

## SHORT REPORTS

### Ultrastructural Studies on Experimental Hair Infections In Vitro Caused by *Trichophyton mentagrophytes* and *Trichophyton rubrum*

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Experimental infections with *Trichophyton mentagrophytes* and *Trichophyton rubrum* were performed on human hair in vitro and studied by conventional light microscopy, scanning (SEM) and transmission (TEM) electron microscopy. The penetrations visible by light microscopy in hair infected with *T. mentagrophytes* appeared at SEM as disrupted surface areas with perforating holes. *T. rubrum*-infected hair displayed minute perforations and less conspicuous cuticular damages seen at SEM and TEM. In both organisms multiple tiny perforating holes were observed at SEM previously unnoticed in experimental hair infections. From a morphological point of view the differences between the fungi investigated in this experimental system seem to be more of a quantitative nature than involving different mechanisms of action. *Key words:* Hair penetration; Dermatophyte infection of hair; Transmission and scanning electron microscopy. (Received March 11, 1985.)

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The ability to penetrate human hair in vitro has been used as an aid differentiating atypical strains of *T. mentagrophytes* and *T. rubrum* (1). However, the reproducibility of the test, despite constant experimental conditions has varied from time to time due to unknown factors. It was therefore of interest to collect morphological information on the penetration of these organisms in vitro using transmission electron microscopy (TEM) as well as scanning electron microscopy (SEM).

*Fig. 1.* Light microphotograph of the characteristic perforation at *T. mentagrophytes* infection (center). Bar 10  $\mu$ m.

*Fig. 2.* Cuticular ridges caused by *T. mentagrophytes* infection. Note openings along ridges. SEM microphotograph. Bar 100  $\mu$ m.

*Fig. 3.* Detail of cuticular ridges and perforations caused by *T. mentagrophytes*. Note fine perforating holes and opening at a ridge.

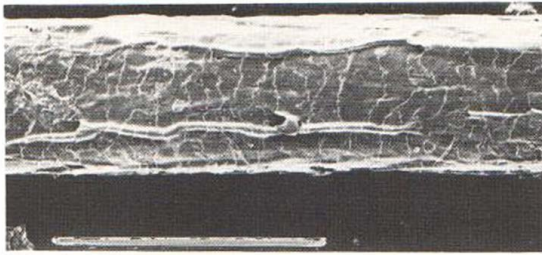
*Fig. 4.* Hair strand infected with *T. rubrum*. To the left the cuticle is lifted by a penetrating hyphae. Conidia adhere to the cuticle. SEM microphotograph. Bar 10  $\mu$ m.

*Fig. 5.* Detail of lifted cuticle at *T. mentagrophytes* infection. The endocuticle (endo) is the focus of attack with little visible morphological change in the exocuticle (exo). Fungus hyphae F Bar 0.5  $\mu$ m. TEM microphotograph.

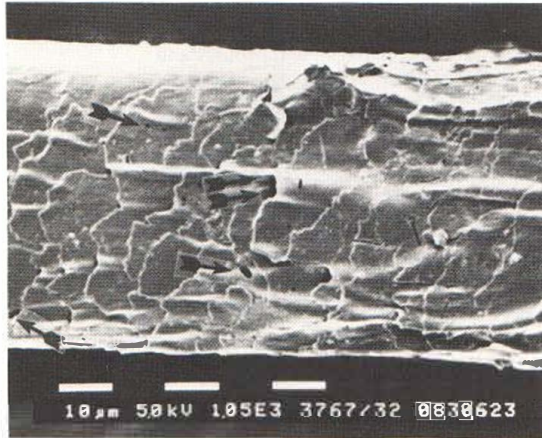
*Fig. 6.* Lifted cuticle in *T. rubrum* infection. The details of the cuticle damage is corresponding to that of *T. mentagrophytes* (Fig. 4). Bar 5  $\mu$ m. TEM microphotograph.

*Fig. 7.* Detail of the morphology at *T. rubrum* attack. Fungus hyphae F TEM microphotograph. Bar 0.5  $\mu$ m.

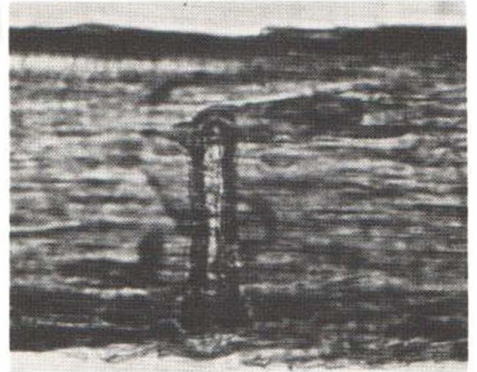
*Fig. 8.* *T. mentagrophytes* perforation of hair. SEM micrograph of specimen in Fig. 1. Bar 100  $\mu$ m. (b) Higher magnification of (a). Bar 10  $\mu$ m.



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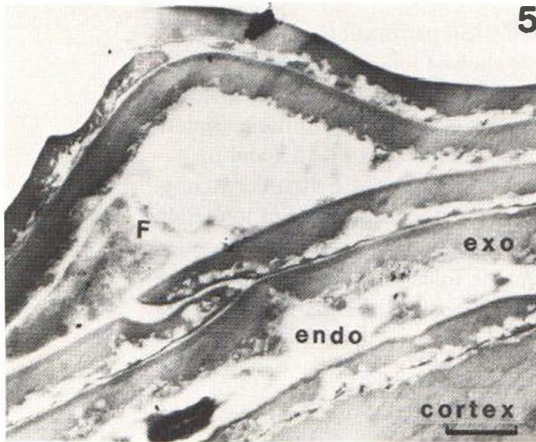
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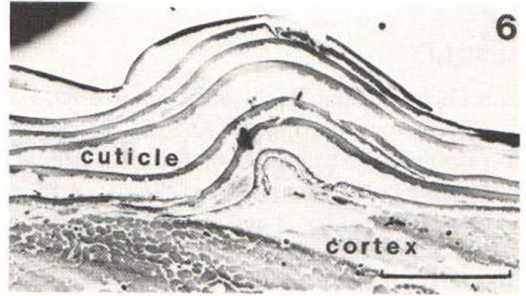
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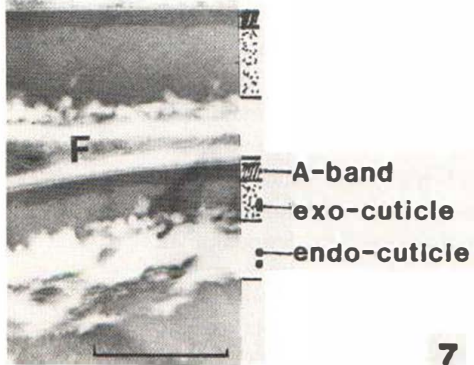
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8a



8b



7

10 μm 50kV 125E3 5503/00 840229

## MATERIAL AND METHODS

### Preparations of hair

Clipped strands of scalp hair from a 2-year-old boy was used. The hair was sterilized at 120°C and placed in a sterile water solution with yeast extract added. The test strains taken from fresh skin lesions and cultured on Sabouraud's agar were incubated at 25°C in a constant temperature box (1). Preliminary experiments were carried out to ensure that characteristic penetrations appeared at light microscopy on several test occasions, when the hair was inoculated with *T. mentagrophytes* of the zoophilic type. No evidence of perforation was found, when these hairs were inoculated with *T. rubrum* in the preliminary experiments.

### Experimental study

Strains from *T. mentagrophytes* (zoophilic strain) and *T. rubrum* were inoculated simultaneously and microscopically examined at regular time intervals. After one week specimen were removed from the medium and inspected in a conventional light microscope. Suitable small strands of infected hair were cut into 5 mm pieces and transferred to 2.5% glutaraldehyde in a phosphate buffer. Dehydration was performed in rising concentrations of alcohol, transfer to propylene oxide and embedding in Epon according to standard procedures. Thin sections (40 nm) cut on an LKB Ultratome were stained with uranyl acetate and lead citrate. For SEM short pieces of hair with fungal colonies were transferred from the absolute alcohol onto double sided Scotch tape on top of SEM specimen stubs. After air drying for 24 hours, specimens were sputtered with gold. In some experiments infected hair were stained with eosin for light microscopic selection of SEM and TEM specimens. These were directly processed according to the previous description. TEM was performed on a Philips EM 3016 at 80 kV, the SEM study on a Philips SEM 505 at 5 kV.

## RESULTS

Hair fibres inoculated with *T. mentagrophytes* showed typical perforations after one week at light microscopy (Fig. 1). At SEM the cuticular cells showed longitudinal folds (Fig. 2) corresponding to triangular shaped structures at TEM (Fig. 5). In some cases conspicuous perforating holes were observed (Fig. 3). A frequent finding is an opening at the margin of a cuticular cell (Figs. 2 and 3). The perforated areas are visualized in Fig. 8a and b.

Hair fibres inoculated with *T. rubrum* showed no perforations at light microscopy. However, a dense mat of hyphae appeared surrounding the hair strand. At SEM longitudinal ridges in the cuticle was observed and in addition fine perforating holes were observed (Fig. 4). At TEM the corresponding structures were cuticle folds of triangular shape with damage of the endocuticle (Figs. 6 and 7). The presence of eosin apparently did not influence the specimens as seen in TEM and SEM.

## DISCUSSION

This study indicates that the structures called perforations, when seen at light microscopy represent superficial disrupted areas with perforating holes. Presumably these holes are openings to more or less developed cavities in the hair shaft, which is in agreement with other studies (3, 5). The areas with lots of fine perforating holes in the cuticle might represent early stages in the keratolytic process. To our knowledge these minor perforating holes have not been observed previously in experimental hair infections with *T. rubrum* and *T. mentagrophytes* but similar holes were documented in dermatophyte infections of the nail (4). Another feature seen in both *T. rubrum* and *T. mentagrophytes*-infected hair is a swelling type of cuticular involvement (2, 3). Generally the cuticular cells swell to produce longitudinal ridges. At TEM these changes are clearly visible as lytic destruction of mainly the inner part of the cuticular cells, i.e. the endocuticle. This is

notably the part of the cuticular cell which contains low amounts of sulphur. The fact that cuticular swelling results in longitudinal ridging is consistent with the fact that the 8 mm filaments of cuticular cells are in a parallel arrangement and aligned along the axis of the fibre. Since the cuticular filaments are straight, not helically arranged, they have less capacity to take elongation stress than has the helically arranged cortical filaments. At swelling the expansion will therefore take place in a direction at right angle to the fibre axes. The fact that eosin staining does not influence the results of SEM and TEM preparation provides for better selection of specimens.

Our results indicate that human hairs may be attacked in vitro not only by *T. mentagrophytes* but also by *T. rubrum*. These organisms are capable of creating apparently lytic changes in the hair-fibre especially involving the cuticula. From the morphological data our interpretation of these findings is that the lesions caused by these two dermatophytes on experimentally infected hair in vitro are likely to represent different degrees of keratolytic break down rather than different mechanisms of action.

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