. Soc. **87**:305–309 (In Japanese.).
12. **Tosti, A., S. Villardita, M. L. Fazzini, and R. Scalici.** 1970. Contribution to the knowledge of dermatophytic invasion of hair. An investigation with the scanning electron microscope. J. Invest. Dermatol. **55**:123–134.
13. **Werner, H. J., C. Catsulis, H. W. Jolly, and C. L. Carpenter.** 1967. Electron microscope observations of the fine structure of *Microsporum gypseum*. J. Invest. Dermatol. **48**:481–484.
14. **Yu, R. J., S. R. Harmon, and F. Blank.** 1969. Hair digestion by keratinase of *Trichophyton mentagrophytes*. J. Invest. Dermatol. **53**:166–171.