

THE HUMAN SCALP AS A HABITAT FOR YEASTS*

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INTRODUCTION AND BACKGROUND

ALMOST from the very moment when Rivolta (1) in Italy in 1873 first isolated and described the yeast organism, *Pityrosporum ovale* from the human scalp, the etiological role of this organism in seborrheic dermatitis and pityriasis capitis has been a matter of much dispute. On the one hand, its universal presence in dandruff scales in numbers proportional to the severity of the condition has been verified by every worker interested in the problem. On the other hand, the lack of convincing evidence to prove pathogenicity through animal or human inoculation has led to the modern supposition that *P. ovale* is an inoffensive saprophyte of man.

Some investigators, however, argue that this organism more nearly behaves as a true parasite. It has been isolated nowhere but from the animal skin. It is extremely fastidious in its growth habits, requiring naturally occurring fatty acids together with a source of nitrogen and glucose. Each of these nutrients is supplied by the skin. Stained smears of the scalp usually reveal many actively budding forms.

In addition to the possible role of *Pityrosporum ovale* in seborrheic dermatitis and pityriasis capitis, an etiological relationship to seborrheic blepharitis and dermatitis of the eyelids has been suggested by Gots (2) and his co-workers. In their studies budding yeast forms morphologically similar to *P. ovale* were found in 100 per cent of 143 cases of seborrheic blepharitis. They were also able to demonstrate sensitization of the organism by intradermal skin tests and have indicated that inflammatory lesions of the conjunctiva are allergic in character, irrespective of the role of the organism in seborrheic dermatitis.

More recently, Gordon (3) has described a new species of *Pityrosporum* which he has named *P. orbiculare* because of its spherical shape. The organism was isolated from 15 of 18 cases diagnosed as tinea versicolor. Because of its lipophilic nature it seems to be related to *P. ovale*. Attempts

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at experimental inoculation have failed to produce tinea versicolor in humans. It is interesting to note that the organism described by Gordon is morphologically identical to the so-called spherical forms of *P. ovale* found by Gots in lid-margin scrapings from cases of seborrheic blepharitis.

The authors of this paper became interested in the yeast flora of the human scalp while working with improved culture media for isolating and growing *P. ovale*. Isolation studies showed that *P. ovale*, while prevalent, is not the only yeast organism of the scalp. The possibility occurred to us that since *P. ovale* could not definitely be linked to dandruff, perhaps some other, as yet undiscovered, yeast could be a contributing factor.

We therefore decided to survey a large number of humans in the hope of relating the scalp yeast flora to possible pathogenicity.

The literature makes many references to the isolation and identification of yeasts from various areas of the body in both normal and pathological conditions but only an occasional reference to the human scalp. Surveys on the yeast flora of the external surface, the nails, the orifices and the alimentary canal have been carried out. At least three factors seem to be responsible for this interest.

1. New and improved media have been formulated for the isolation and study of yeasts, which have resulted, for example, in the realization of the obligate lipophilic nature of *Pityrosporum ovale* (4). The introduction of certain antibiotics into culture media has facilitated the isolation of yeasts without interference by bacterial overgrowth.

2. The monograph of Lodder and Kreger-Van Rij (5) and the contributions of Wickerham (6) have created a better understanding of the taxonomy of yeasts.

3. A number of infections attributed to members of the yeast group have been reported. Many species of *Candida* and *Cryptococcus* are being isolated with increasing frequency from a variety of lesions in man, although *Candida albicans* and *Cryptococcus neoformans* have been considered to be the only pathogenic members of the group. The widespread use of antibiotics is responsible in no small part for the increase in candidiasis and cryptococcosis.

Huxley and Hurd (7) isolated from the skin and described a number of pink yeasts belonging to the genus *Rhodotorula* and *Sporobolomyces*. The skin areas selected for study were those between the toes, between the fingers, and from the lumbar region, the axilla, the inframammary region and the navel. They found the predominant organism to be *Rhodotorula mucilaginosa*. Connell and Skinner (8) determined the occurrence of non-fermenting, nonpigmented yeasts on 250 human subjects. Of 784 isolates of yeasts and yeast-like fungi the following species are recorded as having been isolated. They are given in the order of their frequency: *Cryptococcus diffluens*, *Cr. aerius*, *Cr. albidus*, *Cr. laurentii*, var. *flavescens*, *Cr. rotundatus*,

Candida spp., *Lipomyces starkeyi*, *Candida zeylanoides*, *C. lipolytica* and *C. mesenterica*. Their studies show that qualitatively and quantitatively the yeasts on the body are different from those in the air or from surfaces with which the body comes in contact. They concluded that the skin surface may well be a true habitat for yeasts.

Croft and Black (9) also concluded that yeasts live saprophytically on normal skin. They isolated 29 yeasts from the fingertips of 22 per cent of the persons surveyed. *Candida parapsilosis* was the predominating organism, appearing in 12 cases. Other yeast-like organisms found were *Endomyces* spp., in four cases; *Monilia nigra*, in three; *Cryptococcus*, in two; *Mycoderma*, in one; *Schizosaccharomyces hominis*, in one; unidentified *Monilia*, in three; and unknown colonies, in three.

DiMenna (10) examined several areas of the body. She found that the following nonpathogenic species occurred in approximately equal numbers in the alimentary canal and in the oral cavity: *Saccharomyces* spp., *Candida krusei*, *C. parapsilosis*, and *Torulopsis glabrata*. The pathogenic *C. albicans* was isolated from the oral cavity in 83 per cent of the cases and from the alimentary canal in 67 per cent of the cases. The areas of the skin surveyed by DiMenna were those of the fingernails and of the arms of 120 isolates from 381 subjects. The predominating yeast-like organisms were *Debaryomyces kloekeri*, in 34 cases; *Cryptococcus* spp., in 20 cases; *Rhodotorula* spp., in 27 cases; and the yeast-like phase of *Cladosporium* spp., in 22 cases. In addition, direct smears were made of the scalp to determine the presence of *P. ovale*. This organism was found to be present in 82 of 87 subjects examined. In only one instance was a cultural examination made of the scalp, and this was found to be negative for *P. ovale*. DiMenna considers that there are three possible categories of yeasts, with respect to their habit, isolated from man: 1) those that are contaminants, picked up from their surroundings and incapable of reproduction or of prolonged survival upon the human body; 2) those that are capable of multiplying upon the body as well as apart from it; and 3) those that are obligate parasites upon the human body. *Candida albicans*, *P. ovale*, and very possibly *Torulopsis glabrata* belong in the third category. Contrary to the findings of Connell and Skinner, DiMenna concluded that the yeasts found on the skin (exclusive of *P. ovale*) and in the air are similar, both in proportions of different genera and in kinds of species. Skinner *et al.* (11) stated that yeasts were consistently isolated from the skin of about 50 per cent of the 275 persons who were examined. In another survey Benham and Hopkins (12) isolated 57 strains from 100 fingernail cultures, 62 from toe nails and 41 from toe webs.

Sturde (13) in Germany examined the infected fingernails of 50 patients for the presence of yeasts. In each instance one or more yeasts were isolated. Those predominating were *C. albicans*, *C. parapsilosis*, and *Toru-*

lopsi famata. The most complete study to date on the distribution of yeasts in the human body has been made by Rieth (14) also in Germany. Over 15,000 patients were studied during a four year period. From the skin, hair and nails almost 2000 yeasts were identified. Those predominating were *C. albicans*, *C. parapsilosis*, *Torulopsis famata*, *Trichosporon cutaneum*, *Rhodotorula mucilaginosa* and *Rh. rubra*. Kapica and Blank (15), Fischer (16), Nino *et al.* (17) all claim repeated isolation of *C. parapsilosis* from infected nails showing typical signs of moniliasis. Kapica and Blank furthermore have carried out studies to prove that *C. albicans* (18) and *C. parapsilosis* (15) are both capable of utilizing nitrogen obtained solely from keratin, provided glucose is present as an initial stimulus. These workers admit that while such a biochemical investigation cannot provide an answer to the question of pathogenicity of *C. albicans* and *C. parapsilosis* its results indicate a reason for the pathogenicity. They found that this keratin breakdown occurs between 40 and 56 days.

As far as the references to the scalp flora are concerned Ota and Huang (19) simply report "ordinary" yeasts in addition to the organism they believed to be *P. ovale*. MacKee and Lewis (20) showed that the scalp yields numerous fungi and yeasts as well as bacteria but made no attempt to identify them. Benham (21) isolated a number of yeast-like organisms which she placed in the genus *Cryptococcus*. In several instances, moreover, isolates from the scalp reported to be *P. ovale* were later shown by her also to be members of the genus *Cryptococcus*. Similarly, Spoor *et al.* (22) in their isolation studies of *P. ovale* from normal and seborrheic subjects have isolated not only this organism in approximately 60 per cent of the cases but speak also of unidentified strains which are classified as "yeast-like" types.

EXPERIMENTAL METHODS

Two groups of individuals were studied. They consisted of 98 members of the senior class of the Massachusetts College of Pharmacy from September, 1958, to June, 1959, and 91 members of the senior class from September, 1959, to January, 1960. Included in these groups were 17 females. The members of both groups ranged in age between twenty and thirty years.

The material used in this study from which the isolation of yeasts was made is commonly known as "scurf." Scurf represents desquamated epithelial cells from the scalp together with accumulated secretions, acquired soil, and a mixed microbiological flora. With all subjects it was possible to obtain a sample of scurf by instructing each one to brush his scalp vigorously and to collect the material on appropriate culture media. No attempt was made to record the degree of sloughing off or subsequently to correlate the amount of scurf with the type of organism

isolated. The groups surveyed were considered to be composed of normal young adults.

The culture media used for primary isolation were Littman Oxgall Agar (Difco-pH 7), Littman Oxgall Agar with 2 per cent sesame oil (pH 6.8) and Yeast Morphology Agar (Difco-pH 5). Streptomycin sulfate (30 micrograms per ml.) was added to each of the three media after sterilization and cooling to 45°C. Prior to pouring the plates which were to contain 2 per cent sesame oil, the oil was shaken vigorously with melted Littman Oxgall Agar in order to form an emulsion. Each culture was incubated for three days at 35°C. and then for two weeks at 25°C. The techniques and media used in the determination of the morphological and physiological properties of each yeast isolate are those described by Lodder and Kreger-Van Rij (5).

RESULTS

Yeasts capable of growing on one or more of the media used in this study were isolated from 122 of the 189 individuals participating in the survey. This represents 65 per cent of the total. In some instances more than one yeast was present in the scalp of the same person, resulting in a total of 145 identifiable yeasts which could be maintained in subculture. Three members of the *Dermafiatae* were also isolated. These are sometimes called the "Black Yeasts" because of their color. Since they are believed to represent a yeast-like phase of certain molds they were not included in this survey.

All the yeasts identified in this survey were found to be members of the *Cryptococcaceae*. No member of the ascospore forming family *Endomycetaceae* and the ballistospore forming family *Sporobolomycetaceae* was found. It was also noted that the two yeasts most commonly identified with pathological conditions in man are not scalp inhabitants. These are *C. albicans* and *Cr. neoformans*.

The predominant yeast was *P. ovale* which was found in 46 individuals. Other workers have reported the occurrence of *P. ovale* on the scalp as being between 70 and 94 per cent; but this percentage was reached by a direct smear from the scalp and not by growth on isolation media. The other two most common yeasts were *C. parapsilosis* in 32 individuals and *Rh. mucilaginosa* in 26 individuals. In all, there were fourteen different species of yeasts identified. These are listed in Table 1.

Each of the 14 species of yeasts isolated in this survey were studied to determine which of them had been isolated from the human skin by previous workers and if possible to draw inferences concerning their possible role as scalp pathogens. Table 2 represents a compilation of the work of previous investigators.

It would appear, based upon the frequency of occurrence that only, *P.*

TABLE 1—IDENTIFIABLE YEASTS ISOLATED FROM THE SCALPS OF 189 HUMANS

Organism	Number of Isolates	Frequency Percentage
<i>Pityrosporum ovale</i>	46	31.72
<i>Candida parapsilosis</i>	32	22.07
<i>Rhodotorula mucilaginosa</i>	26	17.93
<i>Cryptococcus diffluens</i>	9	6.20
<i>Torulopsis famata</i>	8	5.52
<i>Cryptococcus albidus</i>	7	4.83
<i>Rhodotorula minuta</i>	4	2.76
<i>Candida mycoderma</i>	3	2.07
<i>Trichosporon cutaneum</i>	3	2.07
<i>Torulopsis inconspicua</i>	2	1.38
<i>Cryptococcus laurentii</i>	2	1.38
<i>Rhodotorula flava</i>	1	0.69
<i>Rhodotorula rubra</i>	1	0.69
<i>Candida scotti</i>	1	0.69
	145	100.00

ovale, *C. parapsilosis*, and *Rh. mucilaginosa* are prevalent enough in the human scalp to warrant serious consideration. The case for and against *P. ovale* has already been discussed. In view of the frequency with which *C. parapsilosis* has been isolated from onychomycotic infections and because of its proven keratinolytic nature this organism could be added to the list of possible pathogens. *Rh. mucilaginosa*, on the other hand, has not been recorded as being associated with any skin pathological condition except in one instance. The monograph of Lodder and Kreger-Van Rij (5) makes a single reference to its isolation by Wolfram and Zach from diseased nails in 1934. Because of the higher incidence of *Rh. mucilaginosa* in the air than upon human skin Connell and Skinner (8) refer to this organism as an adventitious saprophyte.

TABLE 2—COMPARATIVE STUDIES OF YEASTS ISOLATED FROM VARIOUS SKIN SURFACES

Organism	A	B	C	D	E	F	G
<i>P. ovale</i>	*				*		
<i>C. parapsilosis</i>	*			*	*		
<i>Rh. mucilaginosa</i>	*	*	*			*	*
<i>Cr. diffluens</i>	*		*				*
<i>T. famata</i>	*					*	*
<i>Cr. albidus</i>	*		*				
<i>Rh. minuta</i>		*					*
<i>C. mycoderma</i>	*						*
<i>Trich. cutaneum</i>	*					*	*
<i>T. inconspicua</i>	*					*	*
<i>Cr. laurentii</i>	*		*				
<i>Rh. flava</i>							
<i>Rh. rubra</i>	*	*					*
<i>C. scotti</i>							*

A—Lodder and Kreger-Van Rij. B—Huxley and Hurd. C—Connell and Skinner. D—Croft and Black. E—DiMenna. F—Sturde. G—Rieth.

Preliminary studies undertaken by the authors indicate the possibility that both *P. ovale* and *Rh. mucilaginoso* are capable of utilizing keratin as a source of nitrogen following the technique of Kapica and Blank (15, 18). If such is the case the parasitic nature of these organisms would be established.

SUMMARY

1. The yeast flora of the scalps of 189 college seniors has been investigated.
2. From 122 of these individuals (65%) a total of 145 yeasts were isolated by culture and were identified according to the monograph of Lodder and Kreger-Van Rij.
3. The 145 yeasts were represented by 14 species.
4. The three most prevalent yeasts were *Pityrosporum ovale*, *Candida parapsilosis*, and *Rhodotorula mucilaginoso*.
5. A comparative study was made with other surveys of yeasts of skin surfaces, which also showed that *C. parapsilosis* and *Rh. mucilaginoso* were common yeasts found on the scalp and on other skin surfaces.
6. Because of the frequency with which *C. parapsilosis* has been isolated from onychomycotic infections and because of its proved keratinolytic activity an association with pathological scalp conditions is suggested. Preliminary studies indicate a similar activity for *P. ovale* and *Rh. mucilaginoso*.
7. There is no evidence from this paper or from a survey of the literature that any of the other yeasts isolated could be causative of a pathological condition of the scalp.
8. This survey shows that the human scalp harbors yeasts in greater abundance and variety than other areas of the body.
9. The prevalence of *P. ovale* as reported by other workers has been substantiated by this survey.

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