The Human Scalp as a Habitat for Molds^{*}

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Synopsis—Ninety molds, representing 31 different species, including four known pathogens, have been isolated from the scalps of 100 individuals known to have dandruff. The isolation methods, identifying procedures and significance of the findings are discussed.

INTRODUCTION

There has been much interest in the isolation and identification of microorganisms from the human scalp in the last ten years because of their possible association with the scalp condition, "dandruff." It is believed by some investigators that dandruff is caused by a microorganism or group of microorganisms.

The human scalp offers an environment that is favorable for the growth of many types of microorganisms both aerobic and anaerobic. Investigation by Roia (1) has shown that at least 14 separate yeasts are often found on the scalp. Beal (2) isolated 16 aerobic bacteria, and Epstein (3) demonstrated that seven species of bacteria normally associated with the human intestin al tract are also found on the scalp.

Presently, study of the flora of the scalp is concerned primarily with the isolation and identification of the group of fungi known as molds. Although many species of molds are known to be air contaminants, it has not been shown that these molds are parasitic to man. However, some of the molds belong to genera that are known to be pathogenic.

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For 125 years fungi have been known to produce disease in man and animals (4). Before 1930 many fungus diseases were infectants in a small region of the country, but after 1930 an important cause for the spreading of fungus diseases as well as other diseases was the migration of people from one area to another. Some of the reasons for this great migration were economic depression and the great drought that occurred in the Midwest during the 1930's. The Second World War also caused a great deal of moving, both of industries and of the employees of the industrial plants.

The fungi of most concern are the keratinophilic fungi. These fungi are best known because of their relationship to certain dermatomycoses such as athlete's foot and ringworm of the scalp. These fungi are strongly keratinolytic and degrade keratin by an enzyme system (4). This enzyme system is not fully understood, and successful tests of keratin *in vivo* are difficult.

The keratinophilic fungi of the Gymnoascaceae have been studied extensively. Members of this family include *Trichophyton mentagrophytes*, *Microsporum gypseum*, and *Microsporum audouini*. There is little doubt that the infections caused by the keratinophilic fungi are spread from infected to healthy persons by either direct or indirect transfer of arthrospores or keratin that contains the fungus.

The need for knowledge of the microorganisms of the scalp and of their role in dandruff is of great importance to the manufacturers of hair preparations, such as medicated shampoos, hair rinses, and hair dressings. These types of products have been marketed by many companies in recent years. Their value is often questionable and almost always incomplete because of the lack of information about the effectiveness of the product against the organisms that are found on the scalp. It is the purpose of this study to investigate the connection, if any, of the molds found on the scalp with the presence of dandruff.

EXPERIMENTAL

A group of 100 persons was studied. It consisted of members of the senior and third-year classes at the Massachusetts College of Pharmacy between September, 1963, and June, 1964, of members of the faculty, and of people taken at random from outside this College. Twelve of the subjects were females. The subjects were not considered to have any abnormal scalp condition. They ranged in age from 17 to 66 years.

The material used in this study is known as "scurf." A scurf sample was obtained from each subject by having each person scratch his

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scalp with his fingernails and by allowing some scurf to fall upon a culture medium. Each subject selected the area of scalp of his choice. It was assumed that a representative sample from all parts of the scalp was obtained.

IDENTIFICATION OF MOLDS

Culture Technique

The culture medium used for the primary isolation was Sabouraud dextrose agar (Difco pH 5.6). To prevent the growth of yeasts and bacteria, neomycin (3 mg/ml) and nystatin (5 units/ml) were added to this medium.

Primary isolations were made in Petri plates having a diameter of 90 mm. Subsequently subcultures were made in smaller Petri plates (diameter 45 mm). These subcultures were grown on Sabouraud antibiotic agar, except for the molds of the genus *Penicillium*, which were grown on Czapek dox agar (Difco pH 7.3). All mold subcultures were grown from ten to fourteen days in the dark at 25 °C.

The cultural characteristics most useful in identification were the following:

- 1. *Colony Growth:* The rapidity of growth and the size of the colony at maturity is important.
- 2. *Colony Surface:* The colony surface could be velvety, floccose (wooly), funiculose ,or fasciculate. Ridges and furrows oriented in a radiating or concentric manner demarcated the colony into well-defined zones (zonate).
- 3. *Colony Margin:* The colony margin could be undulating or entire.
- 4. *Colony Color:* The chromogenicity of the aerial parts, including hyphae, conidiophores, and conidia, was observed. Sometimes the medium surrounding the colony became colored by soluble pigments, which was an important consideration.
- 5. *Spores:* The degree of sporulation and the spore color were important in designating species.
- 6. *Exudates:* Droplets of liquid appearing on the surface of the colony were often seen. These droplets varied in number, clarity, and pigmentation.
- 7. *Odor:* The odors produced by molds varied considerably and were characteristic.

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8. *Undersurface of Colony:* As the mold hyphae grow into the agar, characteristic colors were sometimes produced which could be observed by examining the underside of the colony.

Microscopic Technique

Wet Mount

A 3 mm plug of agar-containing mold was placed on a slide, 5% KOH was added, sufficient heat was applied to melt the agar, and a cover slip was pressed upon the material before microscopic examination.

Slide Culture

Shoemaker fungus microculture slides were inoculated with all available molds with cornmeal agar (Difco pH 6.0) as the nutrient. In most cultures sporulation was seen, following a ten to fourteen day incubation period at 25 °C.

The microscopic characteristics which were most useful in the identification of the majority of molds, especially the *Penicillium* and the *Aspergillus* were the following:

Head	Hulle cells, perithecia, ascospores,
Foot-cell	and sclerotia
Vesicle	Metulae
Conidiophore or stalk	Chlamydospores
Sterigmata	Stromata
Conidium or spore	Vegetative mycelium

RESULTS

Molds capable of growing on Sabouraud agar were isolated from the scalps of 55 of the 100 subjects tested in the survey. In some cases more than one mold was present in the scalp of the same person, resulting in a total of 90 identifiable molds which could be maintained in subculture.

Thirty-one different species of molds were identified from the scalps of 100 subjects (Table I). Only four of the isolated scalp molds have been reported to be associated with human pathological conditions. These are *Aspergillus versicolor*, *A. fumigatus*, *A. awamori*, and *A. miyckoensis* (5). Pathogenicity studies of mold scalp isolates were not carried out in this research. i

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The most frequent organism, one that was isolated from the scalp of

13 subjects, was *Penicillium notatum*. Other common molds were *Alternaria senecionis*, in the scalps of ten subjects, and *P. brevi-compactum*, in the scalps of six subjects.

Aspergillus awamori and Aspergillus miyakoensis belong to the Aspergillus niger group. Members of this group are commonly isolated from the external ear of man. The black aspergilli are the most common of all aspergilli. They are of world-wide distribution and occur in and upon the greatest variety of substrata, including grains, fabrics, leather, and decaying vegetation in the field.

Identifiable Molds Isolated from the Scal Organism	Number of Isolates
Alternaria senecionis	10
Aspergillus awamori	1
Aspergillus fumigatus	1
Aspergillus miyakoensis	2
Aspergillus terricola var. americana	1
A spergillus versicolor.	1
Cladosporium avellaneum	2
Cladosporium cladosporioides	2
Cladosporium sphaerospermum	4
Helminthosporium halodes	2
Mucor circinelloides	2
Penicillium albidum	2
Penicillium brevi-compactum	6
Penicillium chrysogenum	2
Penicillium citrinum	4
Penicillium commune	2
Penicillium expansum	2
Penicillium frequentans	4
Penicillium implicatum	4
Penicillium lanoso-coeruleum	1
Penicillium lilacinum	1
Penicillium lividum	4
Penicillium nalgiovensis	1
Penicillium notatum	13
Penicillium oxalicum	1
Penicillium paxilli	5
Penicillium roqueforti	2
Penicillium soppi	1
Penicillium vermiculatum	3
Pseudostemphylium lanuginosum	2
Pullularia pullulans	2
Total	$\overline{90}$

Table I

The Aspergillus tamarri series is not widely distributed, nor it is particularly common. It has been isolated from soil and from decaying organic material. It has no pathogenicity and may be regarded only as a saprophyte. The only species isolated belonging to this series was Aspergillus terricola var. americana.

Aspergillus versicolor var. glauca was isolated from human skin showing "ringworm," but pathogenicity was not proved experimentally (5). It is often found upon dried salted lean beef, thus showing its ability to grow upon meat products. It has been isolated from other, "nonmeaty" places, such as bread, cereals, old cheese, rubber, and paraffins. These molds are widely distributed.

SUMMARY

1. The mold flora of the scalps of 100 people has been investigated.

2. From 55 of these people, a total of 90 molds were isolated by culture and were identified according to monographs found in the literature.

3. The 90 molds were represented by 31 species.

4. The three most prevalent molds were *Penicillium notatum*, *Alternaria senecionis*, and *Penicillium brevi-compactum*.

5. Four of the 31 molds isolated are known to be pathogenic to man. The rest are plant saprophytes or parasites as well, responsible for the decomposition of organic materials.

6. There is no evidence in the literature to show that any of the molds isolated may cause any specific pathological condition of the scalp.

7. This survey shows that the human scalp harbors molds in great abundance.

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