IN VITRO HAIR CULTURES FOR DIFFERENTIATING BETWEEN ATYPICAL ISOLATES OF *TRICHOPHYTON MENTAGROPHYTES* AND *TRICHOPHYTON RUBRUM*

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The morphological inconstancy of the dermatophytes has been the source of much taxonomic confusion. Narrow concepts of species limits have led to the creation of over 200 "species", the great majority of which have been defined on tenuous criteria.

However, growing realization that a given dermatophyte may vary considerably in its morphological and biological properties has stimulated taxonomic revision of this group of keratinophilic fungi. Today most medical mycologists recognize only 14 species as valid: *Trichophyton concentricum, T. ferrugineum, T. gallinae, T. megnini, T. mentagrophytes, T. rubrum, A. schoenleini, T. tonsurans, T. verrucosum, T. violaceum, Microsporum audouini, M. canis, M. gypseum, Epidermophyton floccosum.*

Of these, *T. mentagrophytes* and *T. rubrum* are two common dermatophytes with basically distinct cultural characteristics. The first generally produces clavate macroconidia, globose or subglobose microconidia and spirals of tightly wound mycelium. The second gives rise to long macroconidia with parallel side walls and microconidia that are elongate in form. Most isolates of this latter species, in addition, produce a deep red pigment when grown on Sabouraud dextrose agar, while a great many isolates of *T. mentagrophytes* elaborate brownish pigments on the reverse of the colony.

However, the range of cultural variations within these two species is so great that considerable overlapping occurs in their gross and microscopic morphology as well as in pigmentation. It is not unusual to encounter isolates of *T. rubrum* that fail to produce red pigments or conversely some cultures of *T. mentagrophytes* that do elaborate a deep red pigment. Clavate macroconidia are borne by some isolates of *T. rubrum* (Fig. 1A, B), and certain isolates of *T. mentagrophytes* have been found to form pencil-shaped spores (Fig. 2–A, B).

The difficulties inherent in identifying atypical forms of *T. mentagrophytes* and *T. rubrum* have been recognized by several investigators. In a detailed study of the cultural expression of

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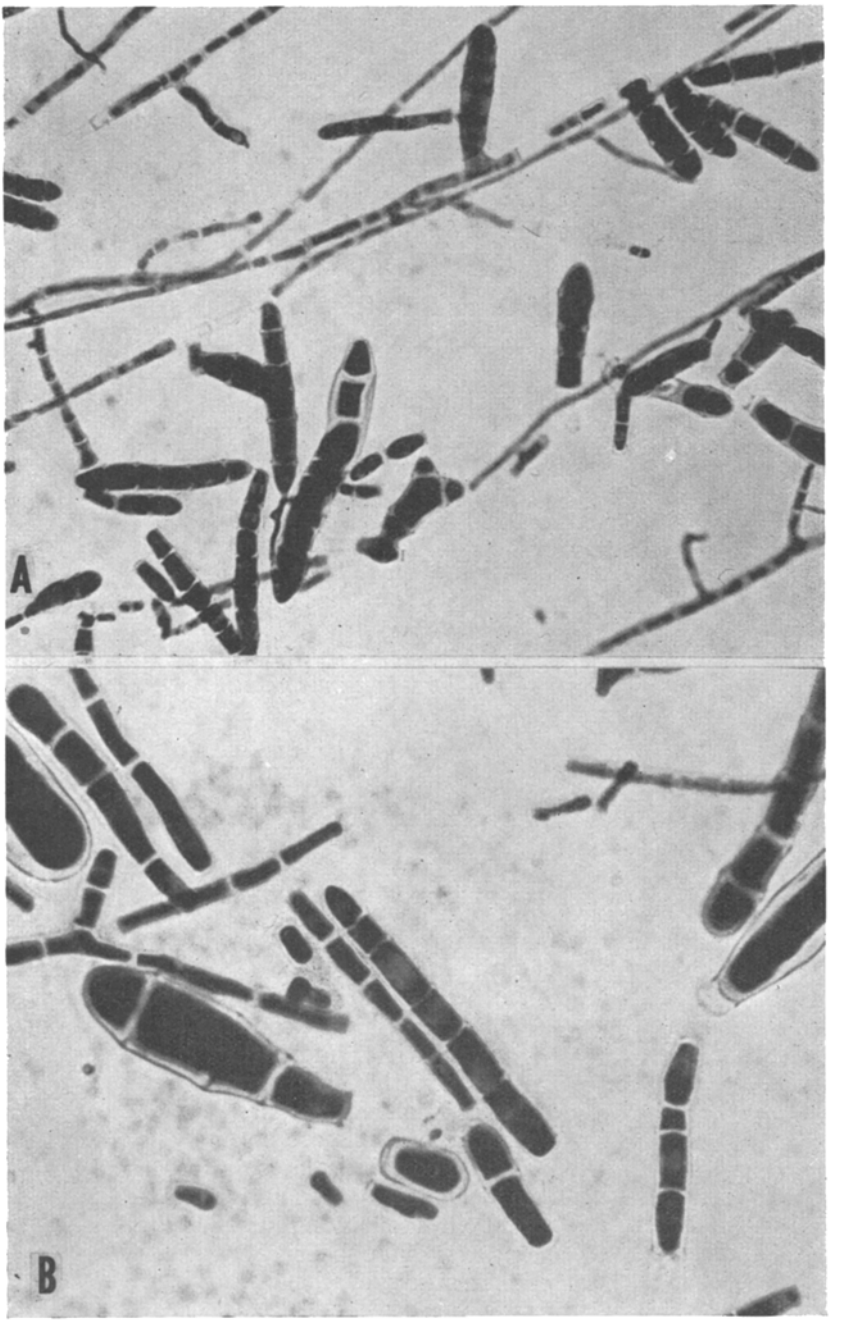

Figure 1. A, B. Group of predominately clavate macroconidia produced by *Trichophyton rubrum* A8. Original magnification (A) × 475; (B) × 970.

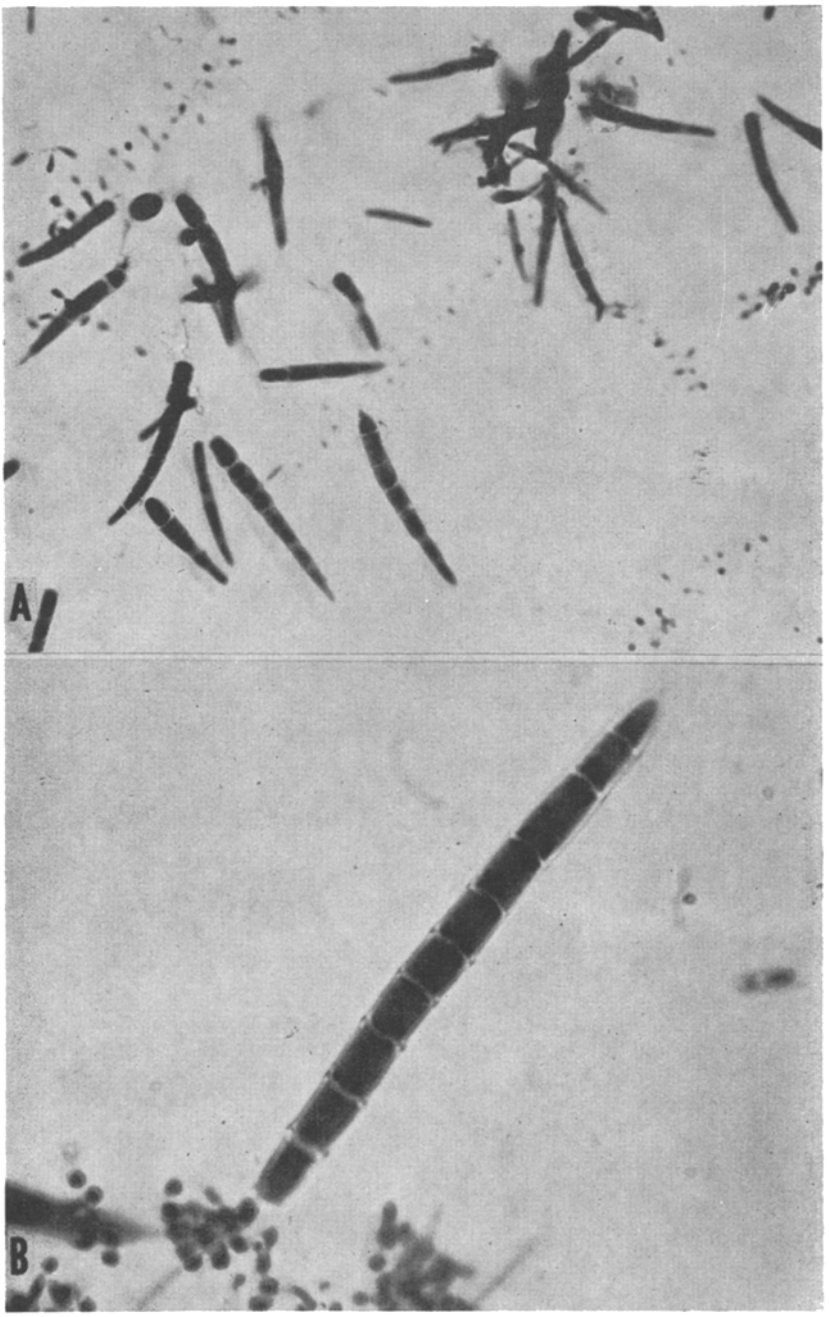

Figure 2. A. Pencil-shaped marcroconidia formed by *T. mentagrophytes* A4. Original magnification × 475.
B. A single pencil-shaped macroconidium. Original magnification × 970.

several isolates of *T. mentagrophytes* and *T. rubrum* on 7 different culture media, EDGECOMBE (1942) concluded that the growth characteristics of these two species on potato dextrose agar were definitive. However, observations made on a large series of isolates of these two dermatophytes grown on this medium have failed to reveal any consistency in pigment formation or spore development.

Heart infusion agar enriched by tryptose was considered by BENHAM (1948) to be of great value in the identification of *T. rubrum*, for on this medium 49 of 50 isolates of *T. rubrum* produced "characteristic macroconidia". But again difficulties have arisen since isolates of *T. rubrum* are encountered from time to time that fail to form macroconidia on that medium.

BOCOBO and BENHAM (1949) recognized the "inconsistent behaviour" of *T. mentagrophytes* and *T. rubrum* and recommended cornmeal agar enriched with 1 % dextrose as an aid in separating the two species. On this medium, *T. rubrum* would develop a pink or red pigment while *T. mentagrophytes* would not develop pigment. Unfortunately, many isolates have failed to conform to this pattern and isolates of *T. mentagrophytes* have been obtained that form pink, red, or yellow pigments on cornmeal dextrose agar; while several isolates of *T. rubrum* have produced yellowish rather than red pigments on that medium. These aberrant forms could be identified as to species only by the combination of structures and/or pigments produced on a wide variety of media.

It occurred to one of us (L.A.) that the manner by which detached filaments of hair were attacked by keratinophilic fungi would be of value in identifying dermatophytes. In 1934 DAVIDSON and GREGORY (1934) noted that hair filaments exposed to *T. mentagrophytes* were radially penetrated by organized groups of hyphae forming wedge-shaped perforations. More recently, VANBREUSEGHEM (1949) demonstrated that the different dermatophyte species varied in the manner in which they attacked hair filaments *in vitro*. The members of one group behaved in the manner described by DAVIDSON and GREGORY for *T. mentagrophytes*, i. e., they formed "perforating organs", while other species brought about a gradual erosion of the hair filaments. *T. rubrum* was one of the fungi that did not perforate hair.

The fundamentally distinct manner by which *T. mentagrophytes* and *T. rubrum* attacked hair thus was considered to be of potential value in distinguishing atypical forms of these two fungi from one another. The manner of testing the value of this diagnostic criterion and the results obtained are the subject of this presentation.

Materials and Methods

The cultures used in this study came from many sources. Some were recent isolates while others had been maintained for varying

periods of time in our culture collection. Thirteen isolates of *T. mentagrophytes*, twelve of *T. rubrum*, and fifteen cultures of forms not readily identifiable as either of these two species were studied.

Morphological observations were made on all the fungi used. Sabouraud dextrose agar [1]) was selected to determine their gross colony features and pigmentation as this medium is generally used as the standard for such descriptions by medical mycologists. Other media, however, including wort agar, potato dextrose agar, cornmeal agar, oat meal agar, and heart infusion tryptose agar (Blood Agar Base, Difco), were used to induce spore production. Cornmeal dextrose agar was used according to the method described by BOCOBO and BENHAM (1949) for pigment production.

The study fungi were identified as far as possible on the basis of the features developed on these media. Isolates that differed in minor points, but whose major characteristics conformed to those of one particular species were identified as that species, but they were designated as being atypical. Where the morphological characteristics of a particular isolate were so aberrant that it could not be equated with either species, its identification was deferred.

The manner by which all these fungi attacked hair *in vitro* was studied as follows: Snort segments of human hair were sterilized in Petri dishes by autoclaving them at 120° C. for ten minutes. Following the addition of 25 ml. of sterile distilled water and 2 or 3 drops of 10 % sterilized yeast extract[1], the plates were inoculated with several fragments of colonies of the test fungi that had been grown in tubes of SABOURAUD dextrose agar. Once inoculated, the plates were incubated at 25° C. in a constant temperature box and examined at regular intervals over a period of 21 days. Hair segments were removed from the dishes with sterile forceps, placed in a drop of lactophenol cotton blue mounting fluid and examined under the microscope to determine whether or not they had been perforated. This procedure is considered to be simpler than the one developed by VANBREUSEGHEM (1949) for determing the effect of fungi growing on hair filaments *in vitro*.

RESULTS

Results of the preliminary morphological observations indicated that of the 40 strains studied, it was possible to clearly identify 13 as *T. mentagrophytes*, and 12 as *T. rubrum*. Even among these "typical" strains, there was considerable variation in morphological characteristics and pigment production, however, in each case the preponderance of the characteristics was referable to one of the two species (Tables I and II). The remaining 15 strains were not so clearly identifiable on morphological characteristics and pigmenta-

[1]) Difco, Difco Laboratories, Inc., Detroit, Michigan. Trade names are used as a means of identifying a product; their use does not constitute endorsement by the U.S. Public Health Service.

TABLE I. *Morphological characteristics of 13 isolates of T. mentagrophytes and their manner of attacking hair in vitro.*

Isolates	GROSS MORPHOLOGY	MICROSCOPIC MORPHOLOGY	Pigment on cornmeal dextrose agar	Morphological Identification	Hair Perforation
	Sabouraud-dextrose agar	*Special Media*			
	1. Topography 2. Surface Texture 3. Surface Pigment 4. Reverse Pigment	1. Microconidia 2. Macroconidia 3. Spirals 4. Others			
M-1	1. Flat 2. Granular 3. White to tan 4. Rose-brown	1. 4+ globose (wort agar) 2. 2+ clavate (wort agar) 3. 3+ tight (wort agar) 4. —	—	*T. mentagrophytes*	+
M-2	1. Flat 2. Downy 3. Cream to tan 4. Rose-brown	1. 4+ globose (wort agar) 2. 3+ clavate (wort agar) 3. None 4. —	—	*T. mentagrophytes*	+
M-3	1. Flat 2. Powdery 3. White to tan 4. No pigment	1. 4+ globose (wort) agar) 2. 1+ clavate 3+ irregular (wort agar) 3. 1+ tight 4. —	pink	*T. mentagrophytes*	+
M-4	1. Flat 2. Granular 3. White to tan 4. Rose-brown	1. 4+ globose (Sab. dex.) 2. 2+clavate (wort agar) 3. 3+tight (Sab. dex) 4. —	—	*T. mentagrophytes*	+
M-5	1. Flat 2. Downy 3. White 4. No pigment	1. 4+globose (Sab. dex.) 2. 1+clavate (wort agar) 3. None 4. —	—	*T. mentagrophytes*	+
M-6	1. Flat 2. Downy 3. White to tan 4. No pigment	1. 4+globose (Sab. dex.) 2. 2+clavate (wort agar) 3. None 4. —	—	*T. mentagrophytes*	+

M-7	1. Flat 2. Downy 3. White to cream 4. No Pigment	1. 3+globose (wort agar) 2. 2+clavate (wort agar) 3. None 4. —	Yellow	*T. mentagrophytes*	+
M-8	1. Flat 2. Downy 3. White to cream 4. No pigment	1. 2+globose (wort agar) 2. 1+clavate (wort agar) 3. 1+tight (cornmeal agar) 4. —	—	*T. mentagrophytes*	+
M-9	1. Flat 2. Downy 3. White to cream 4. Rose-brown	1. 3+globose (wort agar) 2. 1+clavate (wort agar) 3. None 4. —	—	*T. mentagrophytes*	+
M-10	1. Flat 2. Powdery 3. Yellow to deep pink 4. Rose-brown	1. 4+globose (wort agar) 2. 3+clavate 1+pencil shape (wort agar) 3. None 4. —	Yellow	*T. mentagrophytes*	+
M-11	1. Flat 2. Fluffy 3. White 4. None	1. 2+elongate (wort agar) 2. 3+irregular 1+clavate (wort agar) 3. 1+tight (wort agar) 4. —	—	*T. mentagrophytes*	+
M-12	1. Flat 2. Fluffy 3. White 4. None	Sterile mycelium (previously produced spores typical of *T. mentagrophytes*).	—	*T. mentagrophytes* pleomorphic	+
M-13	1. Flat 2. Granular 3. Cream to tan 4. Rose-brown	1. 4+globose (Sab. Dex. agar) 2. 2+clavate 2+irregular (wort agar) 3. 2+tight (wort agar) 4. —	—	*T. mentagrophytes*	+

TABLE II. *Morphological characteristics of 12 T. rubrum isolates and their manner of attacking hair in vitro.*

Isolates	GROSS MORPHOLOGY	MICROSCOPIC MORPHOLOGY	Pigment on cornmeal dextrose agar	Morphological Identification	Hair Perforations
	Sabouraud-Dextrose Agar 1. Topography 2. Surface Texture 3. Surface Pigment 4. Reverse Pigment	*Special Media* 1. Microconidia 2. Macroconidia 3. Spirals			
R-1	1. Flat 2. Fluffy 3. White 4. Deep red	1. 4+elongate (wort agar) 2. 1+pencil shaped (oatmeal agar) 3. None	pink	*T. rubrum*	—
R-2	1. Irregularly heaped 2. Fluffy 3. White to pink 4. Deep red	1. 4+small globose (wort agar) 2. 3+pencil shaped 1+clavate, irregular (blood agar base) 3. None	pink	*T. rubrum*	—
R-3	1. Flat 2. Fluffy 3. White 4. Deep red	1. 4+elongate (wort agar) 2. 3+pencil shaped (blood agar base) 3. None	pink	*T. rubrum*	—
R-4	1. Flat 2. Fluffy 2. White 4. Deep red	1. 4+elongate (wort agar) 2. 1+irregular (blood agar base) 3. None	pink	*T. rubrum*	—
R-5	1. Flat 2. Fluffy 3. White to pink 4. Deep red	1. 4+elongate (Sab. dex agar) 2. 4+pencil shaped (blood agar base) 3. None	pink	*T. rubrum*	—
R-6	1. Flat 2. Fluffy 3. White to pink 4. Deep red	1. 3+elongate (wort agar) 2. 2+pencil shaped (blood agar base) 3. None	pink	*T. rubrum*	—
R-7	1. Flat 2. Fluffy 3. White 4. Deep red	1. 3+elongate (wort agar) 2. 2+pencil shaped (blood agar base) 3. None	pink	*T. rubrum*	—

R-8	1. Flat 2. Fluffy 3. White 4. Deep red	1. 4+elongate (wort agar) 2. 3+pencil shaped (blood agar base) 3. None	pink	*T. rubrum*	—
R-9	1. Flat 2. Fluffy 3. White 4. Light red	1. 3+elongate (wort agar) 2. None found 3. None	yellow	*T. rubrum*	—
R-10	1. Flat-center folded 2. Fluffy 3. White to pink 4. Deep red	1. 3+elongate (wort agar) 2. 2+pencil shaped (blood agar base) 3. None	pink	*T. rubrum*	—
R-11	1. Flat 2. Fluffy 3. White to tan 4. Red	1. 3+elongate (wort agar) 2. None found 3. None	pink	*T. rubrum*	—
R-12	1. Flat 2. Fluffy 3. Pink to tan 4. Deep red	1. 2+globose (wort agar) 2. 2+pencil shaped 1+irregular (wort agar) 3. None	pink	*T. rubrum*	—

tion. In four instances the majority of the characteristics appeared to be referable to *T. rubrum*; these strains were classified as *T. rubrum* atypical. In three cases, where the resemblance was closer to *T. mentagrophytes*, the strains were called *T. mentagrophytes* atypical. In six cases, it was not possible to decide for one species, and these were listed as questionable (Table III).

All the strains were given code numbers, so that the investigators would not be influenced by the morphological identifications, and *in vitro* hair cultures were made of each isolate.

All of the fungi grew well when inoculated in the Petri dishes containing sterile distilled water and hair. Within a few days, wisps of newly formed mycelium could be observed floating in the water or developing around some of the hair segments. Generally by the tenth day the mycelium became matted around the hair and was readily visible to the naked eye (Fig. 3). Hair perforations were detected in some of the preparations by the fifth day following inoculation; usually, however, these were not found in abundance until the tenth to fourteenth day (Fig. 4 A–C). Gentle heating of the lactophenol cotton blue mounts aided the detection of perforations as heat intensified the staining of the mycelium. The tests

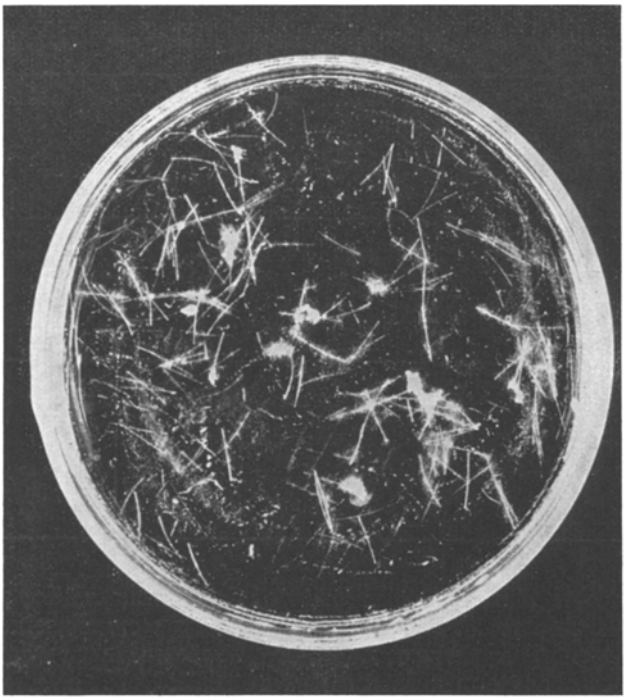

Figure 3. Appearance of *T. mentagrophytes* growing on hair segments in water.

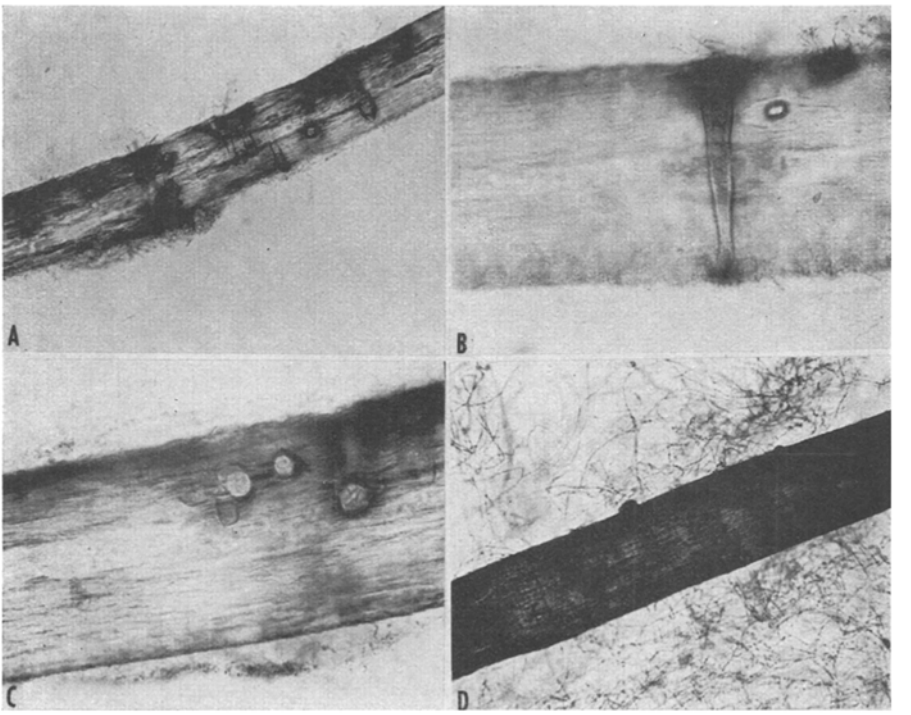

Figure 4. A. Hair segment with multiple perforations caused by *T. mentagrophytes* M10. Original magnification × 220.
B. Median optical view of a perforation extending through the full width of a hair filament. Original magnification × 760.
C. Surface view of a group of perforations. Original magnification × 760.
D. Failure of *T. rubrum* (R5) to perforate hair after 3 weeks of growth. Original magnification × 220.

TABLE III. *Morphology of 15 atypical isolates not readily equated with either T. mentagrophytes or T. rubrum and their manner of attacking in vitro.*

Isolates	Gross Morphology	Microscopic Morphology	Pigment on cornmeal dextrose agar	Morphological Identification	Hair Perforations
	Sabouraud-Dextrose Agar 1. Topography 2. Surface Texture 3. Surface Pigment 4. Reverse Pigment	*Special Media* 1. Microconidia 2. Macroconidia 3. Spirals 4. Others			
A-1	1. Flat 2. Powdery 3. White 4. Red brown	1. 2+globose 2+elongate (wort agar) 2. 2+pencil shaped (blood agar base) 3. 2+spirals loose (wort agar) 3. —	—	*T. rubrum* atypical	—
A-2	1. Folded 2. Downy 3. White 4. Dark red	1. 2+elongate (wort agar) 2. 3+pencil shaped (wort agar) 3. None 4. —	pink	*T. rubrum* atypical	—
A-3	1. Flat 2. Granular 3. White to tan 4. Dark red	1. 4+globose (wort agar) 2. 1+irregular small (wort agar) 3. None 4. —	pink	?	+ *T. mentagrophytes*
A-4	1. Flat 2. Granular 3. Pink 4. Dark red	1. 4+globose (wort agar) 2. 3+pencil shaped 1+irregular (blood agar base) 3. None 4. —	red	?	+ *T. mentagrophytes*
A-5	1. Flat 2. Fluffy 3. White to tan 4. Red	1. 4+globose (wort agar) 2. 2+clavate 3. 3+tight 4. —	pink	*T. mentagrophytes* atypical	+

A-6	1. Flat 2. Downy 3. White 4. Dark red	1. 3+elongate 2. 1+pencil shaped poorly formed (wort agar) 3. None 4. —	pink	*T. rubrum* atypical	—
A-7	1. Flat 2. Fluffy 3. Deep pink 4. Deep red	1. 3+globose 2. 4+pencil shaped (blood agar base) 3. None 4. —	red	*T. rubrum* atypical	—
A-8	1. Flat 2. Powdery 3. Rose to tan 4. Deep red	1. 4+globose 2. 2+irregular, pencil shaped 2+clavate (wort agar) 3. 2+loose (wort agar) 4. —	pink	?	— *T. rubrum*
A-9	1. Flat 2. Fluffy 3. White 4. Deep red	1. 1+elongate 2. 1+irregular, small (wort agar) 3. None 4. —	pink	?	— *T. rubrum*
A-10	1. Folded 2. Downy 3. Cream to pink 4. Deep red	1. 4+globose and elongate (wort agar) 2. 2+pencil shaped 2+clavate (wort agar) 3. None 4. —	pink	?	+ *T. mentagrophytes*
A-11	1. Flat 2. Fluffy 3. White to yellow 4. Yellow to orange	1. 1+elongate (Sab. Dex. agar) 2. None formed (wort agar) 3. None 4. 4+nodular bodies (cornmeal agar)	yellow	?	+ *T. mentagrophytes*
A-12	1. Folded 2. Downy 3. White 4. None	1. 4+globose (wort agar) 2. 3+irregular 1+clavate (wort agar) 3. None 4. —	—	*T. mentagrophytes* atypical	+ few only formation delayed
A-13	1. Folded 2. Downy 3. White 4. None	1. 4+elongate (wort agar) 2. 1+clavate (wort agar) 3. None 4. —	yellow	*T. mentagrophytes* atypical	+

A-14	1. Flat 2. Fluffy 3. White 4. None	1. 2+globose (wort agar) 2. 3+pencil shaped (blood agar base) 3. None 4. —	yellow	*T. rubrum* atypical	—
A-15	1. Heaped and folded 2. Downy 3. Rose 4. Deep red	1. 2+elongate (wort agar) 2. 3+pencil shaped (wort agar) 1+ varied forms 3. None 4. —	pink	*T. rubrum* atypical	—

were considered negative if no perforations were observed following 21 days of growth.

A study of Tables I and II indicates that the 13 strains previously identified as *T. mentagrophytes* perforated the hairs, whereas the 12 strains which had been identified as *T. rubrum* did not. In Table III it can be seen that those strains which most closely resembled *T. mentagrophytes*, but which were atypical, also perforated the hairs; while the atypical *T. rubrum* strains did not (Fig. 4–D). In the cases of those strains which could not be identified on morphological criteria, the behavior of the fungus in the *in vitro* hair culture was the deciding factor in determing its identity.

Discussion

Examination of the tables reveals that only certain isolates developed morphological characteristics that were exclusively possessed by either *T. mentagrophytes* or *T. rubrum*. It will be observed, however, that when morphological identification was possible the mode of development on hair was consistently of one type. That is, perforating organs were produced by all the isolates which could be morphologically identified as *T. mentagrophytes*, and no perforations were formed by those strains which had been identified as *T. rubrum*. For those few strains which could not be identified on morphological criteria alone, the ability or inability to penetrate hair proved to be decisive in their identification.

It is interesting to note that one of the test cultures of *T. mentagrophytes* (M 12), although completely pleomorphic at the time the *in vitro* hair cultures were made, perforated hair in the manner characteristic of actively sporulating isolates of that species.

It should be emphasized that the ability to perforate hair is not unique with *T. mentagrophytes* and consequently this property cannot be utilized as the sole basis for separating that species from *T. rubrum*. Several other fungi form perforating "organs", among

which may be mentioned the saprophyte, *Keratinomyces ajelloi*, the dermatophytes, *M. canis* and *M. gypseum*, and several as yet undescribed fungi (AJELLO) encountered in a wide variety of soils. This phenomenon becomes of value only when correlated with the gross and microscopic features of the fungus undergoing study as manifested on appropriate media.

Summary

Isolates of *T. mentagrophytes* and *T. rubrum* are encountered that cannot be distinguished from each other solely on the basis of morphological criteria.

Since these two species fundamentally differ in the manner in which they attack hair *in vitro*, this property can be used as a diagnostic aid, when correlated with their morphologic characteristics as developed on a variety of media.

T. mentagrophytes radially penetrates hair segments immersed in water forming wedge-shaped perforations. *T. rubrum* does not perforate hair.

The method of determining the ability or inability of *T. mentagrophytes* or *T. rubrum* to perforate hair and the use of this test in identifying those species are described.

Acknowledgment

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