Seborrhoeic Dermatitis and Pityrosporum ovale: Cultural, Immunological and Clinical Studies

Ing-Marie Bergbrant



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From the Department of Dermatology,
University of Göteborg,
Göteborg, Sweden

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ABSTRACT

Seborrhoeic dermatitis is a common skin disease mainly affecting the scalp and face. The etiology of seborrhoeic dermatitis is unknown but a connection with the lipophilic yeast *Pityrosporum ovale* has been found in a number of treatment studies. *P.ovale* belongs to the normal cutaneous flora but is also an oppurtunistic pathogen. The purpose of these studies was to examine how the density of *P.ovale* changes with age, to determine the number of *P.ovale* in seborrhoeic dermatitis compared to controls, to study the immunological functions in patients with seborrhoeic dermatitis, to evaluate different methods of detecting antibodies against *P.ovale* and to describe how the patients experience their disease.

The number of *P.ovale* on clinically normal skin decreases with increasing age. In patients with seborrhoeic dermatitis, the number of *P.ovale* in lesional skin was not increased compared to healthy skin in the patients or in healthy controls.

A reduction of the skin surface lipids was seen in elderly healthy individuals. The lipid content on the skin in patients with seborrhoeic dermatitis was higher than in controls ($p=0.0001$).

Serum IgG antibodies against *P.ovale* measured with indirect immunofluorescence decreased parallel to increasing age in healthy individuals and no difference was found between patients with seborrhoeic dermatitis and healthy controls. ELISA with a *P.ovale* protein extract was the only method that demonstrated a difference in immune response between patients and controls when this method was compared with four other assays ($p=0.03$).

Immunological screening was done in 30 patients with seborrhoeic dermatitis. No major abnormalities in the humoral and local immune system were found but T-cell and NK-cell aberrations were found in several patients with seborrhoeic dermatitis. One-third of the patients had low lymphocyte stimulations with Concanavalin-A and phytohaemagglutinin and almost half of the patients had high frequencies of circulating natural killer-cells.

In a questionnaire answered by 431 patients with seborrhoeic dermatitis, we found indications that heredity, the season, mental stress and the work environment influence the disease.

The investigations suggest that the number of *P.ovale* in seborrhoeic dermatitis is of minor importance. How each individual reacts to *P.ovale* and the amount of skin surface lipids are probably of greater importance in the development of seborrhoeic dermatitis.

Key words: Age variations, cultures, antigens, serum antibodies, lipid content, immunological studies, lymphocyte surface markers, lymphocyte stimulation, heredity, work environment.

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II. Bergbrant I-M, Faergemann J. Seborrhoeic dermatitis and F *Pityrosporum ovale*: a cultural and immunological study. Acta Derm Venereol (Stockh) 1989; 69: 332-335.

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Abbreviations

ATCC	– American type culture collection
BSA	– Bovine serum albumin
CD	– Cluster of determinants
ConA	– Concanavalin-A
ELISA	– Enzyme-linked immunosorbent assay
FACS	– Fluorescence activated cell sorter
FITC	– Fluorescein-isothiocyanate
HLA	– Human leucocyte antigen group A
IIF	– Indirect immunofluorescence
MHC	– Major histocompatibility complex
NK	– Natural killer
PHA	– Phytohaemagglutinin
PBS	– Phosphate buffered saline
RAST	– Radioallergosorbent test

Introduction

A. Pityrosporum ovale

History and nomenclature

Rivolta was the first to describe yeast cells in human epidermis. He noticed double-contoured budding cells in a case of psoriasis and he named the cells *Cryptococcus psoriasis* (Rivolta, 1873). The first one to consider this fungus responsible for scalp scaling was Malassez in 1874. In 1904 Sabouraud described what he called *Pityrosporum Malassezii* in association with dandruff. Castellani and Chalmers (1913) introduced the name *Pityrosporum ovale* and succeeded in culturing the organism (1925). In 1951, Gordon isolated spherical double- contoured cells with buds attached by a narrow isthmus and he proposed the name *Pityrosporum orbiculare*. Since then there has been discussion as to whether *Pityrosporum ovale* and *Pityrosporum orbiculare* are two different species or not. Growth requirements, lipid utilisation and the optimal temperature are practicaly the same for the two species (Caprilli et al, 1973). Similarities in serum antibody response have been found using the immunofluorescent technique (Alexander, 1967; Faergemann et al 1982), the Ouchterlony gel diffusion test (Tanaka & Imamura, 1979) and immunoelectrophoresis (Bruneau & Guinet, 1983). Both forms can develop hyphae (Nazzaro-Porro et al 1977). Succesive subculturing of the species has resulted in progressive conversion from the globose through the ovoid to the cylindrical form, suggesting that *Pityrosporum ovale* and *Pityrosporum orbiculare* are different stages in a cell cycle (Salkin & Gordon, 1977). In two recently published papers, different strains have been described, based on their in vitro cultural characteristics and groupspecific antigens (Midgley, 1989; Cunningham et al, 1990). In a study using the genome comparison technique Guého and Meyer (1989) have shown that the two forms *Pityrosporum orbiculare* and *Pityrosporum ovale* are synomymous.

In this report the name *Pityrosporum ovale* will be used.

Growth requirements

P.ovale is lipophilic and requires addition of lipid substances to the culture medium for maximal growth (Roberts, 1969; Faergemann & Bernander, 1979; Leeming & Notman, 1987). Fatty acids of chain length greater than C12 are required for growth (Nazarro-Porro et al, 1976). *P.ovale* has lipoxygenase activity and is capable of peroxidating unsaturated free fatty acids and unsaturated triglycerides, cholesterol and squalene (Nazarro-Porro et al, 1976). The mycelial form of *P.ovale* which can be seen in vivo can also be produced in vitro using a medium containing cholesterol and cholesterol esters (Nazzaro-Porro et al, 1977) or glycine (Dorn & Roehnert, 1977). Hyphae can also grow on human stratum corneum pieces placed on a culture medium (Faergemann, 1989).

P.ovale is able to grow in the temperature range of 24°C to 37°C, with maximum growth between 30°C and 34°C (Caprilli et al,1973 ; Leeming & Notman, 1987). An incubation temperature of 37°C is superior to 32° for hyphae production (Faergemann, 1989).

Isolation and distribution

P.ovale is a member of the normal cutaneous flora and can be cultured from almost all adults (Roberts, 1969; Faergemann et al, 1979). The yeast can be isolated from infants (Broberg & Faergemann, 1989) and school-children (Noble & Midgley, 1978). The density of *P.ovale* increases in prepubery and puberty (Noble & Midgley, 1978).

The greatest number of *P.ovale* are found on the upper trunk, face and scalp (Roberts, 1969; Faergemann et al; 1983; Leeming et al, 1989).

P.ovale has also been isolated from blood, maxillary sinus, peritoneum, joints and lungs (Gueho et al, 1987; Oberle et al, 1981; Wallace et al, 1983; Wurtz & Knospe, 1988).

Determinations of *P.ovale* can be done by direct visualisation or cultures. Quantitative cultures using a scrub technique (Faergemann, 1984), contact plates (Faergemann, 1987) or a tape method (Wikler et al,1988) have been described.

Pathogenicity

P.ovale is not only a member of the normal human cutaneeous flora, but also the etiological agent of several diseases. In pityriasis versicolor *P.ovale* changes from the yeast form to the mycelial form under the influence of predisposing factors (McGinley et al, 1970; Faergemann & Fredriksson, 1981).

In Pityrosporum folliculitis *P.ovale* is present in the hair follicles and causes itching follicular papules and pustules (Bäck et al, 1985).

A connection between *P.ovale* and seborrhoeic dermatitis hase been clearly demonstrated in a number of treatment studies with antifungal agents (Shuster, 1984, review). Moore et al (1926) and MacLeod and Dowling (1928) have been able to reproduce lesions of seborrhoeic dermatitis by inoculating the skin of humans with *Pityrosporum* cells. It is still unknown how *P.ovale* induces the dermatitis and this will be discussed later in the text.

In patients with atopic dermatitis and especially those with head and neck distribution positive reactions to prick tests and epicutaneous tests with *P.ovale* and a beneficial effect of antimycotic treatment have been reported (Waerstedt and Hjort, 1985; Kieffer et al 1990).

Confluent and reticulate papillomatosis is a rare disease with unclear etiology. However, many patients have been treated successfully with antifungal agents and a parallel disappearance of *P.ovale* was seen (Roberts & Lachapelle, 1969, Faergemann et al, 1980).

In patients receiving intravenous fat emulsions septicemia with *P.ovale* has been reported (Dankner et al, 1987; Garcia et al, 1987; Redline et al, 1985). The yeast has also been found and considered to be the etiological agent in peritonitis (Wallace et al, 1983), sinusitis (Oberle et al, 1981), arthritis (Wurtz & Knospe, 1988) and pulmonitis (Gueho et al, 1987).

Immune reactions to *pityrosporum ovale*

Antibodies against *P.ovale* can be found in serum from both healthy individuals and patients with Pityrosporum-related diseases. Adults have significantly higher IgG antibody titres than children, measured with an indirect immunofluorescence technique (Faergeman, 1983). With ELISA and a *P.ovale* extract, no difference in IgG antibody titres has been found between elderly individuals and young adults, but a reduction in IgM antibodies was found in the elderly (Sohnle et al, 1983). In patients with pityriasis versicolor both raised (Sternberg & Keddie, 1961; DaMert et al, 1980; Wu & Chen, 1985) and normal (Faergemann, 1983) IgG antibody titres against *P.ovale* have been described. Significantly increased titres have been reported in patients with *Pityrosporum* folliculitis compared to patients with pityriasis versicolor and healthy controls (Faergemann et al, 1986). In a study of patients with dandruff, seborrhoeic dermatitis and alopecia areata, the highest titres were found in patients with the greatest amount of dandruff (Alexander, 1967). In a majority of these reports on *P.ovale* serum antibody levels in patients with *Pityrosporum*-related diseases, the IgG antibody titres have been measured with indirect immunofluorescence and whole *P.ovale* cells. Another technique has been used by Midgley and Hay (1988), who found increased titres in patients with seborrhoeic dermatitis when they used a cytoplasmatic extract and ELISA and immuneblotting.

A cell-mediated immune response to *P.ovale*

has been demonstrated in lymphocyte transformation tests in healthy individuals (Sohnle & Collins-Lech, 1980). Patients with pityriasis versicolor have a diminished lymphocyte transformation response compared to healthy individuals, which might be due to a decreased number of antigen responsive cells in peripheral blood (Sohnle & Collins-Lech, 1982). *P.ovale* can activate complement both the classical and the alternative way (Sohnle & Collins-Lech, 1983). Immediate and delayed hypersensitivity to a *P.ovale* protein extract have been found in high numbers among patients with atopic dermatitis with head and neck distribution but not in patients with seborrhoeic dermatitis (Kieffer et al, 1990).

B. Seborrhoeic Dermatitis

History

Seborrhoeic dermatitis is a well-known disease and was first described by Unna in 1887. The term refers to the oily appearance of the skin. Seborrhoeic dermatitis and dandruff are often hard to distinguish. Dandruff is defined as increased scaling in the scalp without inflammation and without skin lesions on other sites of the body. However, no clear-cut distinction can be drawn between dandruff and seborrhoeic dermatitis and we will not distingiush between the two terms. Rivolta (1873) was the first to connect yeasts with skin scaling when he described yeast cells present in scales from his beard, which was affected with sebopsoriasis. One year later, Malassez (1874) described yeast-like cells in stratum corneum and considered the organism responsible for scalp scaling. After that a number of papers have been written about the presence of the yeast in scaling disorders (Sabouraud, 1904; Unna, 1887; Ota and Huang, 1933). For many years dandruff was thought to be a disorder of hyperproliferation and the increased number of *Pityrosporum* a secondary event to increased scaling (Leyden et al, 1976). However, *P.ovale* has clearly been demonstated to be the main cause of the disease in a number of treatment studies (Gosse et al, 1969; Faergemann, 1986; Carr et al, 1987; Green et al, 1987).

Prevalence and incidence

The prevalence of seborrhoeic dermatitis has been studied in the USA and was 2.8% in the general population (Johnson & Roberts, 1977). In 19-year-old air force recruits the incidence was 8% (Garcia, 1976). In a survey of 68.400 dermatological outpatients in Scotland from 1955 to 1964, 2.4% of the patients suffered from seborrhoeic dermatitis (Ratzer, 1969). The seasonal incidence has been reported to be highest in the winter (Booken, 1968; Richter & Kleine-Natrop, 1968), but no seasonal variation was found when 20 patients were examined regulary for 15 months (Van Abbe, 1964). An increased incidence of seborrhoeic dermatitis has been found among identical twins, suggesting a genetic disposition (Niermann, 1967).

Clinical features

The disease usually starts after puberty and the incidence is highest around 40 years of age. In hospital materials the disease is more common in males than in females (Rook et al, 1986; Ratzer 1969). A dominance of women was found in a prevalence study of a sample population in the USA (Johnson & Roberts, 1977). The lesions are located in areas with high numbers of sebaceous glands such as the scalp, face and chest. The lesions are red, scaly and often sharply marginated and covered with greasy scales. The disease tends to be chronic with recurrent flare-up. Seborrhoeic dermatitis of infancy can be seen in the first months of life. The relationship to the adult type is unclear. The hormonal pattern in newborn infants resembles that of puberty (Winter et al, 1975) and that could be one explana-

tion for the increased incidence of *P.ovale* colonisation that has been described (Broberg & Faergemann, 1989).

Association with other diseases

Seborrhoeic dermatitis has been found in a high percentage of cases in two *P.ovale* related diseases, pityriasis versicolor (Faergemann et al, 1979) and *Pityosporum* folliculitis (Bäck et al, 1985; Jacinto-Jamora et al, 1991). Other diseases where seborrhoeic dermatitis occurs frequently are Parkinson's disease (Krestin, 1927; Weidman, 1930), major truncal paralysis (Wilson & Walshe, 1988), mood depression (Maietta et al, 1990) and acquired immunodeficiency syndrome (Mathes & Douglas, 1985; Berger et al, 1988).

Histopathology

The histological picture of seborrhoeic dermatitis is not diagnostic. The horny layer shows focal areas of parakeratosis occasionally containing a few pyknotic neutrophils and the epidermis shows slight to moderate acanthosis and slight to moderate spongiosis (Lever, 1990). A mild chronic inflammatory infiltrate can be found in the dermis. The histology in seborrhoeic dermatitis is similar to changes seen in psoriasis, but spongiosis is not present in psoriasis (Civatte, 1924; Pinkus, 1966).

Microbiology

The microbiology of the scalp in dandruff and seborrhoeic dermatitis has been studied extensively but there are only a few reports on the microbiology on other parts of the body in seborrhoeic dermatitis. The scalp provides a good environment for microorganisms and approximately 25 species of bacteria and 15 species of yeasts have been isolated from the scalp (Roia et al, 1963; Mackee et al, 1938 and 1939). In dandruff and seborrhoeic dermatits Pityrosporum makes up 74% and 83% respectivelyof the total microflora, compared to 46% in normal individuals (McGinley et al, 1975). The number of *P.ovale* in dandruff has been studied by direct microscopy by Van Abbe (1964), who did not find any relation between the number of *P.ovale* and the degree of dandruff, and by Ackerman and Kligman (1969) and Leyden and coworkers (1976), who found an increased number of *P.ovale* in patients with dandruff. Heng and coworkers (1990) have examined skin scrapings from facial lesions by direct microscopy and found a correlation between the density of *P.ovale* and the severity of seborrhoeic dermatitis. Direct counting of *P.ovale* in patients with seborrhoeic dermatitis and controls using a scrub method did not show any difference in number of *P.ovale* between the groups (Clift et al, 1988). Wikler and coworkers (1988) have used a tape method for quantitative cultures and found an increased number of *P.ovale* in leisonal skin in patients with seborrhoeic dermatits compared to healthy-looking skin in patients and controls.

Treatment studies

In half head experiments with yeast-inhibiting treatment with nystatin and bacteria-inhibiting treatment with tetracycline, the yeast flora was found to have a greater influence upon dandruff production than the bacteria flora (VanderWyk and Hechemy, 1967). Gosse and VanderWyke (1969) have been able to show the importanceof the presence and absence of *P.ovale* by inducing relapse of dandruff with a nystatin-resistant *P.ovale* strain in a patient who had already responded to nystatin.

Selenium sulphide and zinc pyrithione shampoos have a good effect on seborrhoeic dermatitis (Marks et al, 1985; Brauer, 1969). They have been classified as antikeratolytic agents but their mode of action has also been reported to be fun-

gicidal (Shuster, 1984; Marks et al, 1985; Butterfield et al, 1987; Van Cutsem et al, 1990). Ketoconazole, an antimycotic drug with pronnounced Pityrosporicidal activity (Van Cutsem et al, 1990), has a good effect on seborrhoeic dermatitis when administrated orally (Ford et al, 1984), as a shampoo (Carr et al, 1987; Green et al 1987; Faergemann, 1990) or as a cream (Skinner et al, 1985). Miconazole, another antimycotic drug, is also very effective in curing seborrhoeic dermatitis, the cure of the disease is paralleled by a fall in numbers of *P.ovale* (Faergemann, 1986). Miconazol was significantly better than hydrocortisone to cure seborrhoeic dermatitis, but the number of *P.ovale* was also significantly reduced by hydrocortisone (Faergemann, 1986).

The role of sebum in seborrhoeic dermatitis

Seborrhoeic dermatitis occurs in areas of skin where the highest density of sebaceous glands is found. No increase in sebum excretion rates (Burton & Pye, 1983) or sebum levels (Hodgson-Jones et al, 1953) has been found in patients with seborrhoeic dermatitis. Only minor abnormalities in the composition of skin lipids have been reported among these patients (Gloor et al, 1972; Hodgson-Jones et al, 1953). Isotretinoin, a drug which reduces sebum production, has been given to patients with seborrhoeic dermatitis and an improvement of the severity of the rash was reported (Cowley et al, 1990). Cowley and coworkers (1990) have suggested that the high incidence of seborrhoeic dermatitis among patients with neurological disorders could be explained by an increased pooling of sebum due to immobility. No evidence of neural control of the sebaceous glands has been found (Thody & Shuster, 1989). In Parkinson's disease, the sebaceous gland secretion is significantly higher compared to controls (Pochi et al, 1962) and a decrease in sebum production has been seen during L-dopa treatment (Kohn et al, 1973). In another study, improvement of seborrhoeic dermatitis was reported in 70% of patients receiving L-dopa (Glick et al, 1971).

Aims of the study

The aims of this investigation were to study:

1. The density of *P.ovale* in healthy adults of different ages and in healthy and lesional skin in patients with seborrhoeic dermatitis (I,II).
2. Serum IgG antibody levels against *P.ovale* in healthy individuals and in patients with seborrhoeic dermatitis (I,II,III,IV).
3. Skin lipid levels in healthy individuals and in patients with seborrhoeic dermatitis (I,II).
4. Immunological status in patients with seborrhoeic dermatitis (III).
5. Methods of detecting serum IgG antibodies against *P.ovale* (IV).
6. Clinical parameters of seborrhoeic dermatitis, such as age, sex, locations, age of onset, duration, continuity of symptoms, relation to work, family history and relation to other skin diseases, in patients attending a dermatology clinic (V).

Patients and control subjects

The volunteers used as healthy controls were not suffering from any skin diseases except for a few of the the older controls who had basalioma, actinic keratosis and small leg ulcers. Seborrhoeic

dermatits was defined as chronic or recurrent red lesions covered with scales and predominantly distributed on the scalp, face and upper trunk. All patients in papers I–IV were culture positive for *P.ovale* on the chest. In addition, inclusion criteria in studies I–IV were no use of topical or systemic antimycotic treatment for a week and no soap or emolient cream for 24 hours before sampling was done.

Paper I

Sixty subjects, 20 males and 40 females, with a mean age of 55 years (range 29–81) were examined. *P.ovale* cultures were taken from all individuals, serum samples from 57 and lipid measurements from 30 of the subjects.

Paper II

Thirty patients with seborrhoeic dermatitis, 24 males and 6 females, with a mean age of 42 years (range 18–80) were investigated and compared with the 60 control subjects included in study I. Concomitant diseases were seen in 4 of the patients with seborrhoeic dermatitis: ankylosing spondylitis (1), rosacea (1), pityriasis versicolor (1) and Mb Crohn (1). Cultures for *P.ovale* and serum samples were taken and lipid measurements performed in all patients.

Paper III

Immunological parameters were investigated in serum samples from 30 patients with seborrhoeic dermatitis, 24 males and 6 females, with a mean age of 46 years (range 19–81). Concomitant diseases were seen in six patients: ankylosing spondylitis (patient no. 9), diabetes mellitus (patient no. 29), rosacea (patient no. 5), *Pityrosporum* folliculitis (patients no. 25 and 29) and pityriasis versicolor (patients no. 1 and 23).

Serum samples were also taken from seven elderly (range 64–83 years) and sex-matched healthy individuals and used as controls for the oldest patients in the study population. This was done due to lack of laboratory normal range values covering this age-group.

Skin biopsies were taken from lesional and healthy-looking skin on the chest in 12 of the patients, 11 males and 1 female, with a mean age of 53.5 years (range 19–81).

Paper IV

Serum IgG antibodies against *P.ovale* were measured in 10 patients with seborrhoeic dermatitis, 8 males and 6 females, with a mean age of 56 years (range 29–80) and in 10 healthy individuals, 7 males and 3 females with a mean age of 56 years (range 28–83). All patients had suffered from the disease for more than one year and were otherwise healthy. Two scores, one based on the condition of the patient's scalp and one based on the condition of the patient's face and chest, were assigned to each patient. The scale ranged from 0 to 3, 0 being healthy, 1 mild erythema, 2 moderate erythema with scaling and papules and 3 severe erythema with scaling, papules and yellowish-brown crusts covering lesions. The average of the two scores was used as the disease severity.

Paper V

A questionnaire was mailed to the 524 patients with seborrhoeic dermatitis who had visited the Department of Dermatology at Sahlgrenska hospital in 1988. Patients under the age of 11 were excluded.

Methods

Quantitative culture of *P.ovale* (I,II)

Scrub method (I, II)

Cultures were taken from healthy skin on the chest from all all subjects in paper I and from all patients in paper II. In paper II cultures were also taken from lesions on the chest (16 pts), face (5 pts) and back (1 pat.). Other culture areas for healthy skin than the central part of the chest had to be included because many patients had lesions in that area. Cultures were taken from the scalp in 23 patients in paper II. 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 in the same skin area. On the scalp the procedure was performed once. Samples (0.1 ml) from each wash were inoculated onto a glucose-neopeptone-yeast extract agar medium containing olive oil (2%), Tween (0.2%) and glycerol monostearate (2.5 g l^{-1}). Plates were incubated at 37°C and examined after 10 days. The technique used has been described earlier (Faergemann, 1984).

Contact plates (II)

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 seconds, 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 has been described in detail earlier (Faergemann, 1987).

Lipid measurement (I, II)

Measurements were done with the Sebumeter SM 410 (Courage-Khazaka electronic, Köln, West Germany) (Dickstein et al, 1987; Schrader, 1974; Schaefer & Kuhn-Bussius, 1970). Briefly, samples were taken from the forehead skin of the volunteers and patients 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 $\mu g\ cm^{-1}$. Lipid measurements were taken from the forehead because it would then be possible to correlate our data with earlier findings.

Indirect immunofluorescens technique on sera (I, II, III, IV)

Circulating IgG antibodies against *P.ovale* were estimated as described earlier (Faergemann et al, 1982). Briefly, *P.ovale* (ATCC 42132) cells in a concentration of 10^6 cells ml^{-1} were washed in phosphate-buffered saline (PBS), pH 7.2, smeared on 10-hole Cooke's glass slides, air dried, gently heat fixed and stored at –20°C until used. Serial two-fold dilutions of serum were added to the dried cells. After incubation in a moist chamber at room temperature for 20 min, the slides were washed in PBS. In a second incubation, fluorescein isothiocyanate (FITC)-labelled sheep antihuman IgG (DAKO, Copenhagen, Denmark, lot 034, F202) was added to each hole for 20 min. After several washes with PBS the preparations were mounted in glycerol with 10% PBS, and examinated in a Zeiss fluorescence microscope. The serum dilution where a fluorescent ring surrounding the majority of the cells was still seen was recorded as the endpoint titre. In study IV the endpoint titre for each sample was divided by the endpoint titre of a pooled reference serum, WHO 67/97, and expressed as a percentage.

Lymphocyte surface markers on peripheral blood lymphocytes (III)

The surface expression of antigens on peripheral blood lymphocytes was determined by quantitative two-colour analysis with a FACS-star (Becton Dickinson, Sunnyvale, Ca, USA). 10,000 events were counted in each case. Forward and right-angle scatter gates were set on lymphocytes, excluding other leukocytes, erythrocytes and debris. Analysis was performed on unseparated heparinised blood using fluorescein isothiocyanate (FITC) or Phycoerythrin- conjugated monoclonal antibodies (Table 1).

Table 1. Monoclonal antibodies used in the study

Antibody[a] (cluster determinant)			Cellular distribution
Peripheral blood		Skin	
Leu 4	(CD3)	Leu-4	Pan T cells
Leu-2a	(CD8)	Leu-2a	"Suppressor/cytotoxic" T cells
Leu-3a	(CD4)	Leu-3a	"Helper/inducer" T cells
Leu-7	(CD57)		Natural killer cells, T and B cells
Leu-11	(CD16)		Fc IgG receptor
Leu-12	(CD19)		B cells
Leu-6	(CD1a)	Leu-6	Langerhans' cells, thymocytes
HLA-DR			HLA-DR antigens
Leu-10			HLA-DQ antigens

[a] Becton Dickinson, Ca, USA

The normal ranges for CD3, CD4, CD8 and CD19 levels and CD4/CD8 ratios were determined in a recent multicentre study including 319 healthy individuals up to the age of 70 years and using the same monoclonal antibodies as in the present study (Reichert et al, 1991). Normal values for DR expressing CD4 and CD8 cells were from the laboratory of Clinical Immunology, University of Gothenburg and the CD57/CD16 levels were compared with previously published levels (Lanier & Philips, 1986).

Lymphocyte stimulation by mitogens (III)

Mononuclear cells were prepared from heparinised venous blood by centrifugation on Ficoll-sodium metrizoate lymphoprep (Nyegard, Denmark). The cells were diluted to a concentration of $5x10^5$ cells/ml in Iscoves modified Dulbeccos medium (Gibco, Europe, Paisley, Scotland), supplemented with 10% pooled heat-inactivated human AB serum, 2 mmol/l L-glutamine and 100 μg/ml gentamicin. The mitogens were Concanavalin-A (Con-A, Miles Yeda, Rehovat, Israel) and phytohaemagglutinin (PHA, Difco Laboratories, Mi, USA). Triplicate cultures containing 0.2 ml of the lymphocyte suspension and 10 μl of 100 μg/ml mitogen were prepared on Linbro Tissue Culture grade microtitre plates and incubated at 37°C in 5% CO_2 in air for 3 days before 1 μCi of H^3-thymidine (Amersham International, UK) was added to each well. After overnight incubation, the incorporation of H^3-thymidine was determined by liquid scintilla-

tion as described (Robbins et al, 1972). The results were expressed as ratios between the counts of the patient sample and the mean count of 3 healthy blood donors analysed simultaneously (stimulation index). A normal stimulation index >0.7 was established in the Laboratory of Clinical Immunology, University of Gothenburg.

Quantification of immunoglobulin classes and subclasses (III)

Immunoglobulin isotype quantification was done by the Mancini technique. To measure IgG, IgA and IgM, heavy chain specific rabbit polyclonal antibodies (DAKO-immunoglobulins, Copenhagen, Denmark) were applied, and as reference sera we used the Behringwerke (AG, Marburg Lahn, FRG) standard human serum as well as the WHO serum 67/97. IgE antibodies were determined by RAST using comercially available Phadebas RAST kits (Pharmacia Diagnostics AB, Uppsala, Sweden). For determination of IgG subclass levels, we utilised subclass-specific monoclonal antibodies (clones JL512, GOM1, ZG4, RJ4, Unipath, Bedford, England) as previously described (Söderström et al. 1986). The WHO serum 67/97 was the reference serum, and age-related normal ranges for IgG subclasses were determined on 227 healthy adults using the same method (Söderström). Previously published normal ranges were used for IgG, IgA, IgM (Joliff et al, 1982) and IgE (Zegers et al, 1975).

Immunohistochemical staining of skin sections (III)

Three millimeter punch biopsies were taken under local anaesthesia from lesions and normal-looking skin on the chest in 12 patients. The biopsies were snap frozen in liquid nitrogen and stored at –70°. Acetone-fixed cryostat sections, 6 μm thick, were processed for the avidin-biotinylated-peroxidase complex (ABC, Vector laboratories, Burlingame, Ca, USA) technique (Hsu, 1981). The monoclonal antibodies (Table 1) Leu 4, Leu 3a and Leu 2a were diluted 1:32, Leu 6 was diluted 1:64 and anti-HLA-DR was diluted 1:128. The sections were counterstained with Mayer's haematoxylin. Omission of the primary antibodies gave no staining. Keratinocytes were considered positively stained when the full circumference of the cells was labelled. Routine haematoxylin and eosin staining was also carried out for each biopsy.

Enzyme-linked immunosorbent assay (ELISA) (IV)

An ELISA was used to measure antibodies against *P.ovale* protein extract (method 1), *P.ovale* carbohydrate extract (method 2) and *P.ovale* (ATCC 42132) whole cells (method 3). The protein extract, Solu-Prick^{R} (ALK, Hörsholm, Denmark), of *P.ovale* was prepared by extraction of the raw material under physiological conditions, as well as dialysis and freeze drying (Löwenstein, 1980). Acidic cell wall carbohydrates were purified using a modified version of the method described by Schneerson et al (1980). Briefly, the cells were suspended in hexadecyltrimethylammonium bromide and extracted with ethyl alcohol. After enzymatic treatment with DNAase, RNAase and pronase, residual protein was removed by phenol extraction and the product dialysed against CaCl2 and freezdried. The wells of Dynatech (Alexandria, Va., USA) microtitre plates (polystyrene for method 1, polyvinyl for method 2) and Titertec PVC microplates (Flow, Herts, England) for method 3 were used. The plates were coated with the extracts overnight at 4°C (10 μg ml^{-1} for protein, 50 μg ml^{-1} for carbohydrate, in PBS, pH 7.2) and with *P.ovale* cells suspended at a concentration of 10^7 cells ml^{-1} in 0.05 M carbonate buffer (pH 9.6) for three hours at 37°C (Johansson and Faergemann, 1990). After each incubation step the wells were washed with PBS with

0.05% Tween 20 (PBS-Tw). The plates coated with *P.ovale* whole cells were blocked with 20 g l^{-1} bovine serum albumin (BSA) in PBS solution by incubation overnight at 4°C. Serum dilutions were added and incubated overnight at 4°C (methods 1 and 2) or for 2.5 hours at 37°C (method 3). Alkalinephosphatase-conjugated swine (methods 1 and 2) or rabbit (method 3) antihuman IgG (Dakopatts) was added and the plates incubated for 4 hours at 30°C (methods 1 and 2) or overnight at 4°C (method 3). Finally, 4-nitrophenyl phosphate (Sigma USA), 1mg ml^{-1} in 1 M diethanolamine buffer pH 9.8 (Merck, West Germany) was added and the optical density was read on a Titertek multiscan MCC photometer (Flow, Helsinki, Finland) after 50 minutes at 405 nm. Absorbance readings were compared with those of the reference serum at the same dilution (1:800) and the percentage activity calculated. The use of different techniques for the three ELISA methods was due to preliminary results indicating that these techniques gave the most optimal results.

Fluorescence activated flow cytometry (IV)

Triplicate samples for analysis by flow cytometry (method 5) were prepared by incubating *P.ovale* cells for 30 minutes at 4°C with serum diluted 1:10. The cells were washed three times in cold PBS and centrifuged. FITC-conjugated rabbit antihuman IgG (Dakopatts) was added to the cell pellet and allowed to react for 30 minutes. The cells were washed as before and analysed immediately on a Becton Dickinson FACStar (Becton Dickinson Inc., Mountain View, Ca., USA). 10,000 events were collected and a gate was set on the population of fungal cells that was of the characteristic size and optical density of a yeast cell using the forward scattered and side scattered light measurements. Fluorescence was plotted in a single parameter histogram and the mean fluorescence determined and expressed as the percentage of the mean fluorescence of the WHO 67/97 reference serum.

Questionnaire (V)

A questionnaire with 32 questions concerning age, sex, age at onset of the disease, duration between onset of the disease and time for the questionnaire, distribution and severity of the disease, influence of the environment, physical and psychological status and family history of skin disease was mailed to 524 patients, together with an explanatory letter. If the questionnaire was not returned within a month, a reminder was sent, consisting of the questionnaire and a new letter.

Statistical methods (I, II, IV, V)

In paper I, the Newman-Keuls test was used to compare the results between the groups and multiple regression analysis to express the associations between age and various parameters measured.

In paper II, multiple regression analysis was used to compare the results between the parameters and between the patients and the controls. Correlations between the parameters were studied with Pearson's correlation coefficient analysis.

In paper IV, multiple linear regression analysis with the age factor taken into account was used to compare the response between healthy controls and seborrhoeic patients. The correlation between the methods was analysed with Pearson's correlation coefficient.

In paper V Pitman's test (Bradley, 1968) was used to test the correlation between the variables. In the special case when both variables are zero-one variables, the test coincides with Fisher's exact test. When comparing work-places, a chi-square test was applied. All test were two-tailed. A comparison between one or two of the parents and the conjugal was performed by a test variable equal to the number of partners with seborrhoeic dermatitis. The test variable was distributed as the sum of the three independent binomially distributed variables with the parameters p equal to 0.67 (when two of

three have seborrhoeic dermatitis), 0.33 (when one of three have seborrhoeic dermatitis) and 0.50 (when information about one parent is lacking and the other two have seborrhoeic dermatitis). The pairs or triplets of one partner and one or two parents where none or all suffered from seborrhoeic dermatitis did not contribute to the comparison.

Results

Quantitative cultures of *P.ovale* (I, II)

The density of *P.ovale* in 60 adult healthy individuals decreases significantly with increasing age (p=0.002,multiple regression analysis) (Fig 1).

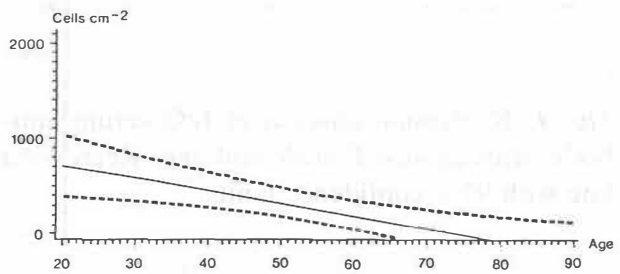


Fig. 1. Regression analysis of cultured *P.ovale* cells and age. Regression line with 95% confidence limits.

The number of organisms found on normal skin on the chest in patients with seborrhoeic dermatitis was lower than the number found on normal skin on the chest of healthy volunteers (Table 2) (p=0.013).

Many patients had lesions on the midchest area and cultures from normal skin were 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. 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 with either the scrub method or the contact plate method (Table 2). The scrub method and the contact plates showed a significant correlation between the number of *P.ovale* on healthy skin and skin lesions (scrub method, p=0.005; contact plates, p=0.0001).

Table 2. Quantitative cultures of *Pityrosporum ovale* in patients with seborrhoeic dermatitis and in healthy controls

P.ovale Mean±SD	Controls	Patients		
		Normal skin	Lesional skin	Scalp
Scrub method (colonies/cm²)	245±584 (n=60)	78±125 (n=30)	203±358 (n=22)	65±101 (n=23)
Contact plates* (colonies/plate)	ND	15±24 (n=23)	23±25 (n=17)	63±59 (n=16)

* Contact plate area 24.6 cm².

Lipid measurements (I,II)

The skin surface lipid levels on the forehead in healthy individuals decreased significantly with age (p=0.0002, regression analysis) (Fig 2).

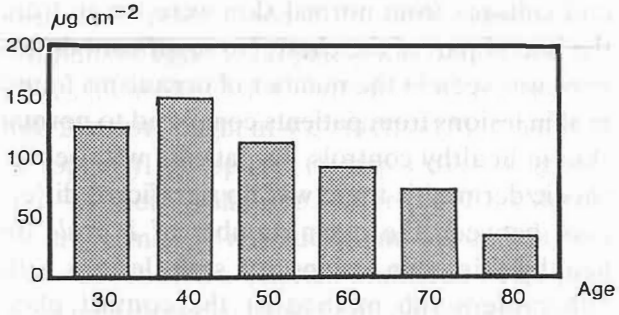


Fig. 2. Lipid content of the skin in various age groups. Five patients in each group.

The amount of lipid on the forehead skin in patients with seborrhoeic dermatitis was significantly higher (p=0.0001) (168 µ±46) than in the controls (95µ±58) (Fig 3).

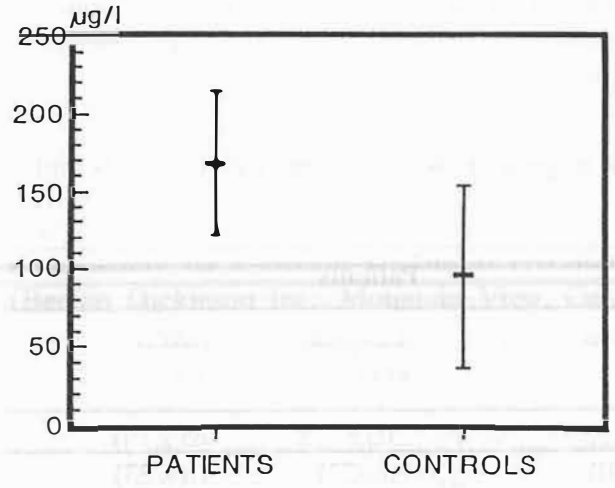


Fig. 3. Lipid content of the skin in patients with seborrhoeic dermatitis (n = 30) and healthy controls (± = 30) (mean ± SD).

In patients with seborrhoeic dermatitis, there was no correlation between the amount of lipid and the number of *P.ovale* on the skin.

Serum IgG antibodies against *P.ovale* estimated with indirect immunofluorescence technique (I, II, III, IV)

In 57 healthy individuals in various ages in paper I the anti-*P.ovale* serum antibody titres decreased with increasing age (p=0.002) (Fig 4).

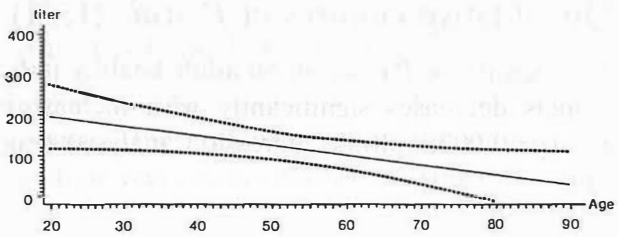


Fig. 4. Regression analysis of IgG serum antibody titres against *P.ovale* and age. Regression line with 95% confidence limits.

The mean serum IgG antibody titre against *P.ovale* in sera from the 30 patients in paper II (202±257) showed no significant difference compared to the 57 healthy individuals in paper I (11 3±136) when consideration was taken to age. There was no correlation between the titres and the number of cultured *P.ovale*. The 30 patients in paper III had a mean titre of 349±333. In paper IV 10 patients were compared with 10 healthy age and sex matched controls and no significant difference was found in antibody titres in relation to WHO serum 67/97.

Immunological parameters (III)

Lymphocytes

The absolute number of circulating lymphocytes was in all patients but two within the reference limits (1.2–3.5 x 10^9/l). Four patients showed a decrease in relative total T-cells as measured by CD3 antibodies. CD8-expressing cells, which are cells with supressor/cytotoxic effect were found in a lower than normal relative count in two patients. The ratio of T-helper/T-supressor cells (CD4/CD8) was above 0.6 in all patients, but 1.0 or lower in 10. Four patients had higher than normal ratios (Fig 5).

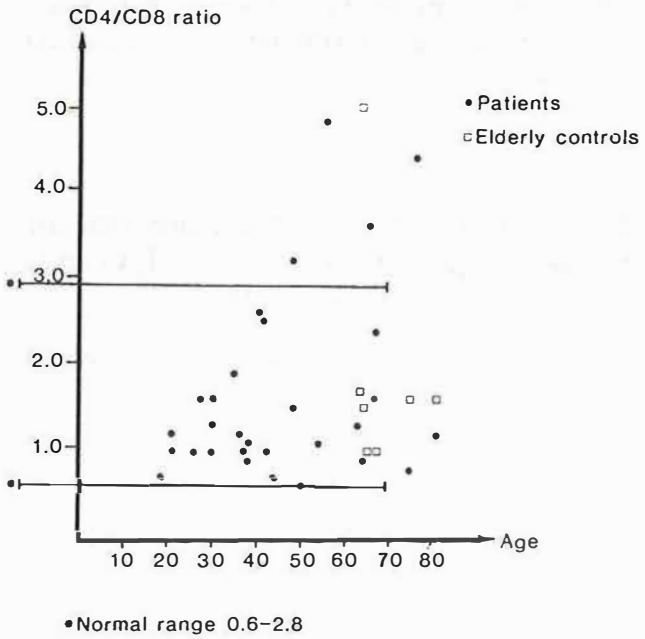


Fig. 5. Ratio of CD4 and CD8 lymphocyte surface markers on peripheral blood lymphocytes in 30 patients with seborrhoeic dermatitis and 7 elderly controls.

Low B-cell (CD19) frequencies were seen in eight patients. Cells with expression of HLA-DR were seen on more than 35% of the lymphocytes in three individuals. The HLA-DQ expression was lower than the HLA-DR in most patients. Of the three individuals with high frequencies of HLA-DR expression, one had a similarly high DQ number whereas the other two showed lower DQ than DR expression. Two patients had very high frequencies of activated CD8-positive lymphocytes (CD8;DR) and two patients showed a high proportion of activated CD4-positive cells (CD4;DR).

Cells associated with natural killer cell activity (NK-cells) measured by Leu 7 and Leu 11, were seen in high frequencies in 14 of 29 patients. Cells positive for both Leu 7 and Leu 11 were also seen.

Mitogen stimulation

The lymphocyte stimulation using ConA was low in 11 patients (Fig 6a). In six of these and in two additional patients, a low PHA stimulation was detected (Fig 6b). The low stimulations were confirmed in a second test.

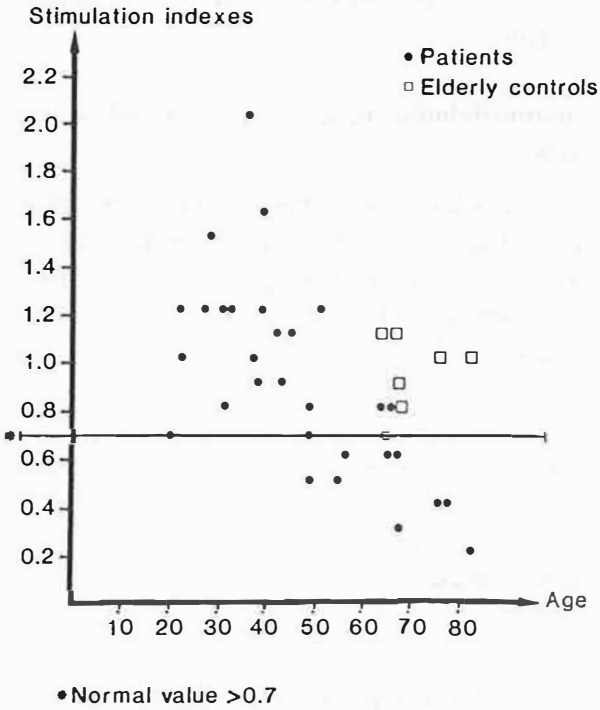


Fig. 6a. Lymphocyte stimulation by ConA in patients with seborrhoeic dermatitis and in elderly controls.

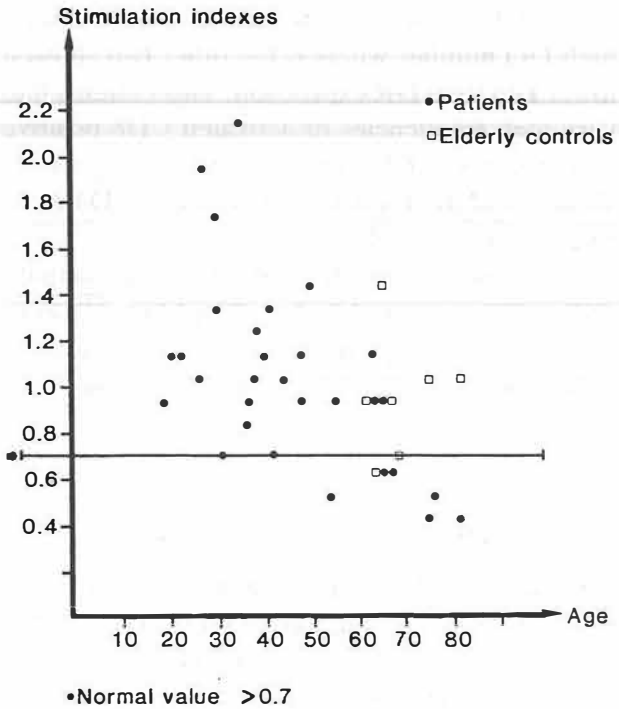


Fig. 6b. Lymphocyte stimulation by PHA in patients with seborrhoeic dermatitis and elderly controls.

Immunoglobulins in serum and IgG subclass levels

Total IgG levels were high in 14 of the 29 patients. Twelve patients showed high IgG subclass levels and two had low IgG4. IgA was higher than normal in 11 and three patients had elevated levels of IgE.

Phenotypic characterisation in situ of inflammatory cells and class II MHC antigens on keratinocytes (III)

In biopsies from lesional skin, large perivascular dermal cell infiltrates were observed in 2 of 12 patients and small to moderate dermal cell infiltrates in 7 of 12. The remaining three did not differ from normal-looking skin. The majority of the infiltrating cells reacted with anti-CD4 antibodies, whereas CD8-expressing cells were few and scattered. There was a similar number and distribution of epidermal anti-CD1a reactive cells in normal and lesional skin. A few epidermal dendritic cells were CD4 positive in the two biopsies with large and in one biopsy with moderate dermal cell infiltrates. Patchy areas of HLA-DR-expressing keratinocytes were found in two of the biopsies with moderate dermal cell infiltrates.

Evaluation of various methods and antigens for detection of serum IgG antibodies against *P.ovale* (IV)

The results obtained with the different methods are shown in Table 3.

Table 3. Comparison of *P.ovale* antibody levels between patients and controls using five different methods.

Method used	Differences between patients and controls	P-value
1. ELISA (protein extract)	–28.66	0.0256
2. ELISA (purified carbohydrate)	– 9.17	0.6037
3. ELISA (whole cells)	7.91	0.6213
4. Glass slide immunofluorescence technique	125.59	0.1773
5. Flow cytometric analysis of immunofluorescence	103.97	0.6159

The only method that demonstrated a significant difference between patients and controls was the ELISA using the protein extract as an antigen (method 1). A lower anti-protein response was observed in the seborrhoeic patients compared to healthy controls ($p = 0.0256$). Patients with the highest severity score had the lowest response. The ELISA with whole cells (method 3) and carbohydrate extract (method 2) as antigens did not show any difference between patients and controls. Neither did indirect immunofluorescence (method 4) and fluorecence activated flow cytometry (method 5) with whole *P.ovale* cells.

A correlation between the results obtained with the five different methods was found for the ELISA using whole cells and indirect immunofluorescence ($p=0.001$). A positive correlation was also found between methods 2 and 3 ($p=0.027$) as well as between methods 4 and 5 ($p=0.041$).

Questionnaire

Eightytwo per cent (431) of the patients answered the questionnaire. Of the 95 who did not answer, nine had died, eight could not be reached by post and one informed us that the initial diagnosis had been changed to contact eczema. Excluding these cases, 75 were nonresponders (14.8%).

Sex and age distribution

The age and sex distribution of the 431 patients who answered the questionnaire is shown in Figure 7. The mean age of all patients was 47.2 years (range 11–94), 46.9 years for men (range 17–94) and 47.5 years for women (range 11–92). There were more men (55%) than women.

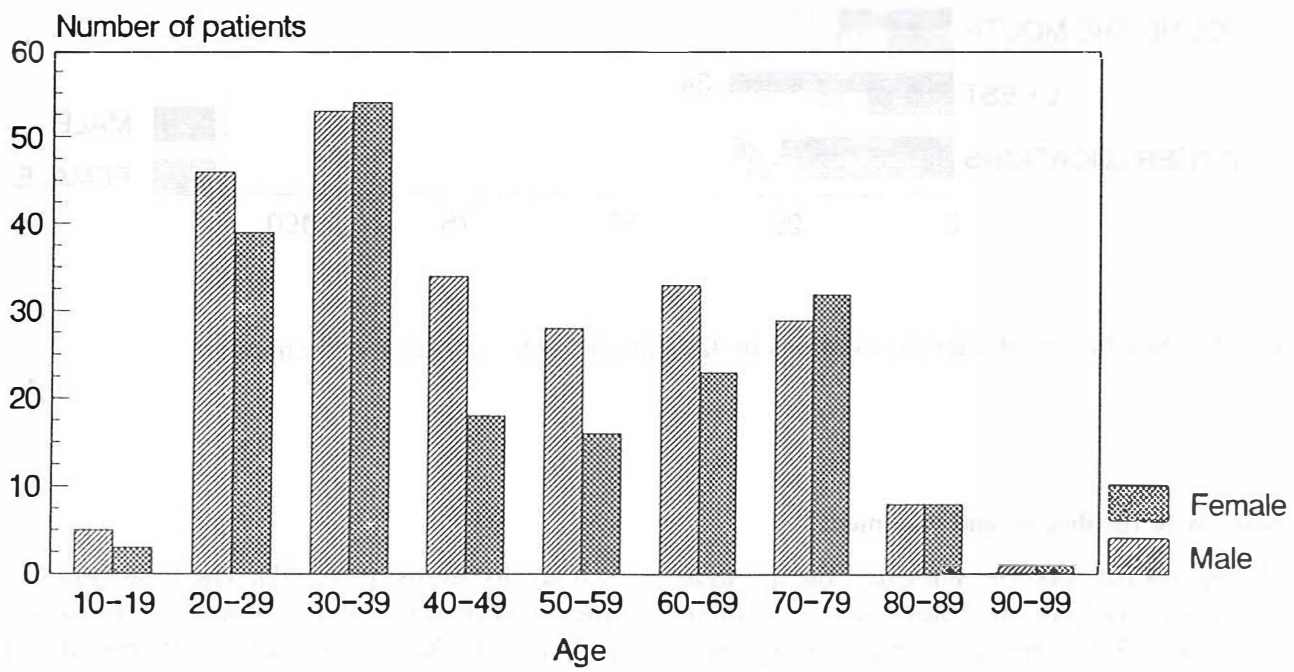


Fig. 7. Sex and age distribution of the 431 patients with seborrhoeic dermatitis included in the survey.

Time of onset and duration

The mean age at the time of onset of the disease was for the whole group 34.5 years, 33.8 years for men and 35.5 years for women. The duration from the onset of the disease to the time for the questionnaire was less than 6 years for 39%, between 6 and 10 years for 21% and more than 10 years for 40% of the patients.

Location

The sites where the skin lesions were usually located were reported by 429 of the patients. The results are shown in Figure 8. The ranking order is almost the same for females and males. The majority (86%) of the patients had lesions at more than one site. Only 20% had lesions at more than 5 sites. The 59 patients who had lesions only at one site had scalp lesions in 57 cases.

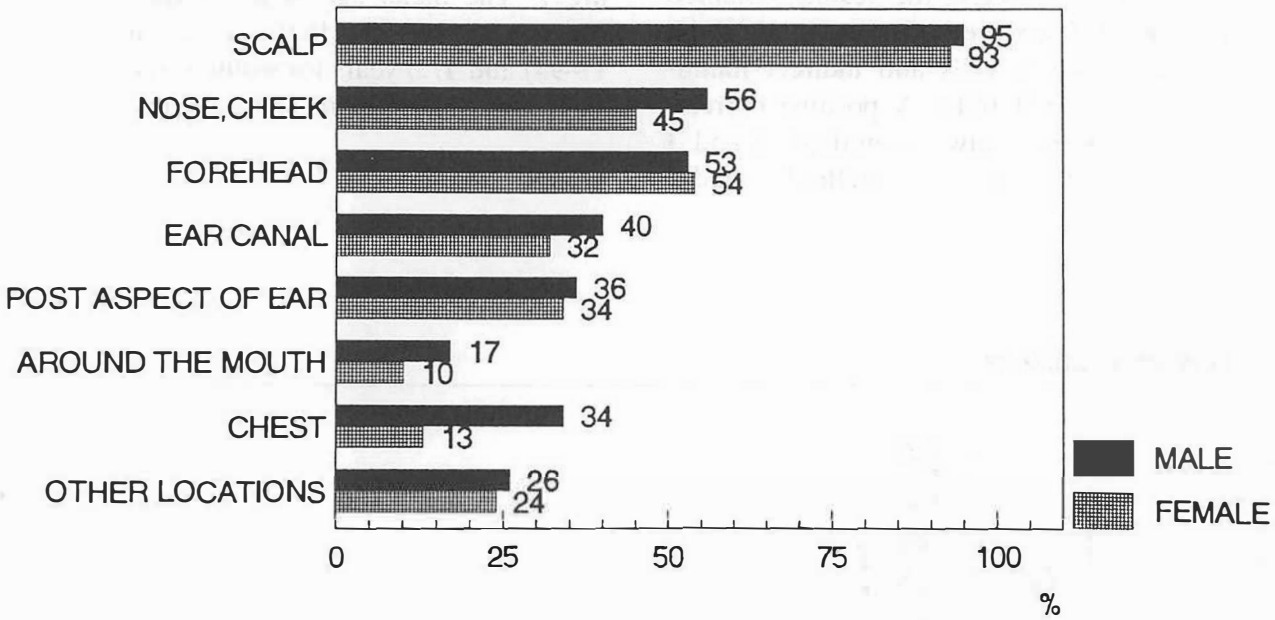


Fig. 8. Distribution of affected locations in 429 patients with seborrhoeic dermatitis.

Severity of the disease and treatment

Of the 414 patients who answered the question about for how long time they could be without symptoms, 36% reported constant symptoms, 19% were free of symptoms for a week or two at a time, 16% one month or more and 29% were without symptoms only when they received treatment. Patients with many affected locations had symptoms more often than patients with only a few locations ($p<0.001$). The treatment frequency is demonstrated in Figure 9. Fiftyeight per cent of the patients treated themselves at least every week. A significant correlation ($p<0.001$) was found between variation of the disease and frequency of treatment. The patients with constant symptoms were under treatment most often.

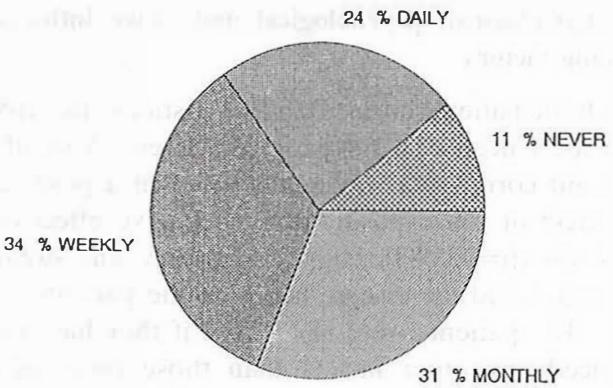


Fig. 9. The rate of treatment in 401 patients with seborrhoeic dermatitis.

The majority of the patients used more than one treatment. Medicated shampoos were used by 55%, steroid solutions by 52%, steroid creams or ointments by 35%, emolients by 32% and topical antimycotics by 31% of the patients.

Occupational distribution and influence

Of the 431 patients in this survey, 81% answered the question about where their work was located and 72% whether their skin lesions were aggravated at work. The answers are shown in Table 4.

Work in an office was most common, followed by a hospital or institution. Eightyfour per cent of the patients reported a deterioration of their disease at work. There was a significant difference between work-places with respect to proportions of deterioration at work ($p<0.05$). The most common reasons for deterioration given by the patients were stress, dry air, pollution, dust and display units.

Table 4. The influence of the working environment on seborrhoeic dermatitis.

Working environment	Number of patients	Number of patients with deterioration of their disease at work
Factory	34	13 (38%)
Office	106	24 (24%)
Hospital or institution	53	20 (38%)
School	39	7 (18%)
Outdoors	9	0
Other places	60	19 (32%)

Seasonal variation and sun exposure

A seasonal variation was experienced by 52% of the patients. Among these patients, the majority had more severe skin problems in the winter (50%) and less in the summer (77%) (Fig 10).

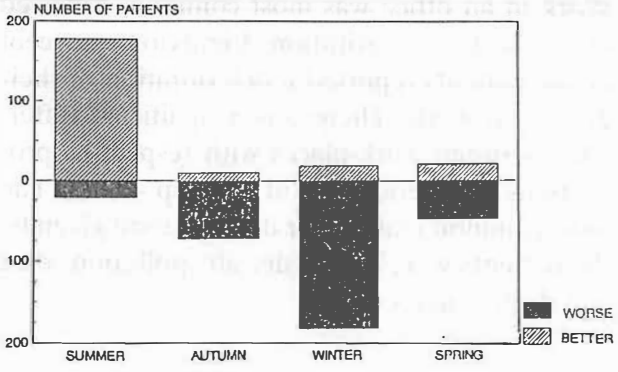


Fig. 10. Seasonal variation experienced by 225 of the 431 patients with seborrhoeic dermatitis.

The question on the effect of sun exposure was answered by 412 patients (Fig 11). Seventyfive per cent of the patients who were better in the summertime experienced a positive effect of sun exposure.

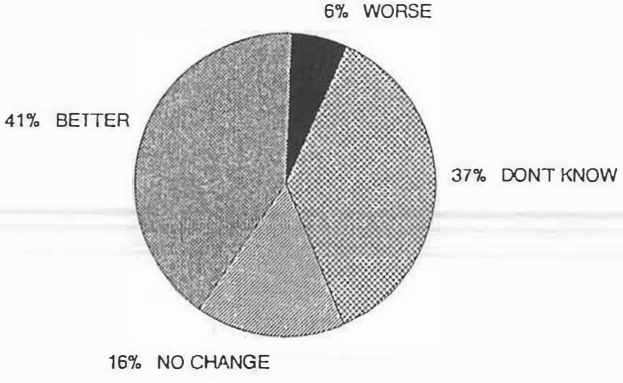


Fig. 11. Effect of sun exposure on seborrhoeic dermatitis (n=412).

Physiological, psychological and other influencing factors

Of the patients answering the questionnaire, 165 experienced a negative effect of stress. A significant correlation was found between a positive effect of sun exposure and a negative effect of stress ($p=0.0088$). High temperature and sweat aggravated the disease in 180 of the patients.

The patients were also asked if they had noticed any other factors than those previously mentioned that had a negative effect on their skin problem. A total of 64 different factors were given by the 119 patients answering this question. The most common answers were haircare products, bathing and showering, alcohol consumption, dry air and dirty hair.

Family history of seborrhoeic dermatitis

Twentyfive per cent of the patients answered that their father and/or mother had seborrhoeic dermatitis and 8% that their spouse had the disease. The probability of finding seborrhoeic dermatitis among the parents was significantly higher ($p<0.001$) compared to spouses. To futher investigate the heridity a special analysis was performed on those parents where information on both were available. A positive correlation between the presence of seborrhoeic dermatitis in the father and mother ($p<0.05$) was found.

Incidence of other skin disorders

The patients were asked whether they had suffered or were suffering from any other skin disease apart from seborrhoeic dermatitis and special attention was paid to acne, psoriasis, pityrasis versicolor and Pityrosporum folicullitis. One or more skin diseases apart from seborrhoeic dermatitis were reported by 147 patients. The skin diagnosis registered in the hospital records for the patients included in the survey was studied and a great discrepancy was found between diagnoses indicated by the patients and the diagnoses registered by the doctors. The diagnoses

most commonly indicated by the patients were acne (n=73), eczema (n=21) and psoriasis (n=18) and by the doctor skin cancer (n=29), rosacea (n=22), fungal infection (n=16), acne (n=11) and eczema (n=11). Pityriasis versicolor was registered as adiagnos for nine and Pityrosporum folliculitis for three patients.

History of atopic disease

Of the 431 patients, 18% reported asthma or hayfever and 13% childhood eczema on some occasion. Among these, 4% had both asthma or hayfever and childhood eczema. Only four of the patients in the survey had been given the diagnosis atopic dermatits in our hospital records 1988.

Incidence of internal disease

Internal diseases were reported by 155 patients and 256 indicated that they were healthy apart from seborrhoeic dermatitis. Oral medical treatment was used by 175 patients. This data is difficult to draw any conclusions from and we have only looked special at the diseases earlier connected with seborrhoeic dermatitis. Four patients were taking anti-parkinson drugs and nine patients took drugs against mood depression.

Discussion

Cultural studies

In healthy individuals we found a reduction in number of *P.ovale* parallel to an increase in age using a scrub method for quantitative cultures. However the individual variation was very high and even though the multiple regression analysis showed a significant decrease the results must be taken with caution. One of the main findings must be that the number of *P.ovale* varies greatly between different individuals.

In patients with seborrhoeic dermatitis, we found no increase in the number of *P.ovale* compared to healthy individuals or between healthy skin and lesional skin. Earlier investigations have mainly been done with direct visualisation of *P.ovale* from the scalp in patients with dandruff. In these studies, increased numbers of *P.ovale* have been found and considered to be a secondary event to increased scaling (Roia & VanderWyk, 1969; Ackerman & Kligman, 1969; Leyden et al, 1976)). Determinations of *P.ovale* in seborrhoeic lesions on other locations than the scalp have shown increased numbers using a tape method for quantitative cultures (Wikler et al, 1988) and when a method employing skin scrapings and direct microscopy was used (Heng et al, 1990). The only study where the results are in agreement with ours is the one by Clift and coworkers (1988), who used direct microscopy to determine the number of *P.ovale* at four different sites of the body and did not find any difference in number of cells between patients with seborrhoeic dermatitis and controls.

In our quantitative culture studies, we have used a scrub method as well as contact plates. The advantages of the wash and scrub method are that superficial cells as well as fungi a few cell layers down and cells from the follicles are removed and only viable cells are counted. The disadvantages are that the method is time consuming and difficult to use in the face and scalp since a flat area is needed. Contact plates or the

tape method are easy to use but only superficial cells can be counted. We think direct microscopy is difficult since the micromorpholgy varies greatly and the viability of the cells cannot be determined. Some authors prefer direct microscopy because they think it gives a more realistic estimate by including non-viable yeasts and allowing the counting of clumped organisms which would only form one colony on culture.

Skin lipid determinations

The determinations of the lipid content of the skin in healthy individuals of different ages in this study showed lower values in the elderly. It is known that the lipid content of the skin decreases in the elderly in both sexes and particulary in females (Cotteril et al, 1972; Kirk, 1948). The highest density of *P.ovale has* been found in the areas of the body where the sebum content is highest (Kirk, 1948; Faergemann et al, 1983). In patients with seborrhoeic dermatitis, we found increased levels of skin surface lipids compared to controls. We have used a new photospectrometrical method and chosen to measure the levels of skin lipids on the forehead at least one hour after the face was washed. The role of sebum in seborrhoeic dermatitis has been denied by some authors (Kligman and Leyden, 1983; Burton and Pye, 1983) because the rate of sebum excretion is not increased in patients with seborrhoeic dermatitis. However, isotretinoin has been given to patients with seborrhoeic dermatitis and the sebum excretion decreased and the rash improved (Cowley et al, 1990). Not the sebum excretion rate, but rather the residual pool of sebum might be of importance. Seborrhoeic dermatitis is common among patients with Parkinson's disease (Krestin, 1927), and an increase in surface lipids in this disease has been reported (Pochi et al, 1962; Burton et al 1973). An increased pooling of sebum due to immobility is believed to explain the high incidence of seborrhoeic dermatitis among patients with other neurological disorders (Thody & Schuster, 1989). Some abnormalities in the composition of skin lipids in patients with seborrhoeic dermatitis have been reported (Gloor et al, 1972, Hodgson-Jones et al, 1953).

Our hypothesis is that patients with seborrhoeic dermatitis have an increased amount of lipids on their skin, maybe with abnormal composition. Together with *P.ovale* lipase activity this might produce degradation products able to cause a skin reaction with inflammation and desquamation. The reaction could be toxic or mediated through the immune system.

Immunological studies

With indirect immunofluorescence (IIF) using whole cells we found decreasing IgG antibody levels against *P.ovale* parallel to increasing age. The number of *P.ovale* on the skin also decreases with increasing age. The antibody levels might just reflect the colonisation of *P.ovale* on the skin. This is also in agreement with the findings that serum antibody titres against *P.ovale* are significantly lower in children than in adults (Faergemann, 1983) and that the colonisation of *P.ovale* starts in puberty (Faergemann & Fredriksson, 1980). In patients with seborrhoeic dermatitis and healthy controls, we found no difference in antibody levels against *P.ovale* detected with IIF. Alexander (1967) has used the same technique in an investigation of patients with dandruff and found the highest titres in patients with the greatest amount of dandruff. However, changes in dandruff were not followed by changes in titres. Midgley and Hay (1988) have found significantly increased titres of IgG but not IgM antibodies in serum of patients with seborrhoeic dermatitis using a cytoplasmatic extract and ELISA and immunoblotting. Our results with IIF and *P.ovale* whole cells are not in agreement with these earlier findings. An explanation for this could be different antigen preparations and methods of detecting serum antibodies.

In our comparative study with various antigens and techniques we found that the only

method that demonstrated a significant difference between patients with seborrhoeic dermatitis and controls was the ELISA with a protein extract as antigen ($p=0.03$). The antibody levels against the protein extract were lower in patients with seborrhoeic dermatitis than in healthy controls. The results obtained with the ELISA using whole cells and IIF correlated significantly ($p=0.001$). Both these methods use whole cells as antigen. The third method using whole cells, FACS, correlated with IIF ($p=0.04$) but not with the ELISA using whole *P.ovale* cells. In flow cytometry fresh fungal cells were used and no fixation was needed. This method measures the fluorescence of each fungus and a mean value based on several thousand cells at a serum dilution 1:10 was calculated. In the other methods, the values are based on how high the concentration of serum antibodies, in a serial dilution, bound to each cell are. A positive correlation was also found between the ELISA using whole cells and the ELISA using a carbohydrate extract as antigen ($p=0.03$). One explanation could be that these antigen preparations have some antigenic determinants in common. The number of patients in our study is small and further studies are needed to evaluate this association between seborrhoeic dermatitis and serum IgG antibodies against a *P.ovale* protein extract.

The humoral immune status was studied in 30 patients with seborrhoeic dermatits. The patients were in good general health and no major dysfunction of their immune status was found. The results of the study of different methods of detecting IgG antibodies in serum gave an indication that the patients with seborrhoeic dermatitis have low levels of antibodies against a *P.ovale* protein extract. In contrast, total serum IgG and IgA levels were high in 14 and 11 of the 30 patients tested. This could be the result of a polyclonal activation in connection with the skin inflammation. High IgA levels could also be the result of antigen exposure along the gastrointestinal tract.

The CD4/CD8 ratios were normal in all patients but lower than 1.0 in 10 patients. It is of interest to note that among patients with atopic dermatitis low numbers of T- supressor cells (Zachary et al, 1983; Faure et al, 1982) and CD4/CD8 ratios around 2:1 have been reported (Lever et al, 1984). About onethird of our patients with seborrhoeic dermatitis and especially the older patients showed low responses in lymphocyte transformation tests. A positive correlation was found between age and lymphocyte stimulation with both ConA and PHA ($p<0.001$). The T-cell dysfunction among our patients could be related to age but a connection with seborrhoeic dermatitis cannot be excluded. High frequencies of cells associated with natural killer-cell activity were seen in 14 of 29 patients. The natural killer cells have a cytotoxic effect as well as an immune modulating function. NK cells can have a suppresive effect on B-cells. In patients with atopic dermatitis the number of NK cells are lower than in controls (Kusaimi et al, 1982). Interferon α has a suppresive effect on IgE production and a stimulating effect on NK cells (Roitt, 1991). A difference in interferon α levels might explain the differences between the NK findings in seborrhoeic dermatits and atopic dermatitis.

Immunohistochemical staining of skin sections was carried out in 12 patients. In nine of the twelve patients investigated perivascular cell infiltrates were observed. A predominance of CD4-positive cells was seen. T-lymphocytes of the "helper/inducer" type are known to dominate in T-cell proliferation in response to soluble antigens in in vitro experiments (Reinherz et al, 1979). The same response with an increase of T-cells with a predominance of the "helper/inducer" phenotype, has been reported for the tuberclin reaction (Scheynius & Tjernlund, 1984), which is often used as an indicator of the overall function of the cell-mediated immunity. A predominance of CD4-positive cells has also been found in skin sections from patients with pityriasis versicolor, *Pityrosporum* folliculitis, *Candida* intertrigo and dermatophytosis (Scheynius et al, 1984; Faergemann et al,1986; Johansson et al, 1986).

In fungal infections, an increase in CD1-positive Langerhans cells has been described (Faer-

gemann, 1986; Emtestam, 1985). This was not seen in our patients. In three biopsies, a few dendritic cells were CD4 positive, presumably Langerhans cells. In tinea cruris gathering of Langerhans' cells and fungal elements has been found and the epidermal Langerhans' cells are believed to be responsible for the antigen uptake in dermatophytosis (Emtestam et al, 1985). In two of our biopsies HLA-DR-positive keratinocytes were found. HLA-DR expression on keratinocytes has been described in skin disorders associated with lymphoid infiltrates in which cellular immune reactions are presumed to take place (Tjernlund, 1978; Lampert, 1984), but also in contact reactions (Scheynius at al 1984). Our results demonstrate that a cellular response is found in seborrhoeic dermatitis, but we do not know how it is induced or if *P.ovale* is involved in the induction.

A T-cell-dependent antibody response can be experimentally induced in two ways; by injection of antigen and evaluation of the delayed reaction or by application of antigens on the skin (epicutaneous test). Among patients with seborrhoeic dermatitis, prick tests with a *P.ovale* protein extract and epicutaneous tests with a *P.ovale* cell suspension did not give any positive reactions (Kieffer et al, 1990). The lack of positive epicutaneous tests among the patients with seborrhoeic dermatitis could be due to an impaired T cell function. It is also possible that patients with seborrhoeic dermatitis do not react to *P.ovale* cells but rather to degradation products derived from *P.ovale* or that the reaction is toxic rather than immune mediated.

Patient survey

Our clinical data on patients with seborrhoeic dermatitis are obtained from a postal questionnaire answered by patients with seborrhoeic dermatitis who had visited the Department of Dermatology in Göteborg 1988.

The problem with this kind of study is that we only reach those individuals who have visited a doctor at our clinic for their skin problem. The severity of the disease is very variable and individuals consulting a doctor are probably those with more severe symptoms. Therefore, no attempt is made to describe this patient group in general. This selection influences the data on sex, age, time of onset, severity and frequency of treatment and occupation. Other data like seasonal variation, location of skin lesions, influence of work and heredity are more reliable. Our intention with this study was to describe a patient group and to find factors which influence the disease. The pathogenesis of seborrhoeic dermatitis is unclear and it will be interesting in the future to study our hypothesis based on this patient survey.

In a large prevalence study in the USA, a higher number of women than men were suffering from seborrhoeic dermatitis (Johnson & Roberts, 1977). In our survey, males dominated (55%), and about the same sex distribution has been found in other hospital materials (Ratzer, 1969; Rook et al, 1986). A reason for the male dominance in hospital materials could be that men have a more severe form of seborrhoeic dermatitis or that they are more troubled by their skin lesions. Among our patients, the variation of disease was almost the same for men and woman, but men were under treatment more frequently ($p = 0{,}002$). Since this is a hospital material, selection of patients with special concern about their skin lesions was likely. Daily treatment was reported by 24% of the patients and weekly by 34%. Medicated shampoos and steroid solutions were the most frequently used treatments.

The age distribution of the patients showed a peak between 20 and 39 years of age and a plateau between 40 and 79 years of age. No conclusions can be drawn from these data since they just reflect the patients comming to the dermatological department.

The skin lesions were mainly located on the scalp, face and in and around the ears. This is in agreement with earlier findings (Rook et al, 1986). These are areas with a rich supply of sebaceous glands.

In this patient survey, we were also interested

in how the environment and psychogenic factors influence seborrhoeic dermatitis. The only environmental factor that previously has been connected with seborrhoeic dermatitis is atmosperic humidity; when the humidity was low, more patients consulted a doctor (Booken, 1968).

Seasonal influence on the symptoms was reported by 52% of the patients, with improvement in the summer and aggravation in the winter. Explanations for the improvement in the summer could be high humidity, seasonal changes in sebum excretion (Piérard-Franchimont et al, 1990), sun exposure and the fact that most people have holidays. Among the 178 patients who had less symptoms in the summer, 75% also reported a positive effect of sun exposure. A significant correlation was found between a positive effect of sun exposure and a negative effect of stress ($p=0.0088$). Altogether 41% of the patients experienced a positive effect of sunshine and 38% a negative effect of stress. UVB is known to inhibit the growth of *P.ovale* in vitro (Faergemann & Larkö, 1987), but the effect of sun-exposure on *P.ovale* in vivo is not studied. The results from the question about stress must be taken with caution. The word stress is widely used in Sweden and has a different meaning for different people. There is also a tendency in our society to blame emotional stress for physical illness.

It is interesting to note that 27% of the patients experienced aggravation of their disease at work and that a significant difference was found between different types of work-places with respect to deterioration. Explanations for the aggravation given by the patients included stress, dry air, pollution, dust and computer display units. These answers indicate that both the environment and emotional stress at work might influence the disease. The patients in this survey mainly represents people living and working in a city and the number of patients with outdoor work was low. Further data are needed to determine whether there really are special factors at work that have a negative influence on seborrhoeic dermatitis.

Seborrhoeic dermatitis among fathers and mothers was reported by 25% and spouses by 8%. The difference was significant ($p<0.001$). A positive correlation was found between the presence of seborrhoeic dermatitis in the father and mother ($p<0.05$).

These findings suggest that hereditary factors, non- genetic as well as genetic, could be involved in the pathogenesis of seborrhoeic dermatitis. Non-genetic familial factors connected with seborrhoeic dermatitis could be dietary and hygiene habits and different strains of *P.ovale*.

Seborrhoeic dermatitis has been associated with other *P.ovale*-related skin diseases such as pityriasis versicolor (Faergemann & Fredriksson, 1979) and Pityrosporum folliculitis (Bäck & Faergemann, 1985; Jacinto-Jamora et al, 1991). In the present study the number of patients registered as having these diagnoses was low, pityriasis versicolor 9 patients and *Pityrosporum* folliculitis 3. The reason why we checked the skin diagnosis reported by the patients with the hospital records was that the names of the diseases are often forgotten by the patients. Our findings do not support the idea that patients with one *P.ovale*-related skin disease are susceptible to other *P.ovale*-related diseases.

The incidence of other skin disease among our patients is difficult to evaluate without sex and age-matched controls. Rosacea was registered as a diagnosis for 5% of the patients. Our incidence of rosacea is higher than the 0.9% found among 232 patients with pityrasis versicolor (Faergemann & Fredriksson, 1979). However, if we compare only the people working in an office we find almost the same incidence of rosacea (9.8%) as in a study of 809 office employees (10%) (Berg et al, 1990).

The information given by the patients about other diseases is difficult to evaluate since the meaning of the word disease varies a lot between different individuals. An interesting finding is that 175 patients reported that they were under medical treatment but only 155 patients indicated that they were suffering from any disease. The answers to the question about the use of drugs are probably more informative and of special interest is the use of drugs for diseases con-

nected with seborrhoeic dermatitis, e.g. mental depression (Maietta et al, 1990) and Parkinson's disease (Weidman, 1930; Glick et al, 1971). In this survey, nine patients reported use of antidepressant drugs and four patients use of anti-Parkinson treatment.

Conclusions

The number of *P.ovale* decreased with increasing age among healthy individuals. In patients with seborrhoeic dermatitis, the number of *P.ovale* in lesional skin was not increased compared to their own healthy skin or the skin of healthy controls. These findings indicate that the number of *P.ovale* is of minor importance in the pathogenesis of seborrhoeic dermatitis. The way different individuals react to *P.ovale* is probably of greater importance.

The patients had increased amounts of skin surface lipids compared to healthy individuals. The size of the pool of lipids on the skin probably has a connection with the development of seborrhoeic dermatitis. It would be of interest to determine if patients with seborrhoeic dermatits have special strains of *P.ovale* and to measure the lipase activity of these strains. Toxic reactions as well as immunological responses to degradation products derived from *P.ovale* and sebum might be of importance in the pathogenesis of seborrhoeic dermatitis.

Serum IgG antibodies against *P.ovale* whole cells did not differ between healthy individuals and patients with seborrhoeic dermatitis. In a study of different methods of detecting IgG antibodies against *P.ovale* in serum, a lower response to a *P.ovale* protein extract was found among patients with seborrhoeic dermatitis compared to controls.

T-cell and NK-cell aberrations were found in serum from patients with seborrhoeic dermatits. These findings might explain the low antibody response to the *P.ovale* protein extract. However, no major abnormalities in the immune system were found and further studies are needed to clarify the role of the immune response in seborrhoeic dermatitis.

The data obtained from the patient questionnaire suggest that hereditary factors are involved in the pathogenesis of seborrhoeic dermatitis. The results also indicates that the work environment and mental stress can influence the disease.

Before we know wheter these theories are correct, we have to study the influence of the following factors: the amount and composition of skin lipids, different strains of *P.ovale,* lipase activity, specific immune reactions against *P.ovale,* heredity, the work environment and mental stress.

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