

6. Beachey EH. Bacterial adherence: Adhesin-receptor interactions mediating the attachment of bacteria to mucosal surfaces. *J Infect Dis* 1981; 143 (3): 325-345.
7. Cole GW, Silverberg NL. The adherence of *Staphylococcus aureus* to human corneocytes. *Arch Dermatol* 1986; 122: 166-169.
8. Lassus A, Geiger JM, Nyblom M, Virrankoski T, Kaartamaa M, Ingervo L. Treatment of severe psoriasis with etretin (Ro 10-1670). *Br J Dermatol* 1987; 117: 333-341.
9. Ellen RP, Gibbons RS. M-protein associated adherence of *Streptococcus pyogenes* to epithelial surfaces. *Acta Derm Venereol (Stockh)* 1972; 5: 826-830.
10. Bruckner-Tuderman L, Sigg C, Geiger JM, Gilardi S. Acitretin in the symptomatic therapy for severe recessive x-linked ichthyosis. *Arch Dermatol* 1988; 124: 529-532.
11. Goldfarb MT, Ellis CN, Gupta AK, Tincoff T, Hamilton TA, Voorhees JJ. Acitretin improves psoriasis in a dose-dependent fashion. *J Am Acad Dermatol* 1988; 18: 655-662.
12. Lauharanta J, Kanerva L, Turjanmaa K, Geiger JM. Clinical and ultrastructural effects of acitretin in Darier's disease. *Acta Derm Venereol (Stockh)* 1988; 68: 492-498.
13. Paravicini U, Camenzind M, Gower M, Geiger JM, Saurat J. Multiple dose pharmacokinetics of Ro 10-1670: the main metabolite of etretinate (Tigason). In: Saurat J, ed. *Retinoids: New trends in research and therapy*. Basel: Karger, 1985; 289-292.
14. Kingston TP, Matt LH, Lowe NJ. Etretin therapy for severe psoriasis: Evaluation of initial clinical responses. *Arch Dermatol* 1987; 123: 55-58.
15. Williams ML, Elias PM. Nature of skin fragility in patients receiving retinoids for systemic effect. *Arch Dermatol* 1981; 117: 611-619.
16. Leyden J, Marples R, Kligman A. *Staphylococcus aureus* in lesions of atopic dermatitis. *Br J Dermatol* 1974; 90: 525-530.

## Seborrhoeic Dermatitis and *Pityrosporum ovale*: A Cultural and Immunological Study

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Seborrhoeic dermatitis is associated with *Pityrosporum ovale*, but the exact role of the organism is not clarified. In order to study this connection we have investigated 30 patients with seborrhoeic dermatitis with quantitative culture for *P. ovale*, serum IgG antibodies against *P. ovale* and lipid measurements. We compared the patients with 60 healthy individuals and found no significant difference in the number of *P. ovale* or serum antibodies. The lipid content on the skin was significantly higher in the patient group ( $p=0.0001$ ). There was no difference in the number of *P. ovale* in lesions compared to healthy skin in the patient group. This study support our theory that an abnormal reaction in the skin to *P. ovale* causes the inflammation and the number of *P. ovale* is of minor importance. **Key words:** *Antibodies; Lipid content.*

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Seborrhoeic dermatitis is characterized by inflammation and desquamation in areas with a rich supply of

sebaceous glands, namely the scalp, face and upper trunk. Several studies indicate that *P. ovale* is associated with seborrhoeic dermatitis, but the exact role of the organism in the disease is still unclear (1-4). Many antimycotics have been effective in treatment (5-7) and cure of seborrhoeic dermatitis is often paralleled by a fall in numbers of *P. ovale* (5-7) and recolonization leads to recurrence of seborrhoeic dermatitis (8). Abnormalities in the composition of skin lipids in seborrhoeic dermatitis have been found (9, 10), but no increase in sebum excretion rate (11) or sebum levels (9) has been reported. There have also been reports of high IgG antibodies against *P. ovale* in sera from patients with seborrhoeic dermatitis (2). *P. ovale* is a member of the normal human cutaneous flora (12). The colonization starts during puberty when the sebaceous glands become active (13). *P. ovale* can be found on the skin of almost all adults (14) and the presence of the organism cannot be the only explanation of the disease.

In this investigation, the presence of *P. ovale* on the skin, IgG serum antibodies against *P. ovale* and skin lipid measurements were studied in patients with seborrhoeic dermatitis.

Table I. Quantitative cultures of *Pityrosporum ovale* in patients with seborrhoeic dermatitis and in healthy controls

P. ovale Mean $\pm$ SD	Controls	Patients		
		Normal skin	Lesional skin	Scalp
Scrub method (colonies/cm <sup>2</sup> )	245 $\pm$ 584 (n=60)	78 $\pm$ 125 (n=30)	202 $\pm$ 358 (n=22)	65 $\pm$ 101 (n=23)
Contact plates <sup>a</sup> (colonies/plate)	ND	15 $\pm$ 24 (n=23)	23 $\pm$ 25 (n=17)	63 $\pm$ 59 (n=16)

<sup>a</sup> Contact plate area 24.6 cm<sup>2</sup>.

## MATERIAL AND METHODS

### Characteristics of subjects

Thirty patients with seborrhoeic dermatitis, 24 males and 6 females, with a mean age of 42 years (range 18–80) were studied. Concomitant diseases were seen in 4 patients: ankylosing spondylitis (1), rosacea (1), pityriasis versicolor (1) and Mb Crohn (1). The patients had not used any topical corticosteroids or antifungal agents for a week and no soap or moistening cream for 24 h before sampling was done. Cultures for *P. ovale*, serum samples and lipid measurements were taken from all patients. Sixty healthy individuals, 20 males and 40 females, with a mean age of 55 years (range 29–81) were included as controls.

### Quantitative culture of *P. ovale*

**Scrub method.** The technique for quantitative cultures was described in detail earlier (15). Cultures were taken from healthy skin on the chest, lesions on the chest (16 pats.), face (5 pats.) and back (1 pat.). Other culture areas than the chest had to be included because not all patients had lesions in that area. Cultures were taken from the scalp in 23 patients. Samples were collected using a stainless steel ring and 0.075 M phosphate buffer, pH 7.9, containing 0.1% Triton-X-100 (1 ml). The skin was gently rubbed with a blunt sterile glass rod for one minute and the procedure was repeated 3 times from the same skin area. From the scalp the procedure was performed once. Samples (0.1 ml) from each wash were inoculated on to a glucose-neopeptone-yeast extract agar medium containing olive oil (2%), Tween (0.2%) and glycerol monostearate (2.5 g l<sup>-1</sup>). Plates were incubated at 37°C and examined after 10 days.

**Contact plates.** Cultures were taken from normal-looking skin of the chest, lesions on the body or face and from the scalp. The plates were pressed against the skin for 30 sec, incubated in a Bio-Bag Cfj at 37°C and read after 6 days. The medium was a glucose-neopeptone-yeast extract agar with addition of olive oil, Tween 80 and glycerol monostearate (PDM *Pityrosporum* Contact Plate, AB Bio Disk, Solna, Sweden). The technique was described in detail earlier (16).

### Indirect immunofluorescence technique on sera

IgG antibodies against *P. ovale* were estimated as earlier described (17) using fluorescein isothiocyanate (FITC)-la-

belled antihuman IgG (DAKO, Copenhagen, Denmark, lot 034, F202). *P. ovale* (ATCC 42132) cells were used as the antigen.

### Lipid measurements

Measurements were made with Sebumeter SM 410 (Courage-Khazaka electronic, Köln, West Germany) (18–20). Briefly, samples were taken from the forehead skin of the patient using a plastic strip pressed firmly (6N) against the skin for 30 sec. Immediately after the samples were taken, results were measured photometrically and expressed as  $\mu\text{g cm}^{-1}$ . Sebum measurements were taken from the forehead because it would be possible to correlate our data with earlier findings.

### Statistics

Multiple regression analysis was used to compare the results between the parameters and between the patients and the controls. Correlation between the parameters was studied with Pearson's correlation coefficient analysis.

## RESULTS

### Quantitative cultures of *P. ovale*

Parallel to the scrub methods and the contact plates, microscopy of *P. ovale* was done in 17 patients. We found this technique difficult since the cells show a great polymorphism and decided not to present these data. In patients with seborrhoeic dermatitis there was no significant difference between the mean number of *P. ovale* on healthy skin, skin lesions and scalp lesions, both with the scrub method and the contact plates (Table I). The scrub method and the contact plates showed a significant correlation between the number of *P. ovale* on healthy skin and skin lesions (scrub methods,  $p=0.005$ ; contact plates,  $p=0.0001$ ). The number of organisms found on normal skin in patients with seborrhoeic dermatitis was lower than the number found on normal skin from healthy volunteers (Table I) ( $p=0.013$ ). However, many patients

had lesions on the midchest area and culture from normal skin was taken from the lateral part of the chest. No significant difference was seen in the number of organisms found in skin lesions from patients compared to normal skin in healthy controls.

#### *IIF-technique*

The mean serum IgG antibody titres against *P. ovale* on sera from patients with seborrhoeic dermatitis ( $202 \pm 257$ ) compared to 30 healthy individuals ( $113 \pm 136$ ) showed no significant difference. There was no correlation between the titres and the number of cultured *P. ovale*.

#### *Lipid measurements*

The amount of lipid on the forehead skin was significantly higher ( $p=0.0001$ ) in the patient group ( $168 \mu\text{g} \pm 46$ ) compared to the controls ( $95 \mu\text{g} \pm 58$ ). There was no correlation between the amount of lipid and the number of *P. ovale* on the skin.

## DISCUSSION

The association between seborrhoeic dermatitis and *P. ovale* has been studied, but is still not clarified. In an earlier study with direct microscopy, McGinley and co-workers found larger numbers of *P. ovale* from the skin in patients with dandruff compared to controls, but not from patients with seborrhoeic dermatitis compared to controls (1). In the present study, we did not find any difference in number of cultured *P. ovale* from the lesions in patients with seborrhoeic dermatitis compared to normal skin and controls. We performed direct microscopy parallel to cultures but found it difficult and frequently unreliable. Culture is the only way to identify the organism seen in skin scales and to get information about the viability of the cells.

Serum IgG antibody titres against *P. ovale* whole cells are significantly higher in adults than in children (13), but decrease with increase in age (14). In patients with Pityrosporum folliculitis, higher serum IgG antibody titres are present (21). The raised titres could be an answer to a stronger stimulation of the immune system, following a deeper colonization in the follicles. In patients with pityriasis versicolor, serum IgG antibodies against *P. ovale* are within normal range (22). High IgG antibodies against *P. ovale* have been found in patients with dandruff (2). The highest titres were found in the patients with the

greatest amount of dandruff, but changes in dandruff were not followed by changes in titres (2).

We found no significant difference in antibody titres in patients with seborrhoeic dermatitis compared to controls. Probably, the serum antibodies only reflect the colonization with *P. ovale*.

Seborrhoeic dermatitis is localized in areas with a rich supply of sebaceous glands. The quantity of sebum in patients with seborrhoeic dermatitis has been found to be within normal limits (9). Minor abnormalities in the composition of surface lipids have been reported (9, 10).

In this study we found a significantly larger amount of lipid on the forehead skin in patients with seborrhoeic dermatitis compared to healthy individuals. We used a Sebumeter, which is a new photospectrometric method.

The present investigation does not indicate that the number of *P. ovale* or the serum IgG antibody titres against *P. ovale* whole cells are of major importance in seborrhoeic dermatitis since the parameters did not differ significantly in patients compared to controls. In earlier studies (unpublished data), we found T-cells abnormalities in patients with seborrhoeic dermatitis and an inverse correlation between the levels of IgG antibodies against a protein antigen from *P. ovale* and the severity of seborrhoeic dermatitis. The response against protein antigens is T-lymphocyte dependent. These results could suggest an immunoweakness in the patients as an explanation of the abnormal reaction in the skin against the saprophyte *P. ovale*.

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## REFERENCES

1. McGinley KJ, Leyden JJ, Marples RR, Kligman AM. Quantitative microbiology of the scalp in non-dandruff, dandruff and seborrhoeic dermatitis. *J Invest Dermatol* 1975; 64: 401-406.
2. Alexander S. Loss of hair and dandruff. *Br J Dermatol* 1968; 79: 549-552.
3. Shuster S. The aetiology of dandruff and the mode of action of therapeutic agents. *Br J Dermatol* 1984; 111: 235-242.

4. Van Abbe NJ, Head D, Reed JV, Murrel EA, Baxter PM. Dandruff: Infection or not? *Int J Cosmetic Science* 1986; 8: 37-44.
5. Ford GP, Farr PM, Ive FA, Shuster S. The response of seborrhoeic dermatitis to ketoconazole. *Br J Dermatol* 1984; 111: 603-607.
6. Skinner RB, Noah PW, Taylor RM, Zanolli MD, West S, Guin JD, Rosenberg EW. Double-blind treatment of seborrhoeic dermatitis with 2% ketoconazole cream. *J Am Acad Dermatol* 1985; 12: 852-856.
7. Faergemann J. Seborrhoeic dermatitis and *Pityrosporum orbiculare*: Treatment of seborrhoeic dermatitis of the scalp with miconazole-hydrocortisone (Daktacort), miconazole and hydrocortisone. *Br J Dermatol* 1986; 114: 695-700.
8. Gosse RM, Vanderwyk RW. The relationship of a nystatin-resistant strain of *Pityrosporum ovale* to dandruff. *J Soc Cosmet Chem* 1969; 20: 603.
9. Hodgson-Jones I, Mackenna RMB, Wheatley VR. The surface fat in seborrhoeic dermatitis. *Br J Dermatol* 1953; 65: 246-251.
10. Gloor M, Wiegand I, Friedrich HC. Über Menge und Zusammensetzung der Hautoberflächenlipide beim sogenannten seborrhoischen Ekzem. *Dermatol Monatsschr* 1972; 158: 759-764.
11. Burton JL, Pye RJ. Seborrhoea is not a feature of seborrhoeic dermatitis. *Br Med J* 1983; 266: 1169-1170.
12. Roberts SOB. *Pityrosporum orbiculare*: Incidence and distribution on clinically normal skin. *Br J Dermatol* 1969; 81: 264-269.
13. Faergemann J, Fredriksson T. Age incidence of *Pityrosporum orbiculare* on human skin. *Acta Derm Venereol (Stockh)* 1980; 60: 531-533.
14. Bergbrant I-M, Faergemann J. Variations of *Pityrosporum orbiculare* in middle-aged and elderly individuals. *Acta Derm Venereol (Stockh)* 1988; 68: 537-540.
15. Faergemann J. Quantitative culture of *Pityrosporum orbiculare*. *Int J Dermatol* 1984; 23: 330-333.
16. Faergemann J. The use of contact plates for quantitative culture of *Pityrosporum orbiculare*. *Mykosen* 1987; 30: 298-304.
17. Faergemann J, Tjernlund U, Scheynius A, et al. Antigenic similarities and differences in genus *Pityrosporum*. *J Invest Dermatol* 1982; 78: 28-31.
18. Dickstein S, Zlotogorski A, Avriel E, Katz M, Harms M. Comparison of the Sebumeter and the Lipometre. *Bioeng Skin* 1987; 30: 197-207.
19. Schrader K. Über eine neues Verfahren zur Messung des Hautoberflächenfettes. *Dragoco-Report* 1974; 171-174.
20. Schaefer H, Kuhn-Bussius H. Methodik zur Quantitativen Bestimmung der menschlicher Talgsekretion. *Arch Klin Exp Dermatol* 1970; 238: 429-435.
21. Faergemann J, Johansson S, Bäck O, Scheynius A. An immunological and cultural study of *Pityrosporum folliculitis*. *J Am Acad Dermatol* 1986; 14: 429-433.
22. DaMert GJ, Kirkpatrick CH, Sohnle PH. Comparison of antibody responses in chronic mucocutaneous candidiasis and tinea versicolor. *Int Arch Allergy Appl Immunol* 1980; 63: 97-104.

## An Evaluation of Broad-spectrum Sunscreens against Topical PUVA-induced Erythema

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**Protection against topical PUVA with broad-spectrum sunscreens was investigated. A protection factor against topical PUVA was established for broad-spectrum sunscreens against topical PUVA-induced erythema. Key words: Photoprotection.**

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For treatment of psoriasis, Kukita et al. (1) reported that oral 8-methoxypsoralen plus ultraviolet A (UVA) chemotherapy (PUVA) was less effective for Japanese than for Caucasians. Topical PUVA or bath PUVA is therefore more common than oral PUVA in Japan

(2). It is necessary to protect the uninvolved skin from both acute harmful effects (erythema, blister) and chronic conditions (pigment freckles, premalignant or malignant skin tumors) resulting from topical or bath PUVA (3). However, the uninvolved skin of psoriatic patients is inappropriate for correctly assessing sunscreens.

In this study, we investigated protection by broad-spectrum sunscreens against topical PUVA-induced erythema in normal skin.

## SUBJECTS AND METHODS

### *Subjects*

Ten healthy Japanese males aged 23 to 26 yrs, who were receiving no medication, participated in this study, which was carried out between February and June 1988. All subjects