BACTERIOLOGICAL AND MYCOLOGICAL INVESTIGATIONS IN ALLERGIC CONTACT, SEBORRHOEIC AND ATOPIC DERMATITIS

by

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SUMMARY

Pityrosporum yeasts are thought to be linked to seborrhoeic dermatitis and also connected with certain patterns of atopic dermatitis. Carriage of *Staphylococcus aureus* has been associated with atopic dermatitis and correlated with disease activity. The mycological and bacteriological flora of seborrhoeic, atopic and allergic contact dermatitis patients, and normal controls, was investigated using the scrub wash technique of Williamson and Kligman for bacterial flora, the scrub wash technique and a contact plate method for *Pityrosporum* yeasts. Immunological responses to *Pityrosporum* whole cells and cytoplasmic antigen were also measured using an ELISA technique for immunoglobulin classes G, M and E in each of the patient groups and controls.

Pityrosporum yeasts were confined mainly to upper body sites and were isolated from all types of dermatitis and controls. For *Pityrosporum* isolation, the contact plate and scrub wash technique showed good agreement.

Staph. aureus was isolated from all body sites in atopic dermatitis and from clinically involved areas in allergic contact dermatitis. Virtually no *Staph. aureus* was seen in seborrhoeic dermatitis or controls.

No significant differences were found between any patient group and controls for IgG or IgM with either whole cells or antigen. With IgE, atopic dermatitis patients showed an increased response against antigen compared to controls. This IgE response was also correlated with disease severity.

These findings suggest that *Pityrosporum* yeasts are not exclusive to seborrhoeic dermatitis. Atopic dermatitis patients were the only group, however, to show

sensitivity to *Pityrosporum*.

Staph. aureus was specific to atopic and allergic contact dermatitis and, hence, does not seem to accompany any skin condition characterised by epidermal excoriation.

1. INTRODUCTION

1.1 *Pityrosporum* YEASTS

1.1.1 History and Classification

Pityrosporum was first described by Rivolta in 1873[1], and, in 1874, Malassez suggested the organism to be responsible for scalp scaling[2]. In 1904, Sabouraud considered *Malassezia ovalis*, now known as *Pityrosporum ovale*, to be responsible for seborrhoeic dermatitis[3].

The genus *Pityrosporum* or *Malassezia* comprises of lipophilic yeasts which are currently members of the heterogenous family of *Cryptococcaceae*[4]. They are probably members of the class Basidiomycetes. This is supposed since they possess such features as positive staining with Diazonium Blue B, their capacity to hydrolyse urea and their multilamellar cell wall structure[5].

There has been, and still is, great debate over the choice of genus name between *Malassezia* and *Pityrosporum*. Confusion arose between the names because it was noted that *Malassezia furfur* was present and produced filaments in the fungal disease pityriasis versicolor, whereas *Pityrosporum*, the yeast associated with seborrhoeic dermatitis, and also present on normal skin, never produced such filaments. However, it is now recognised that both organisms have a common identity[6]. Some authors prefer to use the name *Malassezia* since this term was used originally. In the present study, however, the name *Pityrosporum* will be used. This makes it easier to distinguish the different forms observed - *P. orbiculare* and *P. ovale* Forms 1, 2 and 3[7].

1.1.2 Isolation

These yeasts are found mainly on the skin of warm blooded animals, rarely being isolated from the environment[8]. The species found on human skin, constituting part of the normal cutaneous flora, requires long chain (C12 - C24) fatty acids to enable growth and are able to utilise lipid excretions of the skin. These organisms, hence, need specialised laboratory media for isolation. In humans, most yeasts are found on the upper body sites such as scalp, ear, neck and chest[9]. These sites correspond with areas containing a high number of sebaceous glands.

1.1.3 Pathogenicity

Pityrosporum yeasts are associated with a variety of diseases.

Pityriasis versicolor is a superficial yeast infection, which causes scaling of the stratum corneum. The trunk is the most common area of involvement, although occasionally, the scalp and face may also be affected. The yeasts are present as both blastospores and mycelia[10].

Pityrosporum folliculitis is characterised by erythematous pruritic papules or pustules. They are distributed across the trunk and shoulders. *Pityrosporum* yeasts can be isolated from the infected hair follicles[10].

Pityrosporum yeasts have also been linked with seborrhoeic dermatitis[11], although there is still great controversy as to whether the yeasts are, in fact, the causative factor. This will be discussed in depth later.

Pityrosporum have also been associated with conditions such as infantile seborrhoeic dermatitis[12], confluent and reticulate papillomatosis[13] and septicaemia in infants receiving long-term alimentation with fat emulsions[10].

1.1.4 Immunology

The production of antibodies to *Pityrosporum* has been found in both patients with *Pityrosporum*-related diseases and healthy subjects[14]. Many of such studies have used an indirect immunofluorescence technique to measure humoral antibody responses to *Pityrosporum*[14-16]. With this method, adults were shown to have a significantly higher IgG class response to *Pityrosporum* compared with children[17]. This may correlate with an increased frequency of exposure to the organism. Patients with pityriasis versicolor were found to have normal IgG antibody titres against *Pityrosporum*[17]. However, a significantly increased response was seen in patients with *Pityrosporum* folliculitis compared with both control subjects and patients with pityriasis versicolor[18]. When the immune responses of seborrhoeic dermatitis patients were compared with that of controls, varying results were observed. No differences were found between the two groups when an indirect immunofluorescence technique was used[15,16,19]. When a more sensitive ELISA technique was used, one study found lower IgG responses in seborrhoeic dermatitis patients[20], whereas another study found the opposite[21].

1.2 BACTERIAL SKIN FLORA

1.2.1 History and Classification

Staphylococci were first named by Ogston in the early 1880's, but it was not until 1974 that their association with atopic dermatitis was described[22].

Staphylococci and Micrococci are gram-positive cocci, 0.5-1.5μm in diameter, and belong to the family of *Micrococcaceae*.

The genus *Staphylococcus* is presently composed of 27 species. They are divided

into *Staph. aureus* which is coagulase positive, and the rest of the genus, being coagulase negative. The production of coagulase provides the ability to clot plasma and is, therefore, an extra virulence factor. It is widely used as a differential test to distinguish between *Staph. aureus* and other species of staphylococci.

The genus *Micrococcus*, at present, contains at least eight species.

Diptheroids are pleomorphic gram-positive rods which may belong to one of the genus - *Corynebacterium*, *Brevibacterium* or *Propionibacterium*.

1.2.2 Isolation

At least 18 different species of coagulase negative staphylococci have been recovered from the skin of normal subjects, including *Staph. epidermidis* and *Staph. hominis*. *Staph. epidermidis* constitutes over half of the resident staphylococci, usually colonising sites on the upper body. *Staph. aureus* is not usually found on the skin of healthy individuals.

Micrococci also colonise human skin, although at a lesser rate than staphylococci. *M. luteus* is the most common species isolated from human skin. *M. varians* and *M. lylae* may also be present.

Diptheroids make up a significant amount of the flora of normal human skin. Lipophilic diptheroids are extremely common in the axilla, while non-lipophilic strains are found more commonly on the glabrous skin.

1.2.3 Pathogenicity

Coagulase negative staphylococci are generally considered to be of low pathogenicity. In recent years, however, they have been implicated in an increasing number of

infections. These infections are mainly nosocomial since hospital staff are frequently colonised with antibiotic-resistant organisms. The most common infections are associated with indwelling foreign devices such as artificial heart valves, catheters and prosthetic joints. *Staph. epidermidis* is the major cause of such infections[23]. *Staph. aureus* produces a number of extracellular enzymes and toxins including α, β, γ and δ toxins, lipase, neuraminidase, hyaluronidase and DNAse[24], which can aid the organism in its ability to cause disease. *Staph. aureus* is responsible for a large number and variety of infections. These include boils, wound infections, staphylococcal scalded skin syndrome and toxic shock syndrome. Septicaemia and deep organ involvement can also occur. *Staph. aureus* colonisation also occurs in atopic dermatitis patients. However, it is unclear as to whether it is the primary pathogen with this disease[25]. Studies have focused on the eradication of *Staph. aureus* from these patients and, although clinical improvement was seen, the underlying skin disease was not resolved[26].

Micrococcus species are not normally considered pathogenic, but as contaminants, when isolated from clinical specimens. *M. luteus* has, on occasions, been found to be the causative organism in pneumonia, septic arthritis, septicaemia and meningitis. Other species within the genus have rarely been implicated in infections.

Diptheroids have also been found to be the causative organism in several types of infections such as septicaemia associated with indwelling foreign devices. These organisms have also been associated with such conditions as axillary odour and foot odour.

1.3 SEBORRHOEIC DERMATITIS

1.3.1 History and Epidemiology

The term "seborrhoeal eczema" was first used by Unna in 1887[27] and later by Brocq and Darier.

The prevalence of seborrhoeic dermatitis is estimated between 2-5% of the general population[28], and about 7% of dermatologic outpatients[29]. These figures, however, may be under-estimated since dandruff is possibly a minor manifestation of the disease[29]. In certain groups of patients, seborrhoeic dermatitis has a higher frequency. These include patients with mood depression[29], Parkinson's disease[29] and acquired immunodeficiency syndrome[30].

The disease is rare before puberty and is most common around forty years of age with males more usually affected. Seasonal variations are commonly observed and the incidence is highest in winter[29].

1.3.2 Clinical Features

Classical seborrhoeic dermatitis is characterised by yellowish-greasy scales covering red inflamed skin. The most commonly involved sites are the areas with a high density of sebaceous glands, namely the scalp, retroauricular area, face and upper trunk. Itching is usually moderate, although heat exposure and sweating may cause intense itching. The disease tends to be chronic with recurrent flare-ups.

1.3.3 Microbiology

Pityrosporum yeasts have received most attention in seborrhoeic dermatitis. One study showed that *Pityrosporum* makes up 46% of the total microflora in normal

scalps compared to 74% in dandruff and 83% in seborrhoeic dermatitis[31]. One study which examined skin scrapings by direct microscopy found an increase of *Pityrosporum* in patients with severe seborrhoeic dermatitis[32]. Other studies, however, using scrub wash techniques, do not agree with these findings[16,33].

1.3.4 Therapy

Treatment is usually simple, except in patients infected with HIV[29]. Treatment has to be repeated periodically due to the chronic, relapsing nature of the disease.

Topical corticosteroids have been widely used, particularly because of their non-irritant nature. However, long-term treatment with these compounds should be avoided because of the possibility of side-effects[29]. Other topical agents extensively used include selenium sulphide and zinc pyrithione. The relapse rate for these treatments is high[34]. Topical ketoconazole has been shown to be an effective therapy which is suitable for long-term use[35,36]. Oral ketoconazole should be avoided for such use due to potential problems with hepatotoxicity and interference with testosterone metabolism[35].

1.4 ATOPIC DERMATITIS

1.4.1 History and Epidemiology

This disorder was probably first described in 1808 by Willan as a prurigo-like condition[37]. In 1892, Besnier linked hayfever and asthma with atopic dermatitis and also noted a familial tendency[38]. The term "atopy" was introduced by Coca and Cooke in 1923 to describe the syndrome of asthma, hayfever and eczema[39]. The term "atopic dermatitis" was introduced in 1935 since it suggested the relationship between

the skin manifestations, asthma and hayfever[40].

The prevalence of atopic dermatitis is around 2-3% for children 1-5 years old and 0.7% for all ages. 60% of patients have onset in the first year of life and 90% within the first five years[41].

1.4.2 Clinical Features

Atopic dermatitis is a chronic, recurrent eczematous skin eruption that is itchy, erythematous, flexural and symmetric. It is frequently associated with elevated serum IgE levels and a personal or family history of atopic dermatitis, allergic rhinitis, and/or asthma[28].

Commonly affected body sites include the flexural surfaces of the wrist, the antecubital fossae, the area around the mouth and cheeks, the nape of the neck and the popliteal fossae.

1.4.3 Microbiology

Patients with atopic dermatitis display abnormal carriage of *Staphylococcus aureus*. *Staph. aureus* colonises both dermatitic and clinically normal skin in atopic dermatitis patients whereas in normal subjects, *Staph. aureus* rarely forms part of the cutaneous flora[42]. *Staph. aureus* may be able to preferentially adhere to the damaged stratum corneum of atopic dermatitis patients on the basis of binding or nutrient factors[43]. Respiratory atopics without dermatitis were shown not to have *Staph. aureus* colonisation of their skin[44].

Pityrosporum yeasts have also been found in atopic dermatitis patients, particularly in those affected in the head and neck area[45]. A prick test with an extract of

Pityrosporum is often positive in these patients[46]. This has lead to the suggestion that this group of atopics may be sensitive to *Pityrosporum*.

1.4.4 Therapy

The usual treatment of atopic dermatitis is with topical corticosteroids. Patients are also advised to avoid skin irritants, exertion and overheating[41]. Antibiotics have also been successfully used to reduce the staphylococcal load on the skin[26].

1.5 ALLERGIC CONTACT DERMATITIS

1.5.1 History and Epidemiology

Skin reaction to a chemical was noted in 1889 by Collins[47], although it is generally considered that Jadassohn first established the concept of allergic contact dermatitis in 1895[48].

Allergic contact dermatitis is wide-spread, with its incidence varying among population groups, since it is related to allergen exposure. Little is known about the prevalence of contact allergy in the general population.

1.5.2 Clinical Features

Allergic contact dermatitis is defined as inflammation of the skin characterised by erythematous papules. It usually results from skin contact with a nonprotein, low molecular weight substance or simple chemical. Common sensitizing compounds include nickel, rubber or formaldehyde. Occasionally, ingestion or injection can cause allergic contact dermatitis such as with poison ivy extract, mercury or quinine[28]. Contact dermatitis is a leading cause of industrial illness.

1.5.3 Microbiology

Staph. aureus has also been found in skin lesions in patients with allergic contact dermatitis[49]. The increased adherence of *Staph. aureus* may be by similar mechanisms to that of atopic dermatitis[25].

1.5.4 Therapy

Antibiotics have also been used to remove *Staph. aureus* colonisation[25]. However, the main emphasis on management of allergic contact dermatitis is prevention of further allergen contact and suppression of the skin eruption by the use of corticosteroids[28].

1.6 AIMS OF THE STUDY

The aims of this investigation were to study :

(1) the correlation of *Pityrosporum* isolation between two sampling methods - contact plates and the scrub wash technique. Correlation would allow the faster and more convenient contact plates to be used for extensive sampling studies.

(2) the investigation of *Pityrosporum* isolation from a wide variety of sites in seborrhoeic, atopic and allergic contact dermatitis patients and normal controls. This would determine the exclusivity of the relationship between seborrhoeic dermatitis and *Pityrosporum* yeasts.

(3) the investigation of bacterial flora, in particular *Staph. aureus*, from a wide variety of sites in atopic, seborrhoeic and allergic contact dermatitis patients and normal controls. This would determine the specificity of *Staph. aureus* to atopic dermatitis.

(4) the determination of serum antibody levels of classes IgG, M and E against *Pityrosporum* in healthy individuals and in patients with seborrhoeic, atopic and allergic contact dermatitis.

(5) bacteriophage typing of isolates of *Staph. aureus* to see if any of the classified groups were more prevalent than the others.

2. PATIENTS, MATERIALS AND METHODS

2.1 PATIENTS

All subjects were out-patients of the Dermatology Clinic at the Western Infirmary, Glasgow. Ethical Committee approval was obtained and all subjects gave informed consent. All participants were asked not to wash or apply any topical treatment during the six hours prior to sampling. Any individual who had received any antibiotics or antimycotics, either systemic or topical, in the preceding four weeks was excluded from the study. Corticosteroids had not been used for at least 24 hours before sampling. Apart from their dermatitis, patients were otherwise healthy. Patients were examined to ensure that they had only one type of dermatitis before inclusion in the study.

2.1.1 Subjects with Seborrhoeic Dermatitis

Seborrhoeic dermatitis was defined as chronic or recurrent skin lesions which were red and covered with greasy scales. The group comprised of twenty patients - ten subjects with predominantly face and head involvement, and ten subjects with trunk and limb involvement as well as facial involvement. The group was as detailed below:

Group A - Twenty subjects, eleven males and nine females, with a median age of 42 years (range 21-76).

2.1.2 Subjects with Atopic Dermatitis

Atopic dermatitis is a chronic, intermittent, inflammatory skin condition characterised by erythema, dryness, and severe pruritus. For inclusion in the study, patients had to fulfil the criteria of Hanifin and Rajka[50]. This group was made up of seventeen patients - ten subjects with dermatitis of a general distribution, and seven subjects with mainly head and neck involvement. The group was as follows:

Group B - Seventeen subjects, nine males and eight females, with a median age of 22 years (range 14-62).

2.1.3 Subjects with Allergic Contact Dermatitis

For the purposes of this study, allergic contact dermatitis was defined as inflammation of the skin characterised by erythematous papules and vesicles. All ten patients had active contact dermatitis affecting any part of the body at the time of sampling, which had been patch-test proven. These patients comprised of the group as below:

Group C - Ten subjects, four males and six females, with a median age of 61.5 years (range 21-76).

2.1.4 Control Subjects

Control subjects were healthy volunteers with no history of skin disease. In total, this group comprised of seventeen subjects, eight males and nine females, with a median age of 41 years (range 13-78).

2.2 MATERIALS

2.2.1 Media

Several different media were employed in this study. The various manufacturers' constituents and other ingredients were as follows:

Medium for the Isolation of *Pityrosporum* species

This agar was an adaptation of Dixon medium[51] modified by Midgley[7].

Malt Extract (Boots Chemists)	36.0 g/l
Mycological Peptone (Oxoid)	6.0 g/l
Purified Agar (Oxoid)	10.0 g/l
Ox-bile Desiccated (Sigma)	20.0 g/l
Tween-40 (BDH)	10.0 ml/l
Glycerol (M&B)	2.0 ml/l
Oleic Acid (BDH)	2.0 ml/l
Chloramphenicol (Sigma) stock solution	10.0 ml/l
Acti-dione (Sigma) stock solution	10.0 ml/l

The method for making each stock solution was as detailed below. All ingredients, other than the Acti-dione solution, were added to distilled water and mixed thoroughly. Sterilization was carried out at 121°C for 20 minutes. The medium was allowed to cool to approximately 56°C, the Acti-dione solution was added with a sterile pipette, and plates were poured.

Chloramphenicol Stock Solution

Chloramphenicol (Sigma)	5.0 g/l

The powder was dissolved in 95% alcohol (BDH). Five ml of this solution were added to the medium (500ml), giving a final concentration of 50μg/ml.

Acti-dione Stock Solution

Acti-dione (cycloheximide; Sigma)	50.0 g/l

The powder was dissolved in acetone (BDH). The final concentration of 500μg/ml was achieved by adding 5ml of this solution to the medium (500ml).

Blood Agar

Columbia Blood Agar Base (Oxoid):

Special Peptone	23.0 g/l
Starch	1.0 g/l
Sodium chloride	5.0 g/l
Agar No. 1	10.0 g/l

pH 7.3

39 grams of powder per litre of distilled water was weighed out, boiled and sterilized at 121°C for 15 minutes. This agar was then cooled to 50°C and 5% sterile defibrinated horse blood (Gibco) was added, mixed well and dispensed.

Cystine - Lactose - Electrolyte Deficient Medium

CLED (Oxoid) contains:

Peptone	4.0 g/l
'Lab-Lemco' Powder	3.0 g/l
Tryptone	4.0 g/l
Lactose	10.0 g/l
L-Cystine	0.0128 g/l
Bromothymol blue	0.02 g/l
Agar No. 1	15.0 g/l

pH 7.3

36.2 grams of powder per litre of distilled water was weighed out, boiled and autoclaved at 121°C for 15 minutes. The agar was cooled to 50°C and dispensed.

Nutrient Agar

The formula for this agar (Oxoid) was as follows:

'Lab-Lemco' Powder	1.0 g/l
Yeast Extract	2.0 g/l
Peptone	5.0 g/l
Sodium chloride	5.0 g/l
Agar	15.0 g/l

pH 7.4

28 grams of powder was suspended per litre of distilled water, boiled and sterilized at 121°C for 15 minutes and dispensed.

Glucose - Peptone - Chloramphenicol Agar

Glucose (BDH)	40.0 g/l
Bacteriological Peptone (Oxoid)	10.0 g/l
Purified Agar (Oxoid)	20.0 g/l
Chloramphenicol (Sigma) stock solution	10.0 ml/l

The chloramphenicol stock solution was as detailed previously. All ingredients were added to distilled water, sterilized at 121°C for 15 minutes. The medium was allowed to cool slightly and then poured.

2.2.2 Stains

A variety of stains were used during the course of this project as follows:

Lactophenol Cotton Blue Stain

Lactophenol (Hopkin & Williams)	100 ml
Cotton blue (BDH)	0.075 g

Cotton blue powder was added to lactophenol in the above quantities and allowed to stand for a few days. The resulting mixture was then filtered.

Gram Stain

The traditional Gram Stain involved four stains as detailed below:

Crystal Violet - 0.5g methyl violet (T. Gerrard & Co. Ltd) was dissolved in 100ml distilled water.

Iodine Solution - 2g potassium iodide (BDH) was dissolved in 25ml distilled water. One gram of iodine crystals (BDH) was added and the volume was then made up to 100ml in total.

Destain - absolute alcohol (95%, BDH).

Dilute Carbol Fuchsin - 0.1g basic fuchsin (BDH) was dissolved in 10ml 95% alcohol. This mixture was allowed to stand for 24 hours, then filtered and made up to 100ml volume with distilled water.

The procedure for performing a Gram Stain was as follows:

Step 1 - A drop of sterile distilled water was placed on a clean microscope slide, a small part of the colony to be tested was emulsified and allowed to air-dry. The bacterial suspension was heat-fixed by passing the slide through a bunsen burner flame.

Step 2 - Crystal Violet was flooded on to the slide and left for one minute. The slide

was then washed in running tap water.

Step 3 - Iodine solution was put on to the slide, also for one minute. Running tap water was used to wash the slide.

Step 4 - The slide was flooded with destain for a few seconds. Again, the slide was washed with running tap water.

Step 5 - Dilute Carbol Fuchsin was put on to the slide and left for 30 seconds before washing in running tap water.

Step 6 - The slide was allowed to air-dry and then examined microscopically using x100 magnification with immersion oil (BDH). Bacterial cells which were seen as purple were termed Gram-positive and those which were pink in colour were deemed to be Gram-negative.

2.2.3 Buffers

A wash buffer containing PBS and Triton X-100 was used in two different strengths, as below:

Scrub Wash Buffer

Sodium chloride (Formachem)	8.0 g/l
K_2HPO_4 (BDH)	1.21 g/l
KH_2PO_4 (BDH)	0.34 g/l
Triton X-100 (BDH)	1.0 ml/l

pH 7.9

All ingredients were added to distilled water and sterilized at 121°C for 20 minutes.

Half-strength Scrub Wash Buffer

This was prepared with the same ingredients as Scrub Wash Buffer (see above),

except that the final solution was diluted 1:1 in distilled water.

2.2.4 Materials used in Counterimmunoelectrophoresis

Sodium barbitone-Barbitone Buffer

Barbitone (Sigma)	3.44 g/l
Sodium barbitone (Sigma)	7.57 g/l
Sodium azide (Sigma)	0.5 g/l

pH 8.6

All ingredients were dissolved in distilled water.

Sodium chloride tri-Sodium citrate Buffer

Sodium chloride (Formachem)	20.0 g/l
tri-Sodium citrate (BDH)	50.0 g/l
Sodium azide (Sigma)	0.5 g/l

pH 7.2

Distilled water was used for dissolving all the ingredients.

Saline azide Buffer

Sodium chloride (Formachem)	8.5 g/l
Sodium azide (Sigma)	0.5 g/l

Both constituents were dissolved in distilled water.

Agar

High Electroendo-osmotic Agarose (Sigma)	10.0 g/l

The agarose powder was dissolved in sodium barbitone-barbitone buffer (as described previously), boiled and sterilized at 121°C for 10 minutes and aliquoted into 4ml amounts. This provided a 1% agar.

Coomassie Brilliant Blue Stain

PAGE Blue 83 (BDH)	5.0 g/l

The powder was dissolved in methanolic acetic acid (see below) providing a 0.5% solution.

Methanolic Acetic Acid

This solvent was used to dissolve the dye powder and also as a destain.

Methylated Spirits (BDH)	5 parts
Glacial Acetic Acid (BDH)	1 part
Distilled Water	5 parts

2.2.5 Materials used in Enzyme-Linked Immunosorbent Assay

Phosphate-buffered Saline with Tween

Sodium chloride (Formachem)	8.0 g/l
KH_2PO_4 (BDH)	0.34 g/l
K_2HPO_4 (BDH)	1.21 g/l
Tween 20 (Sigma)	1.0 ml/l

pH 7.2

All ingredients were added to distilled water and dissolved thoroughly.

Sodium carbonate Coating Buffer

Na_2CO_3 (BDH)	1.59 g/l
$NaHCO_3$ (BDH)	2.93 g/l
Sodium azide (Sigma)	0.2 g/l

pH 9.6

Distilled water was used to dissolve all of the ingredients.

Saline azide Buffer

Sodium chloride (Formachem)	8.5 g/l
Sodium azide (Sigma)	0.5 g/l

Both constituents were dissolved in distilled water.

Substrate Buffer

Diethanolamine (Sigma)	97.0 ml
Sodium azide (Sigma)	0.2 g/l

pH 9.8

Diethanolamine was diluted in 800ml of distilled water, pH was adjusted with 1M HCl and then made up to one litre with distilled water. The final solution was kept in the dark at 4°C.

Alkaline-phosphatase Substrate

Phosphatase substrate tablets (Sigma) were used. One 5mg tablet was dissolved in 5ml of substrate buffer (see above).

2.2.6 Antigens

All antigens used in this study were kind gifts from Dr Gillian Midgley, St. John's Dermatological Centre, St. Thomas' Hospital, London. These were as follows:

3488/A *Pityrosporum ovale* Form 2 lyophilised cytoplasmic antigen

199/A *Pityrosporum ovale* Form 3 lyophilised cytoplasmic antigen

3145 *Pityrosporum ovale* Form 3 lyophilised cytoplasmic antigen

2.2.7 Antisera

P. ovale hyperimmune rabbit antisera which had been raised using pooled antigens

199/A and 3145 (50:50) was the kind gift of Dr Gillian Shankland, Regional Mycology Reference Laboratory, Glasgow. All other serum samples were taken from the subjects (patients and controls) involved in this study.

2.2.8 Yeasts

Cultures of *Pityrosporum* used during the course of this study were kind gifts from Dr Gillian Midgley, St. John's Dermatological Centre, St. Thomas' Hospital, London. These were as follows:

GM 101, GM 104 Both *Pityrosporum ovale* Form 1

GM 207 *Pityrosporum ovale* Form 2

GM 308 *Pityrosporum ovale* Form 3

All other cultures of *Pityrosporum* yeasts were clinical isolates from subjects involved in the study.

2.3 METHODS

2.3.1 Skin Sampling Sites

A range of body sites were sampled, chosen to include those frequently affected areas for each different type of dermatitis. As described below, two different sampling techniques were performed at each skin site. Since two techniques were used at one site, directly adjacent areas of skin of similar clinical dermatitic activity were sampled. In addition, a swab was taken from the anterior nares of each subject. Nine standard body sites were examined (see Figure 1) in all subjects. In addition, two extra sampling sites - one involved area and one uninvolved area (as near to the involved area as possible) - were performed in all patients, regardless of the type of

dermatitis.

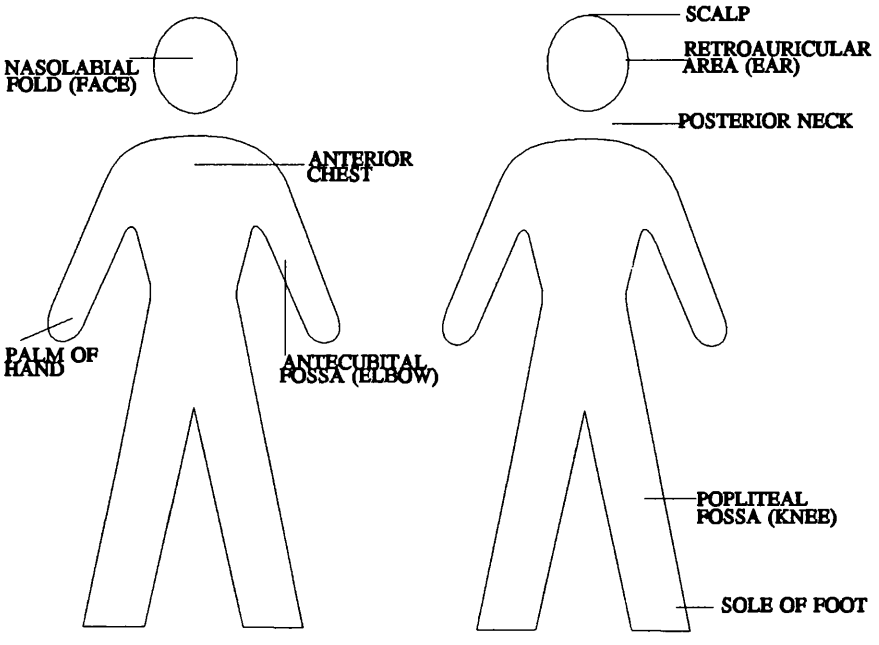


FIGURE 1: STANDARD BODY SAMPLING SITES

2.3.2 Skin Sampling Techniques

Two quantitative procedures, contact plate and scrub wash, were used to evaluate the skin flora of subjects in this study. Swabs, a qualitative method, were also taken from subjects.

Contact Plating Technique

These plates were used to examine the mycological flora of the skin at the body sites detailed above.

Sterile contact plates (Sterilin) measuring 5.7cm in diameter were used. A fifteen-ml amount of sterilized molten agar was aseptically poured into each dish. Once set, this created a slightly convex surface of area 25.5cm^2 which could be directly applied to the skin. The medium used in the plates was *Pityrosporum* Isolation medium as described earlier. This particular agar was chosen because it can support the growth of *Pityrosporum* yeasts very well and has also been reported to differentiate between the various forms of the yeast which then remain stable for several years with frequent transfers[7].

Each body site was sampled by firmly pressing the raised agar surface of the plate against the skin for a count of five. All plates were then incubated at 34°C for 14 days. The plates were subsequently examined and different types of yeasts, in particular *Pityrosporum,* distinguished initially by colony size and morphology, were enumerated and their numbers noted. One representative colony of each of the different forms was then examined microscopically using Lactophenol Cotton Blue stain (see Materials).

Midgley[7] has distinguished *Pityrosporum ovale* from *P. orbiculare*, and has also subdivided *P. ovale* into three distinct forms. In the present study, these different strains and different forms were also observed. The different forms also remained stable upon repeated sub-culture on to the *Pityrosporum* medium, at least for the duration of this project.

The strains seen here were those described by Midgley[7]:

Pityrosporum orbiculare had extremely rough, brittle and slow-growing (on this medium) colonies which are difficult to emulsify. Under microscopy, the cells (2.5μm-8μm in diameter) were spherical and formed large clumps. They produced

buds from a narrow base in comparison to the width of the cell. Cells could also produce short filaments.

Pityrosporum ovale had three variants which all differed from *P. orbiculare* by budding from a broad base. Form 1 had domed, wrinkled colonies which could have a "snowflake" appearance on the agar surface and small cylindrical cells, 2μm-4μm in length. Form 2 had smooth, dull colonies with larger "bottle-shaped" cells, 3μm-8μm long. Form 3 showed smooth, shiny colonies which were easier to emulsify and oval-shaped cells which were 2μm-6μm.

Finally, the ability of any yeast isolated to grow on Glucose-Peptone-Chloramphenicol Agar (see previously) was checked. *Pityrosporum* yeasts described above are unable to grow on any medium without a lipid source.

Using the above procedure, all organisms on the contact plates were classified into the following categories: (1) *Pityrosporum orbiculare*, (2) *Pityrosporum ovale* Forms 1, 2 or 3 and (3) other yeasts or bacteria.

For each type of *Pityrosporum* yeast, the actual number of colony forming units (CFU) per cm^2 of skin was calculated simply by dividing the number of colonies on the contact plate by 25.5 which is the area of skin sampled. This was based on the assumption that one colony on the plate arose from one single cell on the skin. For any contact plate found to have semi-confluent or confluent growth, the values of 1400 and 1800 colonies per plate were assigned, respectively.

Scrub Wash Technique (Williamson and Kligman[52])

This method was used to examine both the bacteriological and the mycological flora at each of the previously described skin sites. The same sample was used to culture for bacteria and yeasts. Sampling by this technique always required two people, one

of which being the doctor.

A teflon ring (3.8cm^2 internal area) was held firmly against the area of skin being sampled. This effectively sealed off the skin site. A one-ml volume of Scrub Wash Buffer containing Triton X-100 (as detailed earlier) was gently pipetted into the ring. A blunt teflon rod, diameter 0.5cm, was used to rub the skin gently to remove organisms adhering to the skin. After one minute, the fluid inside the ring was removed with a pipette and placed in a sterile bijou bottle. This procedure was repeated using fresh Scrub Wash Buffer. The fluid removed on the second occasion was combined with the first solution in the bijou bottle. These two successive washes removed 97-98% of the total organisms present at each skin site[52].

The teflon ring and blunt rod were disinfected between each sampling site by submerging them for one minute in a container of Presept disinfectant (Surgikos) at a concentration of 1000 parts per million. The ring and rod were then thoroughly rinsed with sterile distilled water for one minute, and then air-dried on absorbent tissue before re-use. At the end of sampling, two aliquots of the rinsing water were plated out - one on to blood agar (see Materials) and one on to *Pityrosporum* Isolation agar (see Materials) - to ensure that no carry-over of either bacteria or yeasts occurred between sites.

The amount of scrub wash fluid actually recovered from each site (initial volume being two ml) was measured with a pipette and noted. This fluid was used to examine both the bacteriology and mycology of the individual body site.

Appropriate dilutions (as described below) of fluid recovered from each site were made in half-strength Scrub Wash Buffer (see previously). The detergent, Triton X-100, helped to prevent the organisms in the wash fluid from re-aggregating.

Each sample was processed independently for the culture of yeasts or bacteria, as detailed below:

(a) Mycological Assessment

All samples, whether from control or patient, and regardless of disease grouping, were diluted 10^{-1}. A 200µl aliquot of both neat and the 10^{-1} dilution was pipetted on to *Pityrosporum* Isolation medium and spread with a bent glass rod which had been flamed using absolute alcohol (BDH) and allowed to cool. The agar plates were then incubated at 34°C for 14 days aerobically.

All colonies were identified using the same morphological and microscopic procedures as previously described for the Contact Plating Technique. The number of colony forming units (CFU) per cm^2 of skin for each type of *Pityrosporum* yeast was calculated as follows:

(1) An average of colony counts was obtained from the neat and 10^{-1} dilution plates, remembering to multiply the count from the dilution plate by 10.

(2) This average count was then multiplied by five, accounting for the aliquot cultured (200µl). This value represents the number of CFU's per one-ml of fluid.

(3) This, in turn, has to be multiplied by the amount of actual fluid recovered from the body site sampled. This assumed that the total number of organisms which were on the particular skin area sampled were now concentrated in the fluid recovered. A small amount of Scrub Wash Buffer could be absorbed into the skin, or leakage could occur from the ring during the sampling process. Leakage tended to occur at the beginning of the sampling process if the seal on the skin was not complete.

(4) The area of skin sampled was $3.8cm^2$ (the internal area of the teflon ring). Therefore, to calculate the number of colony forming units (CFU) per cm^2 of skin,

the total number of organisms obtained (at step (3) above) was divided by 3.8.

(b) Bacteriological Assessment

Different dilution series were used depending on whether the sample had been taken from a control subject or a patient, and also which type of dermatitis the patient had. These differences were because, in general, people with dermatitis, particularly atopic dermatitis, tend to have more bacteria on their skin than non-dermatitis controls[53]. Once decided for each type of subject, the same dilution series was used for samples from all body sites of that subject.

The chosen dilutions were as follows:

Control subjects - 10^{-1}, 10^{-2} and 10^{-3}

Seborrhoeic dermatitis patients - 10^{-2}, 10^{-3} and 10^{-4}

Allergic contact dermatitis patients - 10^{-2}, 10^{-3} and 10^{-4}

Atopic dermatitis patients - 10^{-4}, 10^{-5} and 10^{-6}

Aliquots of 100µl from each appropriate dilution were then pipetted on to blood agar plates (as described earlier) and spread using a bent glass rod which had been flamed using absolute alcohol (BDH) and allowed to cool. Blood agar was chosen as a good general purpose medium suitable for the growth of most organisms.

The dilution plates were incubated at 37°C for 24 hours aerobically.

Bacterial colonies which had grown were examined and the numbers of different types, as determined by colony size, morphology and pigmentation, were noted. CLED agar (see Materials) was also used if an extra discrimination step was needed since certain bacteria have clear growth characteristics on this medium. One representative colony of each type was then Gram stained (see Materials). The Gram-stained colonies were then examined microscopically using x100 magnification

with immersion oil (BDH).

Corynebacteria were seen as pleomorphic Gram positive rods occurring in angular arrangements. The cells could range from very long bacillary to short cocco-bacillary forms. It was considered satisfactory in this study to confirm the presence and number of bacteria belonging to the corynebacteria, also called diphtheroid, grouping, but not to differentiate any further.

Gram positive cocci, seen singly, in pairs, tetrads, in short chains of three or four cells or in irregular clusters, were further tested using the "Staphaurex" test (Wellcome). This was a rapid slide agglutination procedure to differentiate staphylococci which possessed coagulase and/or protein A, in particular *Staphylococcus aureus*, from staphylococci which possessed neither of these factors. The manufacturer's instructions were followed to perform this test. Briefly, a drop of latex reagent was dispensed on to the reaction card. A small amount of the culture to be tested was emulsified in the drop of reagent. The card was rotated gently for approximately 20 seconds examining for agglutination. A positive reaction was seen as clearly visible clumping of the sensitised latex particles with clearing of the milky background, which indicated the presence of *Staphylococcus aureus*. If the latex reagent did not clump, it indicated a negative result - termed coagulase negative, thus this suggested that the culture was either coagulase negative staphylococci or micrococci. Both of these classifications contain various species, but none have been suggested as important enough factors in any type of dermatitis examined in this study to warrant the time-consuming biochemical tests necessary for differentiation.

Using the procedure described above, all organisms on the blood agar plates were

classified into the following groupings: (1) *Staphylococcus aureus*, (2) coagulase negative staphylococci and micrococci, (3) corynebacteria (diphtheroids) and, (4) other bacteria or yeasts.

Counts of the bacterial numbers of each grouping per cm^2 of skin at each sample site were obtained as follows:

(1) An average of the counts for one bacterial type was obtained from the dilution plates, remembering to multiply the numbers counted by the appropriate dilution factor.

(2) The average count was multiplied by ten, allowing for the fact that only 100µl was plated out on to each dilution plate.

(3) This value was, in turn, multiplied by the actual amount of fluid recovered from the ring.

(4) The total number of each type of bacteria was divided by the area of skin sampled ($3.8cm^2$) to find out the number of colony forming units (CFU) per cm^2 of skin at each individual sample site.

Any culture of *Staphylococcus aureus* isolated was then inoculated on to nutrient agar slopes and sent off to be bacteriophage typed using the International Basic Set of *Staph. aureus* typing phages. This was performed by Mrs Amanda Wiggins of the Department of Microbiology at Gartnavel General Hospital, Glasgow.

Swab Technique

Plain swabs (Exogen) were taken from the anterior nares of each subject. Each swab was then streaked on to one *Pityrosporum* Isolation medium plate (see Materials) and one blood agar plate (see Materials). The plate containing *Pityrosporum* Isolation medium was incubated at 34°C for 14 days aerobically. Any colonies which had

grown after this time were counted and identified as previously described for the Contact Plating Technique.

The blood agar plate was incubated at 37°C for 24 hours aerobically. Any growth was dealt with in the same manner as detailed above for bacteriological assessment of the scrub wash samples.

Organisms grown on either plate were recorded as numbers obtained per swab.

2.3.3 Severity of Dermatitis

For every patient, regardless of disease type, a subjective score was given to each sample site by the doctor. The scale was as below:

0 - no dermatitis

1 - mild dermatitis

2 - moderate dermatitis

3 - severe dermatitis

Two doctors took part in scoring alternately, however, a good correlation between their estimates had been established prior to this study.

2.3.4 Blood Samples

A ten-ml amount of venous blood was taken from each subject at the time of skin sampling. The blood sample was allowed to clot and then centrifuged for ten minutes. The serum was then removed to a fresh container and frozen at -20°C until use.

2.3.5 Counterimmunoelectrophoresis (CIE)

This procedure was employed to test the reactivity of the serum samples obtained from both patients and controls against antigens 199/A (*Pityrosporum ovale* Form 3) and 3488/A (*Pityrosporum ovale* Form 2). All materials needed have been described previously and the procedure was as follows:

A four-ml aliquot of molten 1% high electroendo-osmotic agarose in sodium barbitone-barbitone buffer was poured onto a 5 x 5 cm glass slide. Once set, wells of 4mm diameter were cut 9mm apart using a well-cutter which had been flamed and cooled prior to use. Ten µl of the appropriate antibody and antigen dilutions were added to the appropriate wells of the gel. Electrophoresis was carried out in sodium barbitone-barbitone buffer with a constant voltage of 30V per slide applied for 90 minutes at room temperature. Subsequently, slides were washed overnight in sodium chloride tri-sodium citrate buffer at 45°C, then carefully rinsed under running tap water. A square of filter paper (No. 1, Whatman) was placed on the top of each gel and gently dried under a stream of hot air. The filter paper was removed by dampening slightly and peeling off. The slide was dried off again under hot air. Staining was carried out for 5-10 minutes using a 0.5% (w/v) Coomassie brilliant blue stain. Destaining of the background followed in the dye-free solvent until the lines of precipitin could be clearly seen. Finally, slides were dried off completely under a stream of hot air. The end-point of antibody titre was taken as the highest dilution of antiserum giving a visible precipitin line with the antigen dilution used. Antigens were used at a concentration of 10 and 1 mg/ml. All antigens were dissolved and diluted in saline azide. Serum samples were used neat and also diluted 1:2 in saline azide.

P. ovale hyperimmune rabbit antisera, diluted 1:8 in saline azide, was used as a positive control.

2.3.6 Enzyme-Linked Immunosorbent Assay (ELISA)

An alkaline-phosphatase indirect ELISA procedure was employed in an attempt to increase sensitivity.

A variety of ELISA procedures were performed in order to detect antibodies of IgG, IgM and IgE classes against both antigen extracts and whole cells of *Pityrosporum ovale* Form 3. This form was chosen since it was most commonly isolated from the skin of patients and controls. The materials needed have been detailed in the previous section. All samples were done in duplicate on each assay plate and every assay was performed on two separate occasions. The mean of all duplicate results was taken for statistical analysis.

Alkaline-Phosphatase Indirect ELISA Procedure using Antigen Extract

Polyvinyl chloride (PVC) microtitre plates (Sterilin Ltd.) were used. The plate was washed 3 times for 3 minutes each using phosphate-buffered saline with Tween (PBS-Tween) between every step. This was done using a well-washer (Denley). After each stage, the plate was incubated, covered with a lid, for 1 hour at 37°C on a shaking incubator (120rpm; Dynatech), except for the substrate which was incubated for 30 minutes in the dark at room temperature.

Step 1 - 30µl of *P. ovale* cytoplasmic antigen (199/A at 10mg/l) was added to 10ml of sodium carbonate coating buffer. 150µl of this solution was added to each well.

Step 2 - 150µl aliquots of the appropriate dilution of serum (as overpage), diluted in saline azide, was added to the appropriate well.

IgG Class - 10^{-2} dilution

IgM Class - 10^{-1} dilution

IgE Class - 10^{-1} dilution

These dilutions were chosen having the shown the best responses from titration curves.

Step 3 - Different conjugates (all from Sigma) were used to detect antibodies belonging to different classes as follows:

IgG Class - Rabbit anti-human IgG (F_c specific) conjugated to alkaline-phosphatase diluted in saline azide to 1:1000.

IgM Class - Rabbit anti-human IgM conjugated to alkaline-phosphatase diluted in saline azide to 1:1000.

IgE Class - Goat anti-human IgE conjugated to alkaline-phosphatase diluted in saline azide to 1:1000.

Aliquots of 150µl were added to each well.

Step 4 - Phosphatase substrate (Sigma) was dissolved in substrate buffer using two tablets for every 10ml of buffer. 150µl was added to each well.

Step 5 - The absorbance at 410nm of each well was determined photometrically by a Dynatech MR950 MicroELISA Minireader using substrate alone as a blank.

Alkaline-Phosphatase Indirect ELISA Procedure using Whole Cells

This procedure was performed similarly to that of the antigen extract, with step 1 being different. The whole cell coating method was as described by Johansson and Faergemann[54].

P. ovale Form 3 (GM 308) was grown on *Pityrosporum* Isolation medium at 34°C aerobically for four days. A suspension of whole yeast cells ($1x10^7$ cells per ml) was

then made in sodium carbonate coating buffer. 150µl of this suspension was added to each well and incubated for 3 hours at 37°C. The plate was then washed 3 times for 3 minutes using PBS-Tween. The binding of yeast cells to the solid phase was checked using an inverted microscope.

All subsequent steps were the same as for the antigen extract procedure.

2.3.7 Statistical Analysis

Statistical analysis was performed by Ms Sally Hollis of the Department of Statistics of the University of Manchester.

Bacterial and Mycological Counts

All statistical analysis was performed on the qualitative carriage of organisms. This was because a high percentage of zero counts was obtained throughout the study. Any attempt at analysing the data quantitatively would have given false results and interpretation would have been extremely difficult. In addition, the *Pityrosporum* counts were very low, so all types of this yeast were combined to give one *Pityrosporum* count before any analysis took place.

However, data on the relative percentages of the different forms of *Pityrosporum* isolated and quantitative estimates of both bacteria and yeasts for each disease and control group can be seen in Appendices 1-8.

Effect of Dermatitis

In order to assess whether the severity of dermatitis affected the bacterial or mycological isolation rates when individual patient and site effects were accounted for, a General Linear Model was fitted to incorporate all three possible effects using the package GLIM (Royal Statistical Society, London). This model was also used

to determine the relative importance of the effect of body site and between individual variation on bacterial or mycological isolation rates compared to the effect of dermatitis activity.

Comparison of Scrub Wash and Contact Plate Techniques

Qualitative comparison was made between paired samples. The M^{c}Nemar Change Test was used to establish whether either of the techniques was significantly better at picking up *Pityrosporum* yeasts than the other. A scattergram was used to examine some quantitative counts visually, but no formal analysis was performed. This was, again, because of the large numbers of zero counts obtained.

ELISA Analysis

All IgM and IgE values obtained against both antigen and whole cells were logged in order to normalise their distribution. IgG values obtained against both antigen and whole cells did not need to be logged as their distribution was already normal. The analysis then consisted of various parts:

(1) The means and 95% confidence intervals were calculated for each disease group and controls for all six assays.

(2) One-way Analysis of Variance was used to examine if there were significant differences between the means of the ELISA values in each of the disease groups and control group for each of the six assay procedures.

(3) Spearman's Rank Correlation was used to examine whether there was any relationship between specific variables and the ELISA results observed within the patient groups. The variables examined were as follows:

(a) the maximum clinical score obtained from each patient.

(b) the average clinical score across the nine standard body sites (not

including involved and uninvolved sites).

(c) the clinical score at the involved skin site.

(d) the *Pityrosporum* yeast results obtained by the Scrub Wash technique at the involved body site.

(e) the *Pityrosporum* yeast results obtained by the Contact Plate technique at the involved body site.

3. RESULTS

3.1 MYCOLOGICAL ISOLATION IN PATIENTS AND CONTROLS

3.1.1 Isolation of *Pityrosporum* Yeasts using the Contact Plating Technique

Figure 2 shows the qualitative carriage of *Pityrosporum* yeasts in each of the patient groups and the control group for all eleven sites.

In all patient groups and the control group, sites 1-4, being scalp, ear, neck and chest, respectively, and site 11, being face, had high percentages of isolation.

Patients with seborrhoeic dermatitis had a high percentage of isolation at both involved (85%) and uninvolved (70%) skin sites (sites 9 and 10, respectively), with involved skin being slightly higher. All remaining sites (5-8 inclusive) had much lower isolation rates.

Patients with atopic dermatitis had lower isolation rates at all remaining sites (5-10 inclusive).

Only one patient with contact dermatitis had a positive isolation at sites 6, 7 and 9 - hand, foot and involved sites, respectively. No contact dermatitis patient had any *Pityrosporum* isolated from sites 5, 8 and 10 - elbow, knee and uninvolved sites, respectively.

In the control group, sites 5 and 6, being elbow and hand, respectively, had higher percentages of isolation than in the patient groups. The remaining sites (7 and 8) had either no, or virtually no, positive isolations.

3.1.2 Isolation of *Pityrosporum* Yeasts using the Scrub Wash Technique

Figure 3 shows the qualitative carriage of *Pityrosporum* yeasts in each of the patient groups and the control group for all eleven sites.

In general, the percentages of isolation obtained using the Scrub Wash Technique were lower than those obtained using the Contact Plating Technique. The comparison between the two techniques will be discussed fully in section 4.5.

In all patient groups and control group, sites 1-4 (scalp, ear, neck and chest, respectively) again had high percentages of isolation. Site 11 (face), however, had lower isolation rates than with the Contact Plating Technique.

Seborrhoeic dermatitis patients, again, had high isolation rates from both involved and uninvolved skin sites, although with this technique, the uninvolved site (80%) had a higher percentage than the involved site (50%). Sites 5 and 6 had higher percentages with this technique.

The other disease groups and control group had similar isolation rates in the remaining sites with this technique.

3.2 BACTERIOLOGICAL ISOLATION IN PATIENTS AND CONTROLS

3.2.1 Isolation of *Staphylococcus aureus* using the Scrub Wash Technique

Figure 4 shows the qualitative carriage of *Staph. aureus* in each of the patient groups and the control group for all eleven sites.

There was a clear divide seen between patients with either atopic dermatitis or contact dermatitis and seborrhoeic dermatitis patients and controls.

Both the seborrhoeic dermatitis patients and the control group had virtually no isolation of *Staph. aureus* at any of the body sites sampled.

Patients with contact dermatitis had higher rates of isolation, particularly in areas of typical involvement such as hand and foot (sites 6 and 7, respectively). Site 9, being involved skin, had a 50% isolation rate whereas site 10, being uninvolved skin, had no isolation at all.

Atopic dermatitis patients had, in general, the highest percentages of isolation. The involved body site had a positive isolation of 76.5%, and the uninvolved body site had a much lower rate of 17.6%. Other typical areas of dermatitic involvement, particularly site 8 (knee), had high levels of isolation, around 40-50%.

3.2.2 Isolation of Coagulase Negative Staphylococci/Micrococci using the Scrub Wash Technique

Figure 5 shows the qualitative carriage of coagulase negative staphylococci/ micrococci in each of the patient groups and the control group for all eleven sites.

In general, high percentages of isolation were seen in all patient groups and control group in all body sites.

Patients with atopic dermatitis tended to have slightly lower levels than the other patient groups and control group.

3.2.3 Isolation of Diptheroids using the Scrub Wash Technique

Figure 6 shows the qualitative carriage of diptheroids in each of the patient groups and the control group for all eleven sites.

The isolation percentages of diptheroids were generally lower than the levels of coagulase negative staphylococci/micrococci.

The control group was seen to have higher levels than in all patient groups.

3.3 RELATIONSHIP BETWEEN ISOLATION RATES AND CLINICAL STATE

3.3.1 Mycological Isolation

The percentages of positive isolation for *Pityrosporum* yeasts obtained using the two techniques can be seen in Table 1.

There was no relationship observed between increasing clinical severity and the percentage of positive isolation with either of the techniques. In fact, the control group had a high percentage of isolation, similar to the patient groups, with both techniques.

3.3.2 Bacterial Isolation

The percentages of positive isolations for all the bacterial groupings obtained using the Scrub Wash Technique are listed, again, in Table 1.

Staph. aureus showed a very obvious increase in the percentage of isolation, with skin sites showing no dermatitic symptoms (score 0) having 7.0% isolation and skin sites severely affected by dermatitis (score 3) showing 74.1% isolation. The control group showed only 1.6% isolation.

The other bacterial groupings showed no relationship between clinical score and percentage of isolation. In both groupings, the control group had similar isolation rates to the patient groups.

3.4 CORRELATION BETWEEN MYCOLOGICAL OR BACTERIAL ISOLATION AND PATIENT GROUPS

Statistical analysis was performed using the package GLIM, and the summary of results obtained are shown in Table 2. These results were interpreted with the aid

of Figures 2-6.

Factors were fitted sequentially so each factor had been adjusted for all previous factors.

Firstly, for each organism, the nine standard body sites were examined. This analysis looked at whether there was any difference in rates of isolation from the different body sites sampled. All organisms, except *Staph. aureus*, showed a significant difference ($p < 0.0001$) in percentages of isolation from the sampling sites.

Staph. aureus was isolated, almost exclusively, from patients with either atopic or contact dermatitis. In these patient groups, particularly the atopic group, site was not very relevant to the percentage of isolation.

For the other bacterial groupings and *Pityrosporum* (isolated by either technique), the isolation rates varied depending on the body site being sampled.

The next factor examined was patients versus controls, after any site differences had been adjusted for. All bacterial groupings showed significant differences between patients and controls. *Pityrosporum* isolation, however, did not show any significant differences between patients and controls with either of the techniques.

Severity of dermatitis was examined, after correcting for both site and patients vs. controls. This analysis split the patient groups and controls into four categories according to the clinical scores obtained from the body sites sampled - these categories being score 0, 1, 2 or 3, and controls. A significant difference was seen with *Staph. aureus* ($p < 0.0001$) and this was confirmed by the isolation percentages seen in Table 1.

Pityrosporum yeasts, isolated by the Contact Plating Technique, only just reached significance at the 5% level ($p = 0.0450$).

No other significant differences were seen when examining the severity of dermatitis. Lastly, the analysis looked at any differences between the patient groups after all of the previous factors had been taken into account. Significant differences were seen with all bacterial groupings and *Pityrosporum* (from either technique).

3.5 CORRELATION BETWEEN THE SCRUB WASH AND CONTACT PLATING TECHNIQUES FOR THE ISOLATION OF *Pityrosporum* YEASTS

3.5.1 Correlation of the Two Techniques using the M°Nemar Change Test

Table 3 shows the comparison of isolation rates by the two techniques.

In four of the eleven body sites - scalp, ear, involved and face, the Contact Plating Technique was significantly better at picking up *Pityrosporum* yeasts than the Scrub Wash Technique. There was no significant difference observed in any of the remaining sites, although the contact plate had higher percentages of isolation in every site, except the uninvolved body site.

3.5.2 Quantitative Counts Obtained by the Two Techniques

Figure 7 shows the counts obtained with either the Scrub Wash or Contact Plating Technique when the other one was negative at the same body site.

The scattergram clearly shows that the vast majority of counts from the contact plate when the scrub wash was negative were less than one CFU per cm^2 of skin. However, the counts from the scrub wash when the contact plate was negative were all greater than one CFU per cm^2 of skin.

3.5.3 Scrub Wash Fluid Recovered from Sampling

Figure 8 shows the amount of fluid recovered from the 2ml sample applied to the skin.

This shows that the majority of recovered samples contained 1.7ml of wash fluid, in fact, 72% of samples measured between 1.6ml and 1.8ml, inclusive. An exception to this was samples obtained from the scalp. Most of these samples only contained 1.3ml of wash fluid.

3.6 ISOLATION FROM NOSE SWABS

Table 4 gives the percentages of each organism isolated from the nose swabs taken from all patient groups and controls.

Staph. aureus had a 94.1% isolation from patients with atopic dermatitis in contrast to 52.9% obtained from controls.

Patients with contact dermatitis had a *Staph. aureus* nasal carriage of 50.0%.

Seborrhoeic dermatitis patients had a carriage rate of 30.0%.

All patient groups and controls, except the atopic group, had high percentages of coagulase negative staphylococci/micrococci around 90-95%. The atopic dermatitis patients had a lesser carriage rate of 41.2%.

The nasal carriage rates of diptheroids were generally much lower, with the highest of 35.0% being from patients with seborrhoeic dermatitis.

The *Pityrosporum* nasal carriage rates varied with, perhaps surprisingly, the lowest of 5.0% being from only one of the 20 seborrhoeic dermatitis patients.

3.7 BACTERIOPHAGE TYPING OF ISOLATES OF *Staph. aureus*

Tables 5-8 show the phage types of *Staph. aureus* obtained from all of the patient groups and controls.

No clear pattern emerged from the analysis, with the isolates from the patient groups and the control group having a different spread amongst the phage groups.

Table 9 demonstrates that the majority of patients, regardless of the type of dermatitis, and controls tended to have only one phage group present on their skin at the time of sampling.

Table 10 shows that, if *Staph. aureus* is present on the body, it tends to be of the same phage type as the nasal isolate.

3.8 IMMUNOLOGICAL ANALYSIS

3.8.1 Counterimmunoelectrophoresis

This procedure did not reveal precipitin lines with any of the sera tested from patients or controls using either of the *P. ovale* antigens. This technique was therefore not sensitive enough to detect any antibodies to *P. ovale* antigens in any of the sera obtained from patients or controls.

3.8.2 ELISA Analysis of Patients and Controls

The antibody values of immunoglobulin classes G, M and E against *P. ovale* cytoplasmic antigen or whole cells for each of the patient groups and control group are illustrated in Figures 9-11.

Table 11 describes the results from the one-way analysis of variance of the antibody values displayed in these figures.

There was no significant difference observed between any of the patient groups or control group for IgG against *P. ovale* antigen or whole cells. The means and 95% confidence intervals for each of the patient groups and controls showed similar trends with both antigen and whole cells.

Again, there was no significant difference seen between any of the patient groups or control group for IgM against *P. ovale* antigen or whole cells. With this antibody class, however, some differences in response were seen between antigen and whole cells for each of the patient groups and control group. The IgM values against antigen had a much bigger spread than against whole cells. The geometric means were mainly lower against *P. ovale* antigen.

The analysis of IgE values against antigen showed that patients with atopic dermatitis mounted a significantly increased response compared to other patient groups and control group. Interestingly, this finding was not observed with IgE values against whole cells, with this analysis showing no significant difference between patient groups or controls. The values obtained against whole cells from patients with atopic dermatitis were very similar to the values obtained against antigen. However, the values from the other patient groups and, particularly the control group, were higher against whole cells than against antigen.

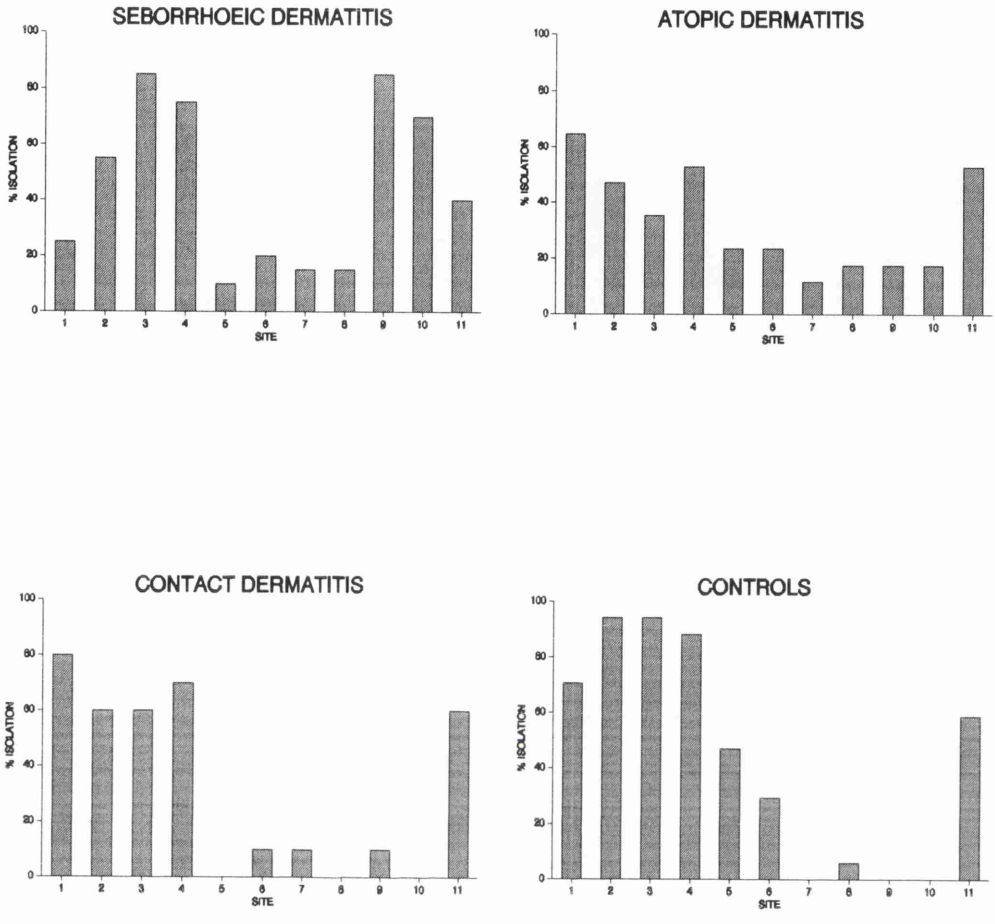
3.8.3 Correlation of ELISA Results with Severity of Dermatitis and *Pityrosporum* Isolation

Tables 12-14 show the correlations between antibody values of immunoglobulin classes G, M and E with clinical scores and *Pityrosporum* isolation rates obtained from each of the patient groups.

For patients with seborrhoeic dermatitis, no significant correlations were found with clinical score or *Pityrosporum* isolation rates. However, some evidence of a relationship ($p < 0.10$) was seen with the clinical scores averaged across all body sites. These relationships were all negative. Some evidence of a relationship was also seen with *Pityrosporum* isolation from the involved body site using the Contact Plating Technique. Again, this relationship was negative.

With the atopic group, significant correlations were found with IgE values against antigen for all measures of clinical score. These correlations were all positive. No correlations were seen with the isolation of *Pityrosporum.*

Contact dermatitis patients showed a significant correlation between the clinical score averaged across all body sites and the IgM response to *P. ovale* whole cells. This correlation was again positive. No other significant correlations were observed, either with the other measures of severity of dermatitis or with *Pityrosporum* isolation.

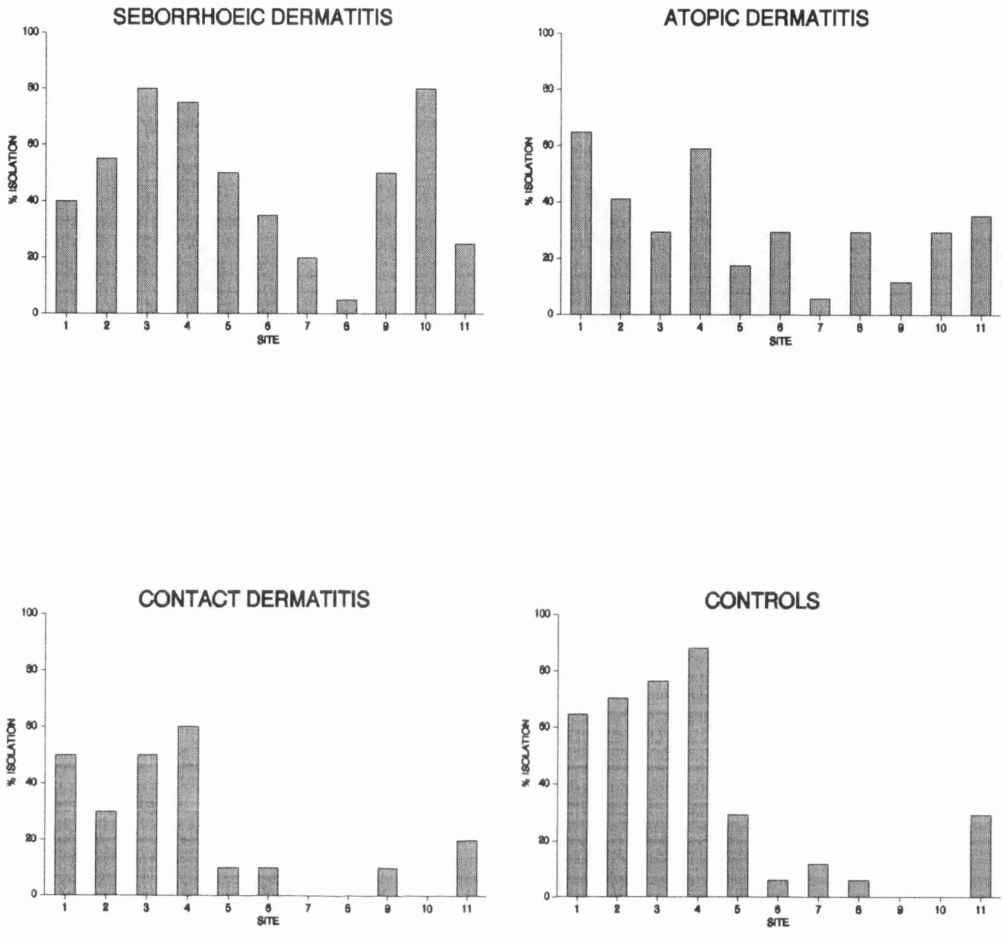


KEY:

Site 1 Scalp	Site 5 Elbow	Site 9 Involved
Site 2 Ear	Site 6 Hand	Site 10 Uninvolved
Site 3 Neck	Site 7 Foot	Site 11 Face
Site 4 Chest	Site 8 Knee	

FIGURE 2: QUALITATIVE CARRIAGE OF *Pityrosporum* YEASTS OF PATIENTS AND CONTROLS AS DETERMINED BY THE CONTACT PLATE TECHNIQUE.

PERCENTAGE OF EACH BODY SITE SAMPLED WITH POSITIVE *Pityrosporum* CARRIAGE.

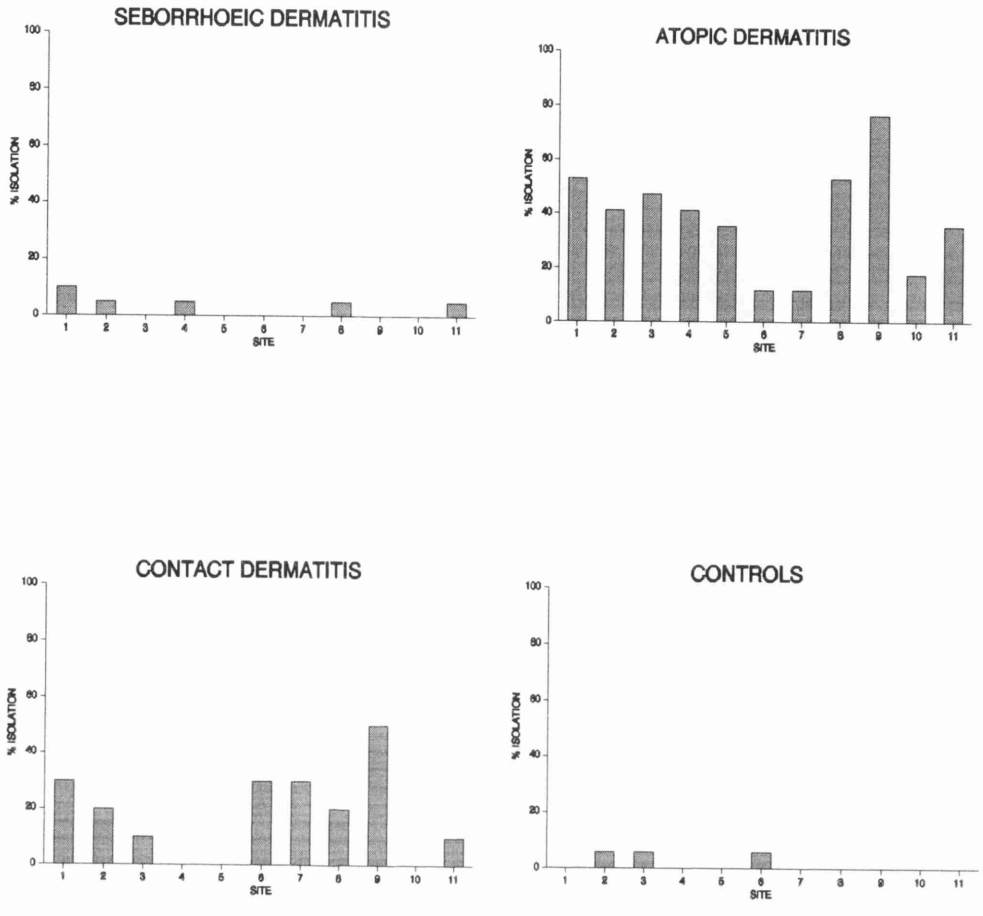


KEY:

Site 1 Scalp	Site 5 Elbow	Site 9 Involved
Site 2 Ear	Site 6 Hand	Site 10 Uninvolved
Site 3 Neck	Site 7 Foot	Site 11 Face
Site 4 Chest	Site 8 Knee	

FIGURE 3: QUALITATIVE CARRIAGE OF *Pityrosporum* YEASTS OF PATIENTS AND CONTROLS AS DETERMINED BY THE SCRUB WASH TECHNIQUE.

PERCENTAGE OF EACH BODY SITE SAMPLED WITH POSITIVE *Pityrosporum* CARRIAGE.

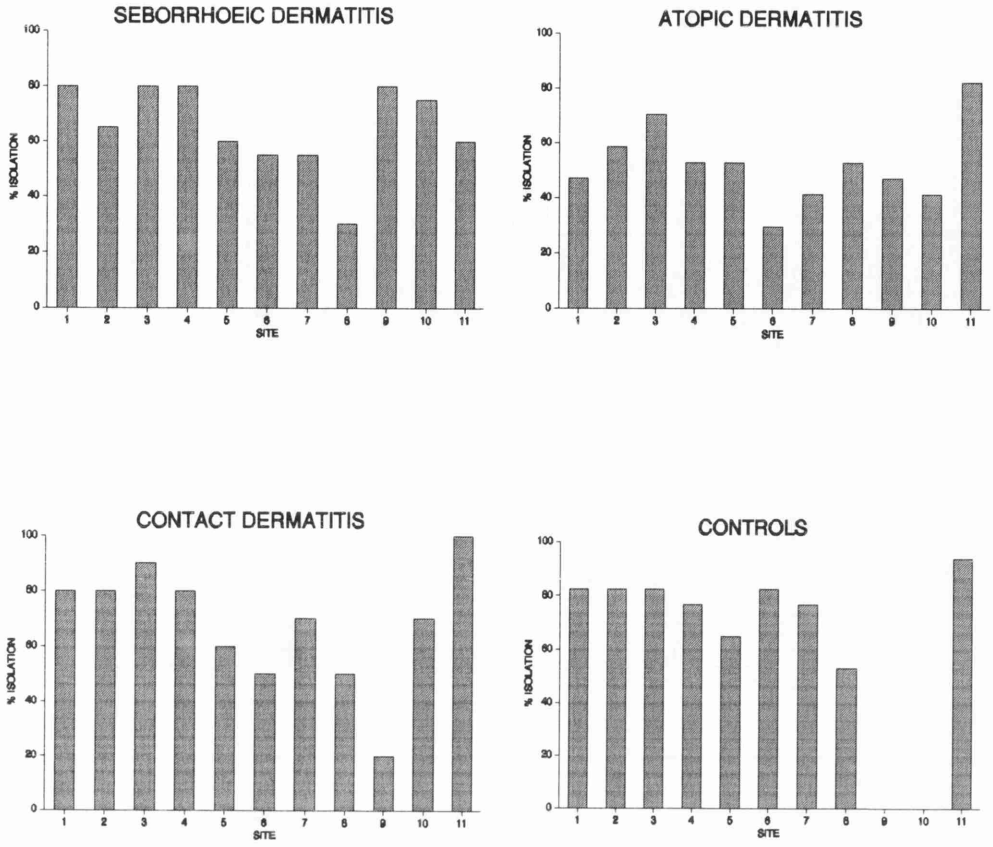


KEY:

Site 1	Scalp	Site 5	Elbow	Site 9	Involved
Site 2	Ear	Site 6	Hand	Site 10	Uninvolved
Site 3	Neck	Site 7	Foot	Site 11	Face
Site 4	Chest	Site 8	Knee		

FIGURE 4: QUALITATIVE CARRIAGE OF *Staphylococcus aureus* OF PATIENTS AND CONTROLS AS DETERMINED BY THE SCRUB WASH TECHNIQUE.

PERCENTAGE OF EACH BODY SITE SAMPLED WITH POSITIVE BACTERIAL CARRIAGE.

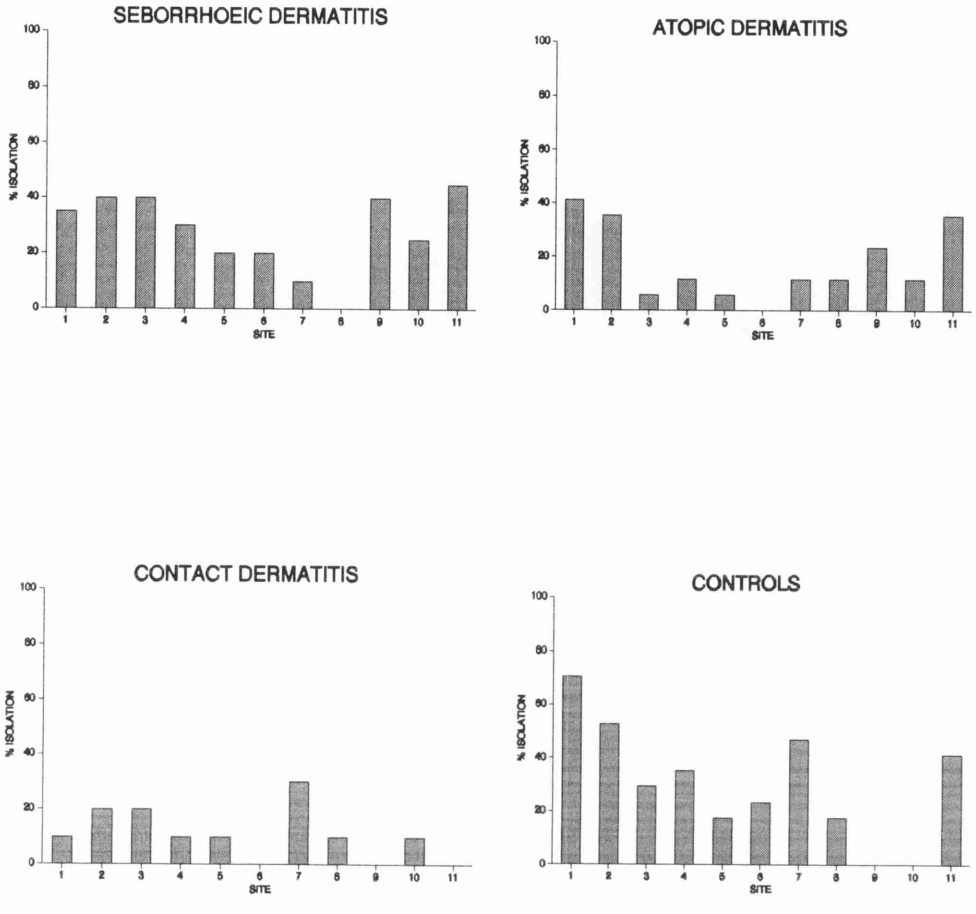


KEY:

Site 1	Scalp	Site 5	Elbow	Site 9	Involved
Site 2	Ear	Site 6	Hand	Site 10	Uninvolved
Site 3	Neck	Site 7	Foot	Site 11	Face
Site 4	Chest	Site 8	Knee		

FIGURE 5: QUALITATIVE CARRIAGE OF COAGULASE NEGATIVE STAPHYLOCOCCI/MICROCOCCI OF PATIENTS AND CONTROLS AS DETERMINED BY THE SCRUB WASH TECHNIQUE.

PERCENTAGE OF EACH BODY SITE SAMPLED WITH POSITIVE BACTERIAL CARRIAGE.



KEY:

Site 1	Scalp	Site 5	Elbow	Site 9	Involved
Site 2	Ear	Site 6	Hand	Site 10	Uninvolved
Site 3	Neck	Site 7	Foot	Site 11	Face
Site 4	Chest	Site 8	Knee		

FIGURE 6: QUALITATIVE CARRIAGE OF DIPTHEROIDS OF PATIENTS AND CONTROLS AS DETERMINED BY THE SCRUB WASH TECHNIQUE.

PERCENTAGE OF EACH BODY SITE SAMPLED WITH POSITIVE BACTERIAL CARRIAGE.

Severity of Dermatitis	Staph. aureus	CNS/MIC	Diptheroids	Pityrosporum from Scrub	Pityrosporum from Plate
	% positive	% positive	% positive	% positive	% positive
Score 0	7.0	60.0	16.5	37.2	38.2
Score 1	22.6	63.5	27.0	40.0	36.5
Score 2	39.0	72.0	28.0	36.6	46.3
Score 3	74.1	51.9	18.5	18.5	29.6
Controls	1.6	63.1	30.5	34.8	44.4

TABLE 1: PERCENTAGE OF ISOLATION OF EACH ORGANISM FOR EACH CLINICAL SCORE OF SEVERITY OF DERMATITIS FOR ALL GROUPS.

VARIABLE	SOURCE	DEVIANCE	DEGREES OF FREEDOM	P-VALUE*
Staph. aureus	Sites	9.7	8	0.29
	Patients v Controls	34.6	1	<0.0001
	Severity	54.7	2	<0.0001
	Patient Groups	63.5	2	<0.0001
CNS/MIC	Sites	48.6	8	<0.0001
	Patients v Controls	10.4	1	0.0013
	Severity	2.2	2	0.33
	Patient Groups	12.3	2	0.0021
Diptheroids	Sites	41.8	8	<0.0001
	Patients v Controls	16.2	1	0.0001
	Severity	2.3	2	0.32
	Patient Groups	11.5	2	0.0032
Pityrosporum from Scrub	Sites	112.2	8	<0.0001
	Patients v Controls	2.2	1	0.14
	Severity	2.9	2	0.23
	Patient Groups	12.8	2	0.0017
Pityrosporum from Plate	Sites	96.1	8	<0.0001
	Patients v Controls	1.5	1	0.22
	Severity	6.2	2	0.0450
	Patient Groups	7.8	2	0.0202

*P-value < 0.05 is statistically significant.

TABLE 2: GLIM ANALYSIS OF SEVERITY OF DERMATITIS FOR ALL ORGANISMS.

SITE	BOTH +VE	BOTH -VE	ONLY SCRUB +VE	ONLY PLATE +VE	P-VALUE*
1-SCALP	33 (51.6%)	16 (25.0%)	2 (3.1%)	13 (20.3%)	0.007
2-EAR	32 (50.0%)	17 (26.6%)	1 (1.6%)	14 (21.9%)	0.001
3-NECK	35 (54.7%)	14 (21.9%)	4 (6.2%)	11 (17.2%)	0.118
4-CHEST	43 (67.2%)	13 (20.3%)	3 (4.7%)	5 (7.8%)	0.727
5-ELBOW	11 (17.2%)	36 (56.2%)	8 (12.5%)	9 (14.1%)	1.000
6-HAND	8 (12.5%)	38 (59.4%)	6 (9.4%)	12 (18.8%)	0.238
7-FOOT	3 (4.7%)	46 (71.9%)	4 (6.2%)	11 (17.2%)	0.118
8-KNEE	5 (7.8%)	53 (82.8%)	2 (3.1%)	4 (6.2%)	0.688
9-INV.	13 (27.6%)	23 (48.9%)	0 (0%)	11 (23.4%)	0.001
10-UNINV.	18 (38.3%)	25 (53.2%)	3 (6.4%)	1 (2.1%)	0.625
11-FACE	17 (26.6%)	25 (39.1%)	1 (1.6%)	21 (32.8%)	<0.001

* P-value < 0.05 is statistically significant.

TABLE 3: QUALITATIVE COMPARISON OF THE TWO TECHNIQUES USING THE M^{C}NEMAR CHANGE TEST.

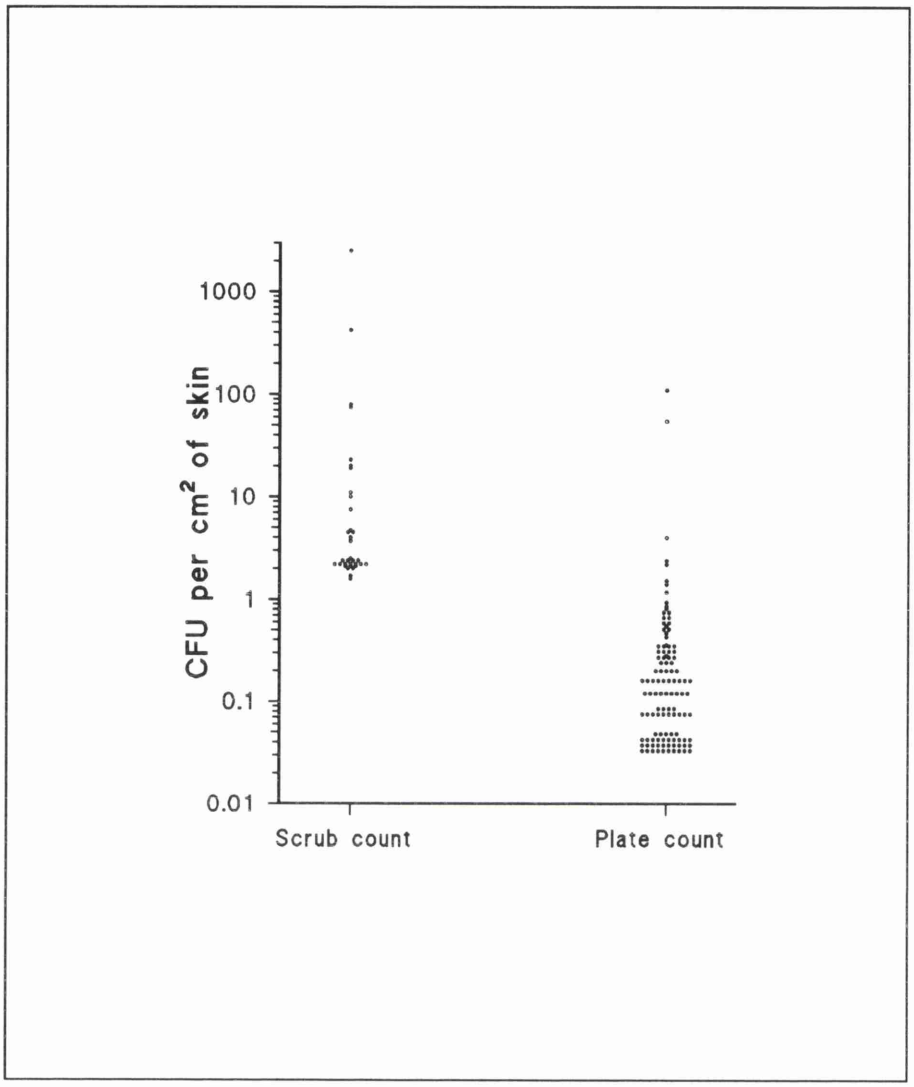


FIGURE 7: SCATTERGRAM INDICATING :-

(i) The counts of *Pityrosporum* yeasts obtained from the scrub wash technique when the contact plate technique gave a negative result.

(ii) The counts of *Pityrosporum* yeasts obtained from the contact plate technique when the scrub wash technique gave a negative result.

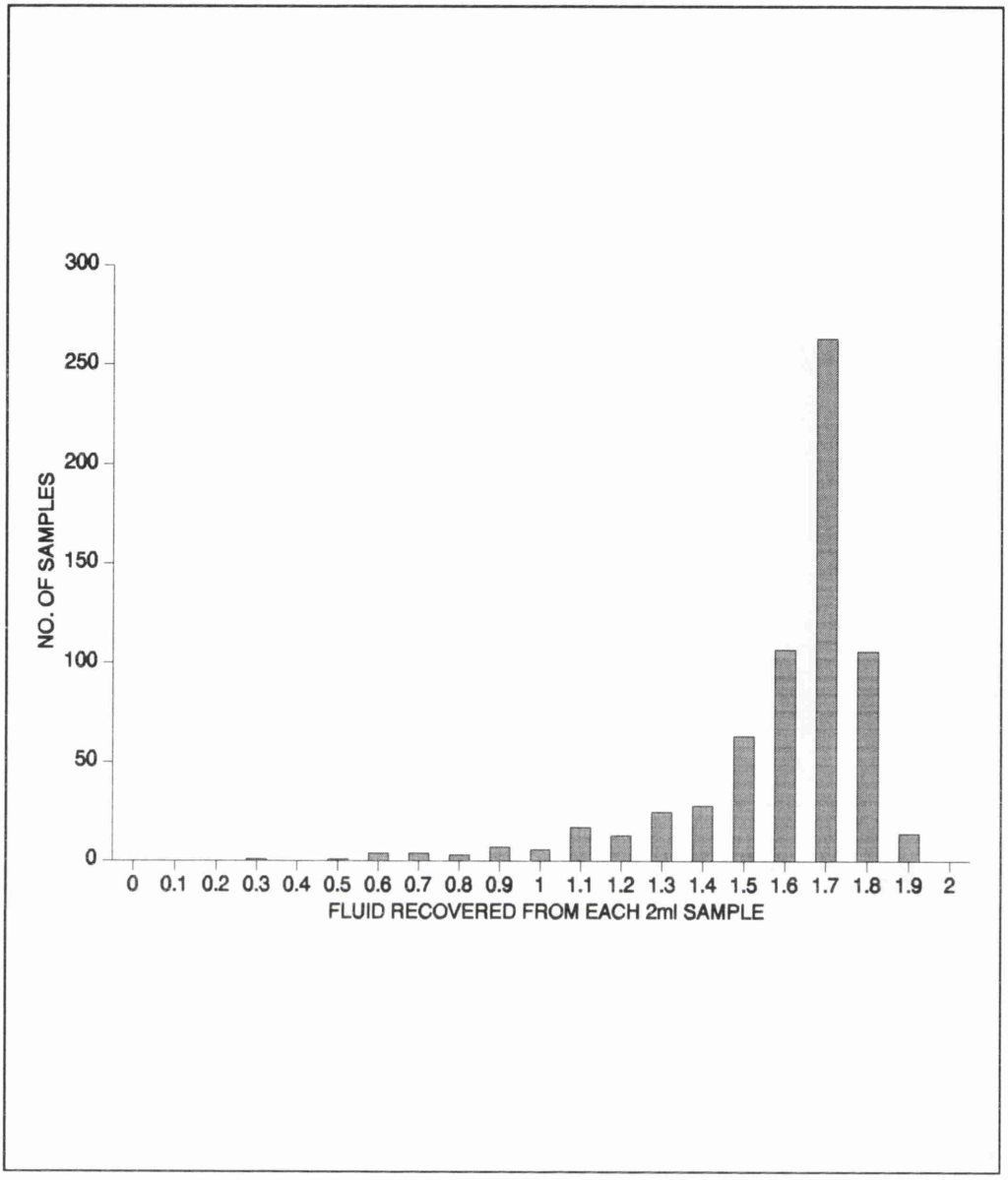


FIGURE 8: FLUID RECOVERED FROM THE SCRUB WASH SAMPLING TECHNIQUE.

GROUP	Staph. aureus		CNS/MIC		Diptheroids		Pityrosporum	
	n	%	n	%	n	%	n	%
SEBORRHOEIC	6	30.0	19	95.0	7	35.0	1	5.0
ATOPIC	16	94.1	7	41.2	1	5.9	7	41.2
CONTACT	5	50.0	9	90.0	1	10.0	3	30.0
CONTROL	9	52.9	16	94.1	2	11.8	3	17.6

TABLE 4: BACTERIAL AND MYCOLOGICAL ISOLATION FROM NOSE SWABS.

PHAGE TYPE	NO. OF ISOLATES	PERCENTAGE (%)
GROUP 1	3	25.0
GROUP 2	0	0
GROUP 3	2	16.7
EXPERIMENTAL GP.	0	0
OTHER (incl. complex)	1	8.3
NON-TYPABLE	6	50.0
CONTAMINATED	0	0

TABLE 5: BACTERIOPHAGE TYPING OF 12 ISOLATES OF *Staph. aureus* (including 6 nasal isolates) FROM 7 PATIENTS WITH SEBORRHOEIC DERMATITIS.

PHAGE TYPE	NO. OF ISOLATES	PERCENTAGE (%)
GROUP 1	10	10.9
GROUP 2	15	16.3
GROUP 3	13	14.1
EXPERIMENTAL GP.	13	14.1
OTHER (incl. complex)	27	29.3
NON-TYPABLE	10	10.9
CONTAMINATED	4	4.3

TABLE 6: BACTERIOPHAGE TYPING OF 92 ISOLATES OF *Staph. aureus* (including 16 nasal isolates) FROM 17 PATIENTS WITH ATOPIC DERMATITIS.

PHAGE TYPE	NO. OF ISOLATES	PERCENTAGE (%)
GROUP 1	9	36.0
GROUP 2	8	32.0
GROUP 3	3	12.0
EXPERIMENTAL GP.	0	0
OTHER (incl. complex)	2	8.0
NON-TYPABLE	2	8.0
CONTAMINATED	1	4.0

TABLE 7: BACTERIOPHAGE TYPING OF 25 ISOLATES OF *Staph. aureus* (including 5 nasal isolates) FROM 7 PATIENTS WITH CONTACT DERMATITIS.

PHAGE TYPE	NO. OF ISOLATES	PERCENTAGE (%)
GROUP 1	2	16.7
GROUP 2	0	0
GROUP 3	2	16.7
EXPERIMENTAL GP.	1	8.3
OTHER (incl. complex)	1	8.3
NON-TYPABLE	4	33.3
CONTAMINATED	2	16.7

TABLE 8: BACTERIOPHAGE TYPING OF 12 ISOLATES OF *Staph. aureus* (including 9 nasal isolates) FROM 10 CONTROLS.

GROUP	ONE TYPE	TWO TYPES	THREE TYPES	UNKNOWN*
SEBORRHOEIC	5	1	1	0
ATOPIC	10	5	1	1
CONTACT	5	1	0	1
CONTROL	7	1	0	2
TOTALS	27 (65.8%)	8 (19.5%)	2 (4.9%)	4 (9.8%)

*Some isolates were not phage typed due to contamination problems.

TABLE 9: PATIENT AND CONTROL GROUPS WITH ONE OR MORE PHAGE TYPE GROUPS.

GROUP	MATCH	DIFFERENT	DIFFERENT -type present on body	UNKNOWN	NASAL ONLY
SEBORR.	1	1	1	0	3
ATOPIC	10	2	3	1	0
CONTACT	3	0	0	1	1
CONTROL	0	1	0	1	7
TOTALS	14 (38.9%)	4 (11.1%)	4 (11.1%)	3 (8.3%)	11 (30.6%)

TABLE 10: COMPARISON OF NASAL *Staph. aureus* PHAGE TYPE WITH THE PREDOMINANT PHAGE TYPE FOUND ON THE OTHER BODY SITES OF PATIENT AND CONTROL GROUPS.

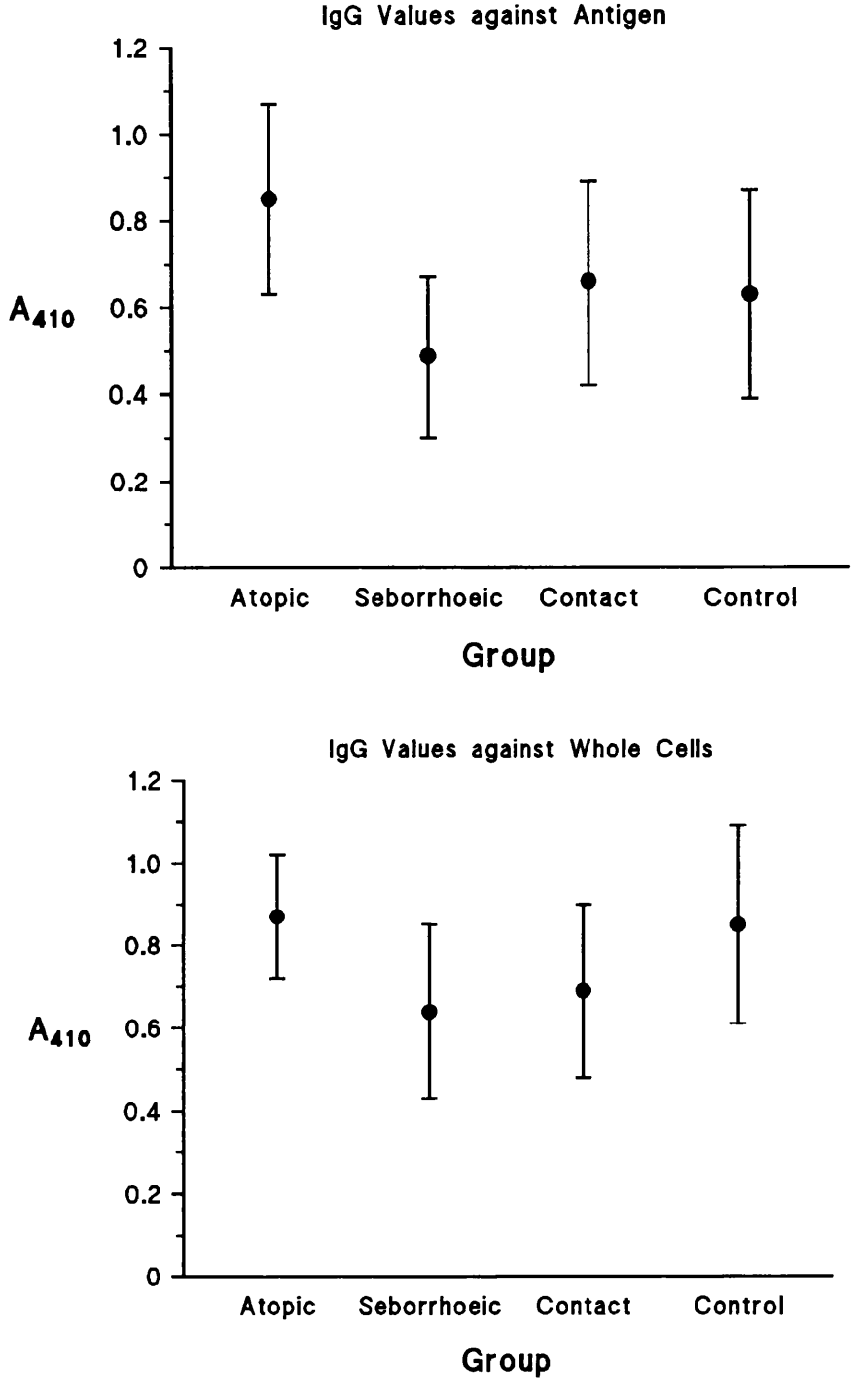


FIGURE 9: THE IgG VALUES (MEANS AND 95% CONFIDENCE INTERVALS) OF THE DIFFERENT DISEASE GROUPS AND CONTROLS TO EITHER *Pityrosporum* CYTOPLASMIC ANTIGEN OR *Pityrosporum* WHOLE CELLS.

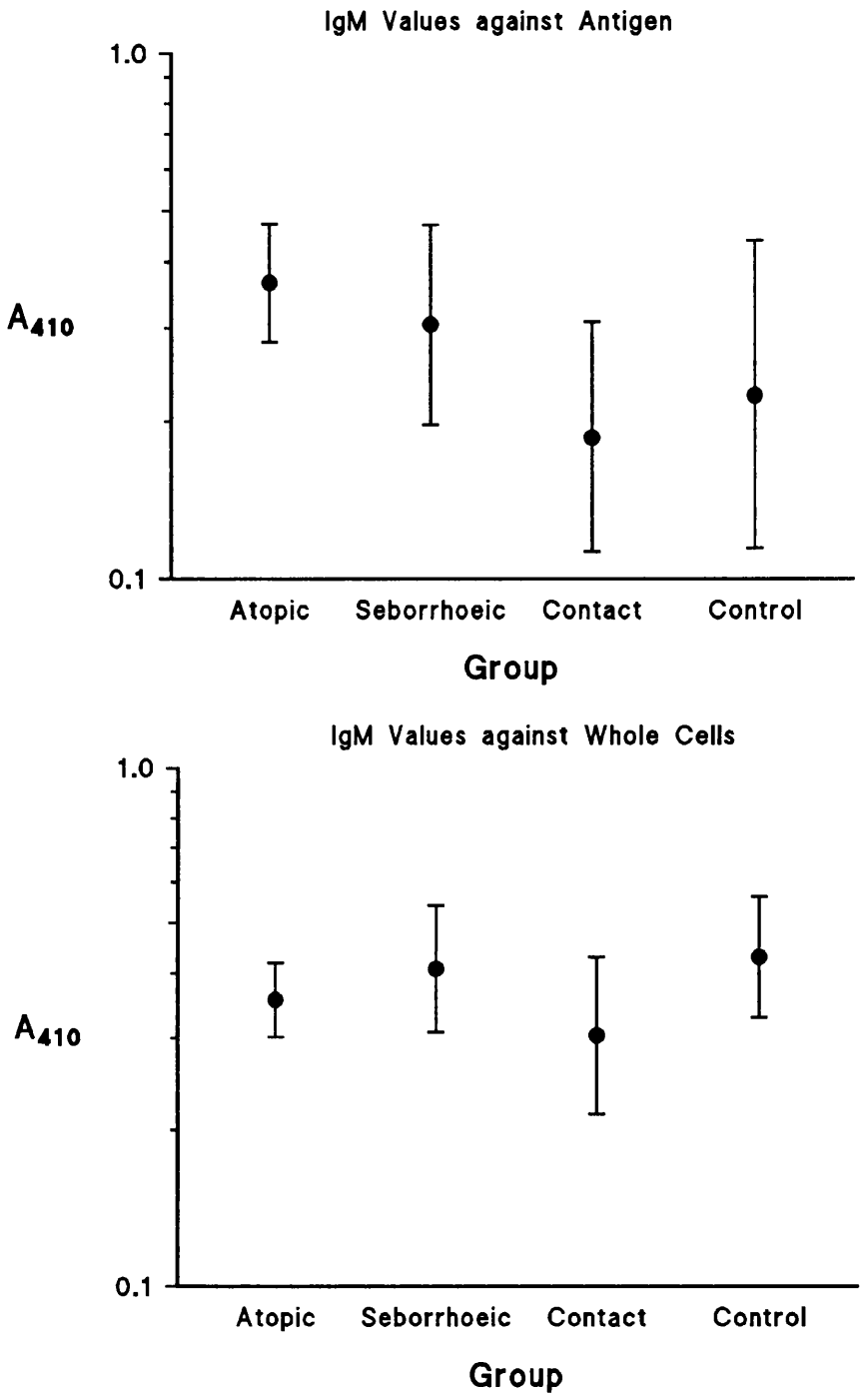


FIGURE 10: THE IgM VALUES (GEOMETRIC MEANS AND 95% CONFIDENCE INTERVALS) OF THE DIFFERENT DISEASE GROUPS AND CONTROLS TO EITHER *Pityrosporum* CYTOPLASMIC ANTIGEN OR *Pityrosporum* WHOLE CELLS.

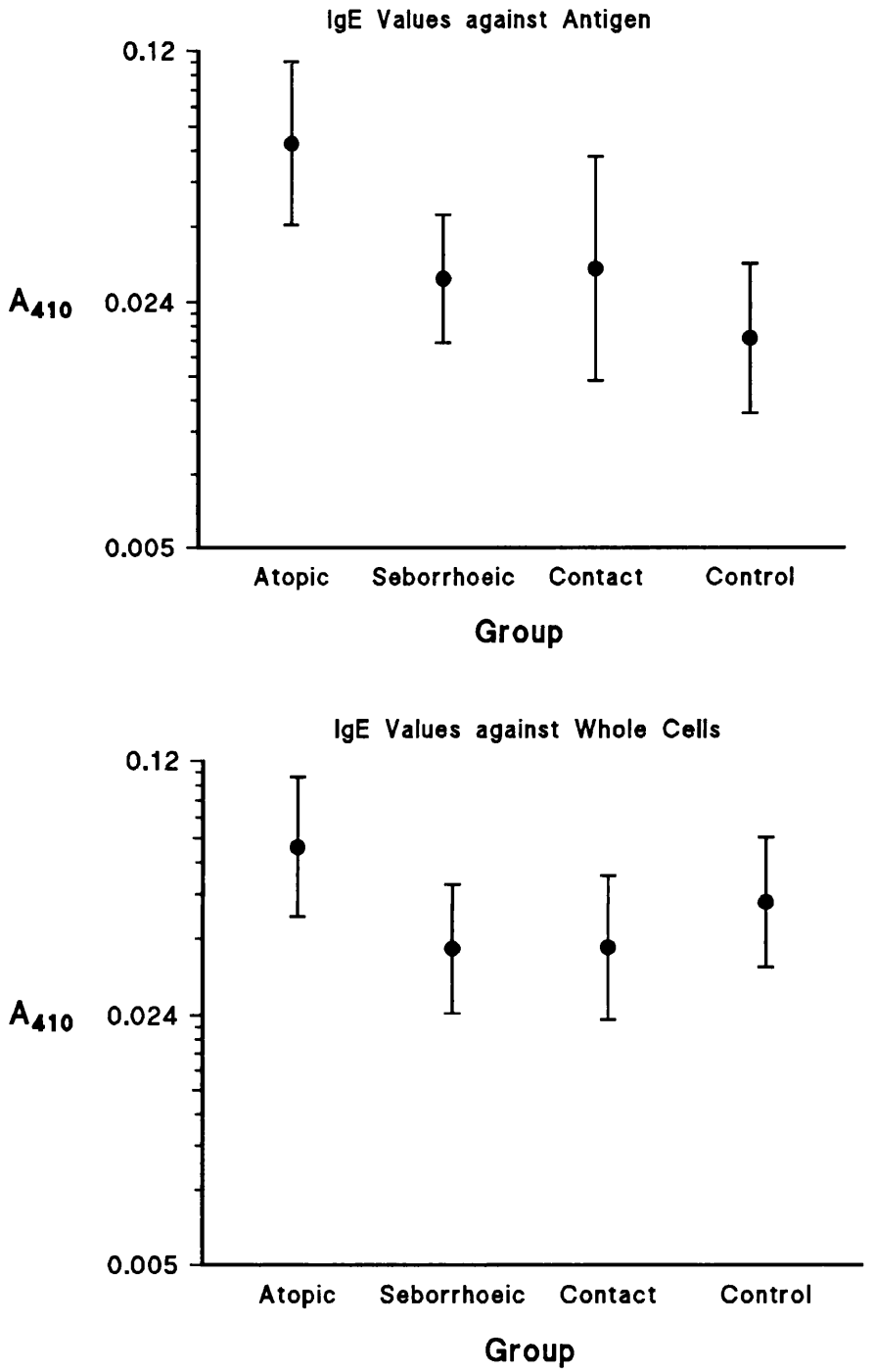


FIGURE 11: THE IgE VALUES (GEOMETRIC MEANS AND 95% CONFIDENCE INTERVALS) OF THE DIFFERENT DISEASE GROUPS AND CONTROLS TO EITHER *Pityrosporum* CYTOPLASMIC ANTIGEN OR *Pityrosporum* WHOLE CELLS.

ELISA PROCEDURE	ONE-WAY ANALYSIS OF VARIANCE
IgG against Antigen	No significant difference between groups ($F_{3,60}$ = 2.20, p= 0.097)
IgG against Whole Cells	No significant difference between groups ($F_{3,60}$ = 1.42, p= 0.246)
IgM against Antigen	No significant difference between groups ($F_{3,56}$ = 1.59, p= 0.202)
IgM against Whole Cells	No significant difference between groups ($F_{3,56}$ = 1.37, p= 0.261)
IgE against Antigen*	SIGNIFICANT difference between groups ($F_{3,56}$ = 5.35, p= 0.003)
IgE against Whole Cells	No significant difference between groups ($F_{3,56}$ = 2.34, p= 0.083)

*The atopic group was shown to be significantly different from all other disease groups and control group by the Multiple Range Test. This test was then modified to correct for multiple comparisons and the atopic group was still shown to be significantly different from the control group at the 0.050 level.

TABLE 11: CORRELATION OF ELISA VALUES TO DISEASE GROUP/CONTROL GROUP USING ONE-WAY ANALYSIS OF VARIANCE.

VARIABLE EXAMINED	SPEARMAN'S RANK CORRELATION
Maximum Clinical Score	No significance seen
Average Clinical Score*	No significance seen
Clinical Score at Involved Site	No significance seen
Pityrosporum Scrub Wash Results at Involved Site	No significance seen
Pityrosporum Contact Plate Results at Involved Site**	No significance seen

*Some evidence of a relationship ($p < 0.10$) was seen with:

IgM against Antigen ($r_s = -0.42$, $n=20$, $p= 0.068$)

IgM against Whole Cells ($r_s = -0.42$, $n=20$, $p= 0.062$)

IgE against Antigen ($r_s = -0.42$, $n=20$, $p= 0.066$)

**Some evidence of a relationship ($p < 0.10$) was seen with:

IgM against Antigen ($r_s = -0.38$, $n=20$, $p= 0.099$)

TABLE 12: SPEARMAN'S RANK CORRELATION OF ELISA VALUES AGAINST SPECIFIC VARIABLES FOR PATIENTS WITH SEBORRHOEIC DERMATITIS.

VARIABLE EXAMINED	SPEARMAN'S RANK CORRELATION
Maximum Clinical Score	IgE against Antigen was the only assay to show significance (r_s = 0.62, n=17, p= 0.008)
Average Clinical Score	IgE against Antigen was the only assay to show significance (r_s = 0.48, n=17, p= 0.048)
Clinical Score at Involved Site	IgE against Antigen was the only assay to show significance (r_s = 0.51, n=17, p= 0.037)
Pityrosporum Scrub Wash Results at Involved Site	No significance seen
Pityrosporum Contact Plate Results at Involved Site	No significance seen

TABLE 13: SPEARMAN'S RANK CORRELATION OF ELISA VALUES AGAINST SPECIFIC VARIABLES FOR PATIENTS WITH ATOPIC DERMATITIS.

VARIABLE EXAMINED	SPEARMAN'S RANK CORRELATION
Maximum Clinical Score	No significance seen
Average Clinical Score	IgM against Whole Cells was the only assay to show significance (r_s = 0.68, n=10, p= 0.030)
Clinical Score at Involved Site	No significance seen
Pityrosporum Scrub Wash Results at Involved Site	No significance seen
Pityrosporum Contact Plate Results at Involved Site	No significance seen

TABLE 14: SPEARMAN'S RANK CORRELATION OF ELISA VALUES AGAINST SPECIFIC VARIABLES FOR PATIENTS WITH CONTACT DERMATITIS.

4. DISCUSSION AND CONCLUSIONS

4.1 MYCOLOGICAL ISOLATION IN PATIENTS AND CONTROLS

The high levels of *Pityrosporum* seen, with either technique, in the upper body sites, in particular scalp, ear, neck and chest, could perhaps be expected since these sites also correspond with areas of the body that have a high number of sebaceous glands, and *Pityrosporum* yeasts which are found on human skin are dependent on lipids[7]. Interestingly, isolation from the face differed between the two techniques with the Scrub Wash Technique yielding a lesser rate of *Pityrosporum* than the Contact Plating Technique. This could be perhaps because the face was a difficult area to sample with this technique and fluid was sometimes lost from the ring.

Patients with seborrhoeic dermatitis had high isolation rates using either technique from both involved and uninvolved skin sites, indicating that *Pityrosporum* does not only colonise dermatitic skin. The high levels of isolation seen here could be mainly because the involved and uninvolved skin sites were usually upper body sites, because of the very nature of the disease, and hence with many sebaceous glands. Involved skin sites were also difficult to sample with the Scrub Wash Technique, hence a possible reason for the slightly lower rate of isolation seen in seborrhoeic dermatitis patients. Extreme care had to be taken not to irritate badly affected skin. For this reason, the Contact Plating Technique was actually preferred for sampling severe eczematous lesions. It was also much quicker to use and, therefore, kept patient discomfort to a minimum.

Pityrosporum would not appear to be associated with either atopic or contact dermatitis since high percentages of isolation were confined to upper body sites only.

Similar findings were observed in control subjects.

4.2 BACTERIOLOGICAL ISOLATION IN PATIENTS AND CONTROLS

Patients with seborrhoeic dermatitis had practically no *Staph. aureus* present on their skin. This suggests that adult patients with seborrhoeic dermatitis seem to differ from children affected by infantile seborrhoeic dermatitis. One study found significantly more *Staph. aureus* in children with infantile seborrhoeic dermatitis compared to controls[12].

In contrast, patients with atopic dermatitis appeared to have all-over colonisation with *Staph. aureus*, whereas patients with contact dermatitis tended to only have the organism present at affected body areas. This is most likely to be due to the nature of the two diseases since contact dermatitis did seem to be confined to much smaller areas, whereas atopic dermatitis was usually more wide-spread. It has also been suggested that patients with atopic dermatitis experience generalised severe pruritis and the associated constant scratching may lead to the spread of *Staph. aureus* to the clinically normal-looking skin[49].

Coagulase negative staphylococci/micrococci were, in general, present in much higher percentages and much more wide-spread than *Staph. aureus*. An exception to this was seen in patients with atopic dermatitis. The lower rate seen here may be due to competition of organisms, particularly *Staph. aureus*, on the skin. This confirms results previously reported from atopic dermatitis patients[49].

Diptheroids were also relatively wide-spread across all body sites, although in generally lower percentages. Control subjects had noticeably higher levels than the patient groups, particularly the atopic and allergic contact dermatitis groups. It has

previously been suggested that the presence of diptheroids indicate normal healthy skin[55].

4.3 RELATIONSHIP BETWEEN ISOLATION RATES AND CLINICAL STATE

Pityrosporum does not appear to be related to the severity of dermatitis since control subjects showed similar percentages of isolation to the patient groups with either technique.

Coagulase negative staphylococci/micrococci and diptheroids showed similar findings to that of *Pityrosporum*. This confirms previous findings in patients with atopic dermatitis[42].

In contrast, however, *Staph. aureus* appears to be very closely linked to the severity of dermatitis. A linear increase in *Staph. aureus* counts with increasing dermatitis activity has been previously reported[42].

4.4 CORRELATION BETWEEN MYCOLOGICAL OR BACTERIAL ISOLATION AND PATIENT GROUPS

Confirmation that *Staph. aureus* was wide-spread on the body, especially in patients with atopic dermatitis was found when no significant differences were seen from body site to body site. For the other organisms, differences between body sites were found as would perhaps be expected since different areas of the body provide different environments and therefore allow the growth of different organisms, or in differing amounts. This is particularly the case with *Pityrosporum* because of their lipophilic nature.

After these observed site differences were accounted for, significant differences were

still seen between patients and controls for all bacterial isolates. This seems to suggest that the bacterial skin flora of patients with dermatitis is made up differently to subjects without dermatitis. A previous study, however, found that there was no significant difference in the population densities of *Micrococcaceae* between patients with seborrhoeic dermatitis and controls for any site sampled[56]. A possible explanation for the difference in these studies could be the adjustment for site. The reported study had simply compared the appropriate sites from patients and controls. Confirmation that *Pityrosporum* only colonises greasy areas of the body, regardless of whether the subjects have dermatitis or not, was obtained when no significant differences were found between patients and controls.

Even after both site and patients vs. controls factors were adjusted for, severity differences were still observed with *Staph. aureus*. This is confirmation of previous results obtained.

The final analysis examining differences between the patient groups, after all other factors had been adjusted for, showed significant differences between all organisms. This would, again, seem to suggest that each type of dermatitis has a particular skin flora, which differs from the other types of dermatitis studied. This could possibly be due to differences in nutrients available on the skin surface and differences in ability to adhere to the skin. Also, competition between different organisms for binding sites and nutrients could exist.

4.5 CORRELATION BETWEEN THE SCRUB WASH AND CONTACT PLATING TECHNIQUES FOR THE ISOLATION OF *Pityrosporum* YEASTS

The contact plate method has previously been reported as reliable and easy to use[57,58].

However, no correlation was made with the Scrub Wash Technique. In the present study, previous results were confirmed with the Contact Plating Technique performing extremely well. Possible reasons to explain why the Contact Plating Technique was better at picking up *Pityrosporum* yeasts are as follows:

(1) adjacent areas of skin were sampled and hence the sample site was not exactly the same thus differences could perhaps be found. This, however, seems unlikely since it would be more feasible if the two techniques had equal preference.

(2) there was a difference in the size of the area of skin sampled, with the larger area being sampled by the contact plate, and hence more chance of picking up yeasts.

(3) the detergent, Triton X-100, used in the Scrub Wash Buffer could, perhaps, to some extent, be fungicidal. It has been shown that this detergent is somewhat bactericidal[59] and it has been recommended that bacterial samples only be left for 30 minutes before inoculation on to agar. In this study, the sampling procedure for each person took approximately 45 minutes, after which, each sample was diluted in half-strength Scrub Wash Buffer before being transferred onto agar plates. If the Triton X-100 had a similar effect on yeasts as it has on bacteria, then it could be that the isolation rates of *Pityrosporum* were slightly under-estimated. However, it has been shown that Triton X-100 (0.1%) had only a slight inhibitory effect when the yeast was in the Scrub Wash Buffer for less than two hours[60].

(4) the contact plate had the advantage of instant nutrients being available for any yeasts picked up from the skin.

(5) some of the body sites were difficult to sample, either because of their awkward position or because of the extent of their dermatitis. Some scrub wash fluid could perhaps be lost, taking with it some yeasts.

The isolation percentages suggested that the Scrub Wash Technique missed a large amount of *Pityrosporum*, however, the true picture can be seen when the quantitative counts are visualised. The scattergram suggests that, although the Scrub Wash Technique appeared to be worse, the missed counts were mainly very low. When the Contact Plating Technique missed yeasts, the counts obtained by the scrub wash procedure were generally higher than when the situation was reversed. However, even these counts were usually less than five CFU per cm^2 of skin. These results suggest that the two techniques are generally comparable for the isolation of *Pityrosporum* from the skin of dermatitic patients and control subjects.

One potential problem with the Scrub Wash Technique is the loss of wash fluid. However, recovered samples tended to contain the majority of the starting volume and also tended to be very similar. This suggests that the number of yeasts calculated from each sample were generally comparable. One body area which disagreed with this finding was the scalp. This was due to a number of reasons:

(1) it was harder to get a good seal on this body site.

(2) more fluid could perhaps be absorbed into the hair.

(3) the wash fluid became frothy whilst it was rubbed around the hair because of the Triton X-100 contained within it.

4.6 ISOLATION FROM NOSE SWABS

In the present study, *Staph. aureus* was isolated from almost all patients with atopic dermatitis. It has previously been shown that the anterior nares of atopic dermatitis patients have a *Staph. aureus* carriage rate of 79%[55]. *Staph. aureus* nasal carriage rates in the normal healthy population vary greatly, but are generally between 19-

40%[61]. The nasal carriage rate of *Staph. aureus* in normal subjects has previously been found to be 53%[42]. This higher value was attributed to the inclusion of hospital staff as control subjects. The similarly high value obtained in this study could also be explained by the use of hospital staff as some of the control group.

Half of all patients with contact dermatitis were found to carry *Staph. aureus* in the anterior nares. This is possibly due to their higher isolation rates of *Staph. aureus* from other body sites, or perhaps the nares act as a reservoir for skin transmittal.

Isolation of *Staph. aureus* from the nares of seborrhoeic dermatitis patients was within the reported range for the normal population.

Isolation of coagulase negative staphylococci/micrococci from the anterior nares seemed to follow the trend of the isolation percentages seen in the patient groups and controls from the other body sites, with atopic dermatitis patients having a lower rate.

4.7 BACTERIOPHAGE TYPING OF ISOLATES OF *Staph. aureus*

It has been previously found that the predominant phage type in atopic dermatitis patients was group 3[26,42]. The present study did not confirm this finding, with the *Staph. aureus* isolates from the atopic group being spread equally amongst all of the phage groups. No particular phage group seemed to be associated with any one type of dermatitis, although the numbers of isolates in this study were small. However, it was noted that isolates of *Staph. aureus* from the skin and nose of an individual patient, regardless of the type of dermatitis, tended to be of the same phage type. This is consistent with the suggestion that *Staph. aureus* present on the skin occurs via constant dissemination from the nares[62].

4.8 IMMUNOLOGICAL ANALYSIS

For IgG antibodies, no noticeable differences were seen between patients and controls for either *P. ovale* whole cells or antigen. However, with IgM, although no patients vs. controls differences were seen, lower responses were seen against *P. ovale* antigen. This might suggest that whole cells generally invoke a stronger primary immunological response than cytoplasmic antigen. IgE responses to *P. ovale* whole cells for seborrhoeic and allergic contact dermatitis patients were again higher than those obtained against antigen. This could possibly suggest that in individuals, other than patients with atopic dermatitis, and in particular, normal subjects, whole cells of *P. ovale* cause a greater response than antigen.

For patients with seborrhoeic dermatitis, the immunological analysis suggested that as clinical score became greater, IgM and IgE responses were lower. It has previously been found that lower IgM titres to *Pityrosporum* are present in elderly individuals[63,64]. It could possibly be that the average clinical scores increased with age and the IgM values, therefore, decreased. It has been suggested that the immune system of elderly individuals does not respond well to primary stimulation by *Pityrosporum*[63]. It was also observed that in seborrhoeic dermatitis patients, the higher the isolation rate of *Pityrosporum*, the lower the IgM response.

For atopic dermatitis patients, it was seen that as severity of dermatitis rose, so did the IgE response.

4.9 SPECULATIONS ON SEBORRHOEIC DERMATITIS PATHOLOGY

The exact role played by *Pityrosporum* yeasts in seborrhoeic dermatitis remains controversial.

Some studies have shown a significant difference in the numbers of *Pityrosporum* yeasts seen in patients with seborrhoeic dermatitis compared with normal individuals[11,32,65] and some have also demonstrated a correlation between the density of *P. ovale* and the clinical severity of dermatitis[32]. These studies tend to centre around techniques such as direct microscopy of skin scales or tape-stripping the skin rather than Contact Plating or Scrub Wash Techniques. It has been suggested that more corneocytes and microorganisms are removed in the patient group because of the increased scaling observed in these patients[66]. Others have found that, when the population densities of *Pityrosporum* on seborrhoeic dermatitis affected skin were compared to normal skin, no significant difference was seen[16,33,56]. These studies used the Contact Plating or Scrub Wash Techniques for sampling patients and controls. It was found in the present study that quantitative analysis was not possible due to the number of zero counts obtained throughout. However, qualitative analysis suggested that there was no significant difference in *Pityrosporum* isolation rates between seborrhoeic dermatitis patients and controls. Also, there was no difference observed between the clinically involved and normal skin of patients.

However, *Pityrosporum* yeasts are undoubtedly present upon the skin, particularly on upper body sites, of patients with seborrhoeic dermatitis. Therefore, many studies have investigated the role played by antipityrosporal drugs in the control of seborrhoeic dermatitis. Many therapies have been tried, the only common link between them being their activity against *Pityrosporum* yeasts. Compounds used include selenium sulphide (2.5%), zinc pyrithione (1%), corticosteroids, nystatin (100,000U/g), propylene glycol (5-40%) and imidazoles[67]. Some of these compounds also have anti-inflammatory, anti-keratolytic and antiseptic properties. However,

these are viewed as having less importance than their antipityrosporal effect[67].

An antifungal drug which has received much attention in the treatment of seborrhoeic dermatitis is ketoconazole. It has been shown *in vitro* to be the most active member of the imidazole group against *Pityrosporum*, with a minimum inhibitory concentration of only 0.01 mg/l[68]. Ketoconazole is available in various formulations - tablet, cream, shampoo and scalp gel. The treatment of choice tends to be topical as systemic formulations are not usually indicated since seborrhoeic dermatitis usually affects relatively small areas of the body. It can also frequently relapse and long-term treatment is generally required. Topical formulations also avoid any potential problems of systemic toxicity[69]. Many studies have reported successful clinical use of topical ketoconazole in the treatment of seborrhoeic dermatitis[35,36,70.71] - some have also correlated this clinical improvement with a reduction in the number of *Pityrosporum* present on the body[36,70,71]. Ketoconazole also has some anti-inflammatory effect, due to the blockage of leukotriene production *in vitro* and *in vivo*[72]. This may account for the superiority of this drug over other imidazoles. It is unlikely that this additional action should account by itself for the results achieved in the treatment of seborrhoeic dermatitis, but could be considered as complementary[36].

Since the relationship between *Pityrosporum* yeasts and seborrhoeic dermatitis does not appear to be direct, yet the removal of the yeast results in clinical improvement, an indirect effect seems possible.

Many studies have examined whether this effect is mediated via immunological mechanisms. Any interaction between *Pityrosporum* and the immune system may be of particular importance since patients with acquired immunodeficiency syndrome

seem to have an increased incidence of the disease[30]. It is known that antibodies against *Pityrosporum* can be found in serum from healthy individuals[14]. Therefore, much interest has focused on serological responses to *Pityrosporum* in patients with seborrhoeic dermatitis. Some studies have demonstrated an increase in antibodies to *Pityrosporum*[21,73]. This increase was found to be due to IgG but not IgM and was only detected after a five-fold concentration of the sera. Others have not found this increase. Studies, using an indirect immunofluorescence technique, found no significant difference in antibody titres against *P. ovale* whole cells in patients with seborrhoeic dermatitis compared to controls[15,16,19]. However, in a further study, where an ELISA technique using a cell-wall protein extract from *P. ovale* as an antigen source, was used, seborrhoeic dermatitis patients were found to have lower levels of serum IgG antibodies compared to controls[20]. In the present study using an ELISA method with whole cells or cytoplasmic antigen, no significant difference was found between patients with seborrhoeic dermatitis and controls for any of the class-specific antibodies tested (IgG, IgM and IgE). The discrepancies found between the results of the numerous studies could possibly be due to differences in techniques used, in particular, preparation of the antigens and the method used to measure the antibody responses[14].

Cellular immunological mechanisms have also been considered. One study found several seborrhoeic dermatitis patients to have a depressed T-cell function with increased frequencies of natural killer cells and subnormal mitogen stimulation responses[15]. It was concluded that the impaired cell-mediated immunity may allow *Pityrosporum* to survive on the skin. This lessened immunity could also account for the lower serum antibody titres observed against *P. ovale* cell-wall protein[20] since this

antibody response is likely to be T-cell dependent[15]. Lipase[74] and lipoxygenase[34] activities of *Pityrosporum* have been described *in vitro*. If these activities were also present *in vivo*, derived by-products could provoke an inflammatory response which would not be down-regulated. This inflammatory reaction could trigger the dermatitis[15].

Another factor of this disease to be considered is the determination of the lipid content of the skin since *Pityrosporum* yeasts are found particularly in areas of the body with a high number of sebaceous glands. Increased levels of skin surface lipids in patients with seborrhoeic dermatitis have been reported[14]. Others, however, have found no difference in the rate of sebum excretion in seborrhoeic dermatitis patients[75,76]. It has, therefore, been suggested that the residual pool of sebum might be important rather than the actual excretion rate[14]. The composition of sebum has also been studied. Only minor abnormalities were found - increased cholesterol levels and decreased concentrations of squalene and unsaturated fatty acids[77]. The nutritional factors of the sebum on the skin surface may encourage the growth of *Pityrosporum* which, in turn, may result in an increase in degradation products, hence causing more irritation.

Further work is needed before the true relationship between *Pityrosporum* yeasts and seborrhoeic dermatitis is elucidated. Studies should concentrate on analysing the host immune response to *Pityrosporum* and also the activities of the yeast *in vivo*.

4.10 SPECULATIONS ON ATOPIC DERMATITIS PATHOLOGY

The role of *Staphylococcus aureus* in atopic dermatitis has been examined extensively. *Staph aureus* has been found to be the most common organism in

patients with atopic dermatitis[22]. One study showed *Staph. aureus* to colonise skin lesions in >90% of atopic dermatitis patients, and the noninvolved skin of the same patients had *Staph. aureus* colonisation of approximately 80%[55]. Only 10% of healthy controls showed any *Staph. aureus*. Other studies have shown similarly high rates of isolation of *Staph. aureus* from eczematous skin of atopic dermatitis patients[42,49]. In the present study, the involved skin of atopic dermatitis patients had approximately 80% colonisation with *Staph. aureus* whereas uninvolved skin showed a rate of about 50%. These values are in agreement with previous studies. Control subjects showed a similarly low level to the previously reported values. The densities of *Staph. aureus* has previously been shown to vary in proportion to the severity of dermatitis of the skin site sampled, with areas of skin with exudative atopic dermatitis showing the highest density of *Staph. aureus*[42]. The present study confirmed this finding with the isolation rates of *Staph. aureus* increasing with the severity of dermatitis. This increased colonisation by *Staph. aureus* seen in patients with atopic dermatitis may be due to enhanced adherence of *Staph. aureus* to skin and mucous membranes[78-80]. Adherence may be due to the presence of receptors on the *Staph. aureus* cell wall for fibronectin[81] and laminin[82] which are present in the tissues of atopic dermatitis patients with eczematous lesions.

Two mechanisms have recently been proposed for staphylococcal damage in atopic dermatitis[25]. The first proposal suggests that *Staph. aureus* has a direct inflammatory effect on the already damaged skin of patients with atopic dermatitis. A cycle is therefore established : itch - scratch - skin damage - inflammation - infection. One study has shown that the protein A component of the cell wall of *Staph. aureus* directly causes inflammation of damaged skin[83]. The second proposed mechanism

is indirect and based upon immunological processes. Various studies have examined the level of IgE responses to staphylococcal antigens. Antistaphylococcal IgE antibodies, if present, could induce the release of the inflammatory mediators from mast cells. This, in turn, could aggravate the dermatitis. However, the role played by IgE antibodies remains unclear. Studies have shown high levels of antistaphylococcal IgE in atopic dermatitis patients, but high levels were also seen in those patients who were atopic but did not have eczema[84]. Other work has revealed much lower levels of antistaphylococcal antibodies in patients with atopic dermatitis[85]. These variable results may be due, in part, to problems which are associated with measuring antistaphylococcal antibodies - specificity of antigens used and specificity of IgE measured in the presence of very high total IgE levels[25].

Cell-mediated immune responses have also been examined. It has been shown that platelets from atopic dermatitis patients have an increased preactivation compared to those from normal donors and also show an enhanced responsiveness to *Staph. aureus*[86]. Polymorphonuclear leukocytes have also been shown to be hyperreactive in patients with atopic dermatitis, perhaps contributing to chronic skin damage[87].

Whether either of these mechanisms are correct, much benefit is seen with the reduction and/or removal of *Staph. aureus* colonisation in atopic dermatitis patients. Therefore, many studies have focused upon therapy for the reduction of *Staph. aureus* load. Antistaphylococcal antibiotics, such as fucidic acid, mupirocin and clindamycin, have all been used as long term topical agents[88]. Studies have shown clinical improvement of atopic dermatitis coupled with a reduction in the extent of *Staph. aureus* colonisation by the use of topical mupirocin ointment[26]. Mupirocin has also been shown to completely eradicate *Staph. aureus* colonisation from the skin and

anterior nares of atopic dermatitis patients after three days of therapy[25]. Clinical improvement was also observed in these patients. After therapy was stopped, *Staph. aureus* slowly recolonised the patients' skin which preceded clinical deterioration. Another study has shown the eradication of a multiple-resistant strain of *Staph. aureus* in a patient with atopic dermatitis by the use of mupirocin immersion[89]. Various problems could potentially be associated with the prolonged use of systemic or topical antibiotics such as the selection of resistant strains, systemic side-effects or contact allergy.

Other treatments have therefore also been considered. Topical antiseptics would avoid potential problems of the development of resistance and systemic side-effects. However, these preparations could have a tendency to be irritant to the skin of atopic dermatitis patients. Good clinical tolerance has been observed in one study with two different topical antiseptics - chlorhexidine and $KMnO_4$[90]. Clinical improvement was observed with both antiseptics, however, since patients were also receiving topical corticosteroids, it is difficult to assess the effect of the antiseptics alone. Topical corticosteroids, used as monotherapy, have been shown to reduce the density of *Staph. aureus* and improve the clinical effects of atopic dermatitis[91]. It would be presumed that with this therapy the underlying disease that allows *Staph. aureus* to colonise is being treated, but the actual mechanism involved remains unclear[25].

From these studies, it is therefore apparent that, although *Staph. aureus* is always associated with atopic dermatitis and the removal of *Staph. aureus* results in clinical improvement, the exact role of the organism is unknown.

Further work examining the mechanism of damage of *Staph. aureus* in atopic dermatitis is therefore needed. Studies should concentrate on the host immunological

responses to *Staph. aureus* and also to examine the use of agents to eradicate *Staph. aureus* colonisation completely from the body for a long period of time.

There has also been much interest in the role played by *Pityrosporum* yeasts in atopic dermatitis, particularly affecting the head and neck area. Studies have shown similar colonisation rates of *Pityrosporum* between patients with atopic dermatitis and normal subjects[45,92]. Similar findings were observed in the present study. However, it has been shown that a specific IgE response to *Pityrosporum* occurs more frequently in patients with atopic dermatitis compared to those with other types of atopic disease. This specific IgE response was only seen in patients with atopy and not in controls[19,92]. The present study confirms the finding of a high IgE response to *Pityrosporum* in patients with atopic dermatitis. It has also been shown that specific IgE responses to *Pityrosporum* correlate with the severity of dermatitis[46,93]. This was also found in the present study. Other work has revealed a high incidence of contact sensitivity to *Pityrosporum* in patients with atopic dermatitis[94]. It has therefore been suggested that an immunological response to *Pityrosporum* yeasts should be considered a possible pathogenic factor, particularly in patients with atopic dermatitis involving the seborrhoeic areas.

Ketoconazole has been shown to significantly improve skin lesions in patients with atopic dermatitis, especially in those with head and neck lesions[95]. However, ketoconazole has some anti-inflammatory effect as well as being active against *Pityrosporum*.

Further work is needed examining the usefulness of antifungal agents in atopic dermatitis, particularly with head and neck involvement.

4.11 SPECULATIONS ON CONTACT DERMATITIS PATHOLOGY

Staphylococcus aureus colonisation has also been reported in patients with allergic contact dermatitis[25,49]. In the present study, *Staph. aureus* was isolated in approximately 50% of samples from the involved skin of patients with allergic contact dermatitis. This is similar to published reports[25,49]. *Staph. aureus* could, therefore, induce similar damage in patients with allergic contact dermatitis as has already been proposed for atopic dermatitis patients. *Staph. aureus* also shows an increased adherence to corneocytes obtained from skin lesions in allergic contact dermatitis[25]. This is also similar to that seen in atopic dermatitis patients. Antibiotic treatment has also been used successfully in allergic contact dermatitis patients. Further work is required to confirm whether similar mechanisms to those proposed for patients with atopic dermatitis are present in allergic contact dermatitis patients. It is widely accepted that allergic contact dermatitis is due to the development of an inflammatory reaction to certain reactive compounds[96]. This reaction is caused by an upset in the normal immunologic balance between a protection of self and a destruction of non-self[97]. The mechanisms involved can be divided into two main stages : (1) the inductive or sensitization phase, and (2) the elicitation phase[98]. Briefly, the phase of sensitization requires a hapten, which is a reactive substance capable of causing an immune response. The hapten binds to antigen-presenting cells (Langerhans cells) in the skin. Keratinocytes are also now thought to play a part in the initiation and propagation of allergic contact dermatitis complementary to that of Langerhans cells[99]. This is the antigen-recognition phase. The Langerhans cells, loaded with antigen, then migrate to the regional lymph nodes and present antigen to T lymphocytes causing sensitization. Activated T cells migrate to the affected area

of skin and leave the circulation[100]. The elicitation phase occurs when antigen is introduced into an individual who has already been sensitized. The antigen is recognised. The previously sensitized T cells then come into contact with the antigen at the site of hapten application. A local inflammatory reaction then develops[98]. The immunological processes involved in allergic contact dermatitis are extremely complicated, with many of the mechanisms still poorly understood. A full immunological discussion of these mechanisms is beyond the scope of this study.

4.12 FUTURE DIRECTIONS

In order to clarify the role that *Pityrosporum* yeasts play in seborrhoeic dermatitis, further studies should examine, in detail, the behaviour of the yeast upon the skin of such patients. Investigation of the biochemistry of *Pityrosporum in vivo*, coupled with analysis of the host responses, should be undertaken. This would help reveal any factors which could enable this normal skin organism to be an initiating or perpetuating factor in seborrhoeic dermatitis.

With atopic dermatitis, the main emphasis has been placed upon *Staph. aureus*. Host immune responses to this organism have not yet been clearly established. Investigation into humoral and cellular immunological mechanisms could elucidate the effect caused by the presence of *Staph. aureus* on these patients' skin. Also, if this organism could be removed completely from the skin almost indefinitely, the disease process could be studied independently. This would establish how big a part *Staph. aureus* plays in the pathogenesis of atopic dermatitis.

Staph. aureus may also have a role in allergic contact dermatitis. Studies investigating the process of staphylococcal damage in atopic dermatitis could be

applicable to allergic contact dermatitis also. Parallel immunological and treatment studies should, therefore, be performed to establish if the mechanisms involved in atopic and allergic contact dermatitis are similar.

5. REFERENCES

1. Rivolta S. Parassiti vegetali. 1st ed. Torino: di Giulio Speirani, F. Figglo. 1873; pp 469-479. (cited by Ive FA, Rowland Payne CME. Seborrhoeic dermatitis. In: Verbov J, ed. Superficial Fungal Infections. Boston: MTP Press Limited, 1986: 73-87).
2. Malassez L. Note sur le champignon du pityriasis simple. Arch Physiol 1874; 1: 451. (cited by Ive FA, Rowland Payne CME. Seborrhoeic dermatitis. In: Verbov J, ed. Superficial Fungal Infections. Boston: MTP Press Limited, 1986: 73-87).
3. Sabouraud R. Pityriasis et alopécies peliculaire. Les maladies desquamatives. 1st ed. Paris: Masson et Cie, 1904: 295. (cited by Ive FA, Rowland Payne CME. Seborrhoeic dermatitis. In: Verbov J, ed. Superficial Fungal Infections. Boston: MTP Press Limited, 1986: 73-87).
4. Barnett JA, Payne RW, Yarrow D. Yeasts: characteristics and identification. Cambridge: Cambridge University Press, 1983. (cited by Midgley[7]).
5. Simmons RB, Ahearn DG. Cell wall ultrastructure and diazonium blue B reaction of *Sporopachydermia quercum*, *Bullera tsugae* and *Malassezia* spp. Mycologia 1987; 79: 38-43. (cited by Midgley[7]).
6. Slooff WCh. Genus 6 *Pityrosporum* Sabouraud. In: Lodder J, ed. The yeasts. A taxonomic study. 2nd ed. Amsterdam: North-Holland Publ Co, 1970: 1167-1186.
7. Midgley G. The diversity of *Pityrosporum* (*Malassezia*) yeasts *in vivo* and *in vitro*. Mycopathologia 1989; 106: 143-153.

8. Marcelou-Kinti O, Hadzivassiliou M, Kapetanakis I, Papavassiliou I. Isolation of yeast-like organisms of the genus *Pityrosporum* from the sand. Acta Microbiol Hellenica 1973; 18: 293-297. (cited by Midgley[7]).

9. Leeming JP, Notman FH, Holland KT. The distribution and ecology of *Malassezia furfur* and cutaneous bacteria on human skin. J Appl Bacteriol 1989; 67: 47-52.

10. Roth RR, James WD. Microbial ecology of the skin. Ann Rev Microbiol 1988; 42: 441-464.

11. Shuster S. The aetiology of dandruff and the mode of action of therapeutic agents. Br J Dermatol 1984; 111: 235-242.

12. Broberg A, Faergemann J. Infantile seborrhoeic dermatitis and *Pityrosporum ovale*. Br J Dermatol 1989; 120: 359-362.

13. Faergemann J, Fredriksson T, Nathorst-Windahl G. One case of confluent and reticulate papillomatosis (Gougerot-Carteaud). Acta Derm Venereol (Stockh) 1980; 60: 269-271.

14. Bergbrant I-M. Seborrhoeic dermatitis and *Pityrosporum ovale* : cultural, immunological and clinical studies. Acta Derm Venereol (Stockh) Suppl 1991; 167: 1-36.

15. Bergbrant I-M, Johansson S, Robbins D, Scheynius A, Faergemann J, Söderström T. An immunological study in patients with seborrhoeic dermatitis. Clin Exp Dermatol 1991; 16: 331-338.

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

17. Faergemann J. Antibodies to *Pityrosporum orbiculare* in patients with tinea versicolor and controls of various ages. J Invest Dermatol 1983; 80: 133-135.

18. Faergemann J, Johansson S, Bäck O, Scheynius A. An immunologic and cultural study of *Pityrosporum* folliculitis. J Am Acad Dermatol 1986; 14: 429-433.

19. Kieffer M, Bergbrant I-M, Faergemann J, *et al.* Immune reactions to *Pityrosporum ovale* in adult patients with atopic and seborrhoeic dermatitis. J Am Acad Dermatol 1990; 22: 739-742.

20. Bergbrant I-M, Johansson S, Robbins D, *et al.* The evaluation of various methods and antigens for the detection of antibodies against *Pityrosporum ovale* in patients with seborrhoeic dermatitis. Clin Exp Dermatol 1991; 16: 339-343.

21. Midgley G, Hay RJ. Serological responses to *Pityrosporum* (*Malassezia*) in seborrhoeic dermatitis demonstrated by ELISA and western blotting. Bull Soc Fr Mycol Méd 1988; 17: 267-276.

22. Leyden JJ, Marples RR, Kligman AM. *Staphylococcus aureus* in the lesions of atopic dermatitis. Br J Dermatol 1974; 90: 525-530.

23. Lennette EH, Balows A, Hausler WJ Jnr, Shadomy HJ, eds. Manual of clinical microbiology. 4th ed. Washington DC: American Society for Microbiology, 1985.

24. Stephen J, Pietrowski RA. Bacterial toxins. 2nd ed. Wokingham: Van Nostrand Reinhold, 1986: 79-83.

25. Williams REA, MacKie RM. The staphylococci. Importance of their control in the management of skin disease. Dermatologic Clinics 1993; 11: 201-206.

26. Lever R, Hadley K, Downey D, MacKie RM. Staphylococcal colonisation in atopic dermatitis and the effect of topical mupirocin therapy. Br J Dermatol 1988; 119: 189-198.

27. Unna PG. Seborrhoeal eczema. J Cutan Dis 1887; 5: 449-453. (cited by Ive FA, Rowland Payne CME. Seborrhoeic dermatitis. In: Verbov J, ed. Superficial Fungal Infections. Boston: MTP Press Limited, 1986: 73-87).

28. Fitzpatrick TB. Dermatology in general medicine. 3rd ed. New York: M^{c}Graw-Hill Book Company, 1987.

29. Rebora A, Rongioletti F. The red face: seborrhoeic dermatitis. Clinics in Dermatol 1993; 11: 243-251.

30. Mathes BM, Douglass MC. Seborrhoeic dermatitis in patients with acquired immunodeficiency syndrome. J Am Acad Dermatol 1985; 13: 947-951.

31. M^{c}Ginley KJ, Leyden JJ, Marples RR, Kligman AM. Quantitative microbiology of the scalp in non-dandruff, dandruff and seborrhoeic dermatitis. J Invest Dermatol 1975; 64: 401-405.

32. Heng MCY, Henderson CL, Barker DC, Haberfelde G. Correlation of *Pityrosporum ovale* density with clinical severity of seborrhoeic dermatitis as assessed by a simplified technique. J Am Acad Dermatol 1990; 23: 82-86.

33. Clift DC, Dodd HJ, Kirby JDT, Midgley G, Noble WC. Seborrhoeic dermatitis and malignancy. An investigation of the skin flora. Acta Derm Venereol (Stockh) 1988; 68: 48-52.

34. Ingham E, Cunningham AC. *Malassezia furfur.* J Med Vet Mycol 1993; 31: 265-288.

35. Carr MM, Pryce DM, Ive FA. Treatment of seborrhoeic dermatitis with ketoconazole : I. Response of seborrhoeic dermatitis of the scalp to topical ketoconazole. Br J Dermatol 1987; 116: 213-216.

36. Piérard GE, Piérard-Franchimont C, Van Cutsem J, Rurangirwa A, Hoppenbrouwers ML, Schrooten P. Ketoconazole 2% emulsion in the treatment of seborrhoeic dermatitis. Int J Dermatol 1991; 30: 806-809.

37. Willan R. On cutaneous diseases. London: Johnson, 1808. (cited by Fitzpatrick[28]).

38. Besnier E. Première note et observations préliminaires pour servir d'introduction à l'étude diathesique. Ann Dermatol Syphiligr (Paris) 1892; 4: 634. (cited by Fitzpatrick[28]).

39. Coca AF, Cooke RA. On the classification of the phenomena of hypersensitiveness. J Immunol 1923; 8: 163. (cited by Fitzpatrick[28]).

40. Hill LW, Sulzberger MB. Evolution of atopic dermatitis. Arch Dermatol 1935; 32: 451. (cited by Fitzpatrick[28]).

41. The Committee on Prevalence, Morbidity and Cost of Dermatological Disease. Eczematous and immunologic diseases. J Invest Dermatol 1979; 73: 414-433.

42. Williams REA, Gibson AG, Aitchison TC, Lever R, MacKie RM. Assessment of a contact-plate sampling technique and subsequent quantitative bacterial studies in atopic dermatitis. Br J Dermatol 1990; 123: 493-501.

43. Dahl MV. *Staphylococcus aureus* and atopic dermatitis. Arch Dermatol 1983; 119: 840-846.

44. Morton F, Lever RS, Hadley KM, MacKie RM. The importance of staphylococcal colonisation in atopic dermatitis. Br J Dermatol 1988; 118: 280-281.

45. Svejgaard E. The role of microorganisms in atopic dermatitis. Seminars in Dermatol 1990; 9: 255-261.

46. Young E, Koers WJ, Berrens L. Intracutaneous tests with *Pityrosporum* extract in atopic dermatitis. Acta Derm Venereol (Stockh) Suppl 1989; 144: 122-124.

47. Collins ET. Atropine irritation. R Lond Ophthal Hosp Rep 1889; 12: 164. (cited by Fitzpatrick[28]).

48. Jadassohn J. Zur Kenntnis der Medikamentosen Dermatosen. Verh Dtsch Dermatol Ges V Kong 1895; 103. (cited by Fitzpatrick[28]).

49. Masenga J, Garbe C, Wagner J, Orfanos CE. *Staphylococcus aureus* in atopic dermatitis and in nonatopic dermatitis. Int J Dermatol 1990; 29: 579-582.

50. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol (Stockh) Suppl 1980; 92: 44-47.

51. Van Abbe, NJ. The investigation of dandruff. J Soc Cosmetic Chemists 1964; 15: 609-630.

52. Williamson P, Kligman AM. A new method for the quantitative investigation of cutaneous bacteria. J Invest Dermatol 1965; 45: 498-503.

53. Gibson AG. *Staphylococcus aureus* and atopic dermatitis. MSc Thesis. Uni. of Glasgow: Dept. of Microbiology, 1989.

54. Johansson S, Faergemann J. Enzyme-linked immunosorbent assay (ELISA) for detection of antibodies against *Pityrosporum orbiculare*. J Med Vet Mycol 1990; 28: 257-260.

55. Aly R, Maibach HI, Shinefield HR. Microbial flora of atopic dermatitis. Arch Dermatol 1977; 113: 780-782.

56. Ashbee HR, Ingham E, Holland KT, Cunliffe WJ. The carriage of *Malassezia furfur* serovars A, B and C in patients with pityriasis versicolor, seborrhoeic dermatitis and controls. Br J Dermatol 1993; 129: 533-540.

57. Faergemann J. The use of contact plates for quantitative culture of *Pityrosporum orbiculare*. Mykosen 1987; 30: 298-304.

58. Bergbrant I-M, Igerud A, Nordin P. An improved method for quantitative culture of *Malassezia furfur*. Res Microbiol 1992; 143: 731-735.

59. Bloom E, Aly R, Maibach HI. Quantitation of skin bacteria : lethality of the wash solution used to remove bacteria. Acta Dermatovener (Stockh) 1979; 59: 460-463.

60. Faergemann J. Quantitative culture of *Pityrosporum orbiculare*. Int J Dermatol 1984; 23: 330-333.

61. Noble WC, Valkenburg HA, Wolters CHL. Carriage of *Staphylococcus aureus* in random samples of a normal population. J Hygiene (Cambridge) 1967; 65: 567-573.

62. White A, Smith J. Nasal reservoir as a source of extranasal staphylococci. Antimicrob Agents Chemother 1963; 5: 679-683.

63. Cunningham AC, Ingham E, Gowland G. Humoral responses to *Malassezia furfur* serovars A, B and C in normal individuals of various ages. Br J Dermatol 1992; 127: 476-481.

64. Sohnle PG, Collins-Lech C, Huhta KE. Class-specific antibodies in young and aged humans against organisms producing superficial fungal infections. Br J Dermatol 1983; 108: 69-76.

65. Wikler JR, de Haan P, Nieboer C. The "tape-method": a new and simple method for quantitative culture of *Pityrosporum* yeasts. Acta Derm Venereol (Stockh) 1988; 68: 445-449.

66. Bergbrant I-M, Faergemann J. The role of *Pityrosporum ovale* in seborrhoeic dermatitis. Seminars in Dermatol 1990; 9: 262-268.

67. M^{c}Grath J, Murphy GM. The control of seborrhoeic dermatitis and dandruff by antipityrosporal drugs. Drugs 1991; 41: 178-184.

68. Faergemann J. *In vitro* and *in vivo* activities of ketoconazole and itraconazole against *Pityrosporum ovale*. Antimicrob Agents Chemother 1984; 26: 773-775.

69. Ive FA. The treatment of seborrhoeic dermatitis and dandruff with topical ketoconazole. In: Shuster S, Blatchford N, eds. Seborrhoeic dermatitis and dandruff - a fungal disease. Royal Society of Medicine International Congress and Symposium Series No. 132: Royal Society of Medicine Services Limited, 1988: 21-27.

70. Katsambas A, Antoniou CH, Frangouli E, Avgerinou G, Michailidis D, Stratigos J. A double-blind trial of treatment of seborrhoeic dermatitis with 2% ketoconazole cream compared with 1% hydrocortisone cream. Br J Dermatol 1989; 121: 353-357.

71. Skinner RB Jnr, Noah PW, Taylor RM, *et al.* Double-blind treatment of seborrhoeic dermatitis with 2% ketoconazole cream. J Am Acad Dermatol 1985; 12: 852-856.

72. Cauwenbergh G. International experience with ketoconazole shampoo in the treatment of seborrhoeic dermatitis and dandruff. In: Shuster S, Blatchford N, eds. Seborrhoeic dermatitis and dandruff - a fungal disease. Royal Society of Medicine International Congress and Symposium Series No. 132: Royal Society of Medicine Services Limited, 1988: 35-45.

73. Midgley G. Studies on the antigens of *Pityrosporum* (*Malassezia*) species. In: Drouhet E, ed. Fungal antigens - isolation, purification and detection. Plenum Publishing, 1988: 342.

74. Ran Y, Yoshiike T, Ogawa H. Lipase of *Malassezia furfur*: some properties and their relationship to cell growth. J Med Vet Mycol 1993; 31: 77-85.

75. Kligman AM, Leyden JJ. Seborrhoeic dermatitis. Seminars in Dermatol 1983; 2: 57-59.

76. Burton JL, Pye RJ. Seborrhoea is not a feature of seborrhoeic dermatitis. Br Med J 1983; 286: 1169-1170.

77. Hodgson-Jones I, Mackenna RMS, Wheatley VR. The surface fat in seborrhoeic dermatitis. Br J Dermatol 1953; 65: 246-251.

78. Cole GW, Silverberg NL. The adherence of *Staphylococcus aureus* to human corneocytes. Arch Dermatol 1986; 122: 166-169.

79. Bibel DJ, Aly R, Shinefield HR, Maibach HI, Strauss WG. Importance of the keratinized epithelial cell in bacterial adherence. J Invest Dermatol 1982; 79: 250-253.

80. Miyake Y, Sugai M, Kohada A, Minagi S, Suginaka H. Adherence of *Staphylococcus aureus* to cultured epidermal cells during differentiation. J Med Microbiol 1990; 32: 9-14.

81. Bibel DJ, Aly R, Shinefield HR, Maibach HI. The *Staphylococcus aureus* receptor for fibronectin. J Invest Dermatol 1983; 80: 494-496.

82. Lopes JD, Reis MDos, Brentani RR. Presence of laminin receptors in *Staphylococcus aureus*. Science 1985; 229: 275-277.

83. White MI, Noble WC. Skin responses to protein A. Proc R Soc Edinburgh 1980; 79B: 43-46.

84. Abramson JS, Dahl MV, Walsh G, Blumenthal MN, Douglas SD, Quie PG. Antistaphylococcal IgE in patients with atopic dermatitis. J Am Acad Dermatol 1982; 7: 105-110.

85. Friedman SJ, Schroeter AL, Homburger HA. IgE antibodies to *Staphylococcus aureus*: prevalence in patients with atopic dermatitis. Arch Dermatol 1985; 121: 869.

86. Neuber K, Hilger RA, Konig W. Differential increase in 12-HETE release and CD29/CD49f expression of platelets from normal donors and from patients with atopic dermatitis by *Staphylococcus aureus*. Int Arch Allergy Immunol (Switzerland) 1992; 98: 339-342.

87. Hoeger PH, Niggemann B, Schroeder C. Enhanced basal and stimulated PMN chemiluminescence activity in children with atopic dermatitis: stimulatory role of colonizing staphylococci? Acta Paediatr 1992; 81: 542-546.

88. Saurat JH, Schmied C. Antimicrobial treatment of atopic dermatitis. Abstract No. 165; Clinical Dermatology in the Year 2000: London, 1990.

89. Luber H, Amornsiripanitch S, Lucky AW. Mupirocin and the eradication of *Staphylococcus aureus* in atopic dermatitis. Arch Dermatol 1988; 124: 853.

90. Stalder JF, Fleury M, Sourisse M, *et al.* Comparative effects of two topical antiseptics (chlorhexidine vs $KMnO_4$) on bacterial skin flora in atopic dermatitis. Acta Derm Venereol (Stockh) Suppl 1992; 176: 132-134.

91. Nilsson EJ, Henning CG, Magnusson J. Topical corticosteroids and *Staphylococcus aureus* in atopic dermatitis. J Am Acad Dermatol 1992; 27: 29-34.

92. Broberg A, Faergemann J, Johansson S, Johansson SGO, Strannegård I-L, Svejgaard E. *Pityrosporum ovale* and atopic dermatitis in children and young adults. Acta Derm Venereol (Stockh) 1992; 72: 187-192.

93. Ring J, Abeck D, Neuber K. Atopic eczema: role of microorganisms on the skin surface. Allergy 1992; 47: 265-269.

94. Rokugo M, Tagami H, Usuba Y, Tomita Y. Contact sensitivity to *Pityrosporum ovale* in patients with atopic dermatitis. Arch Dermatol 1990; 126: 627-632.

95. Clemmensen OJ, Hjorth N. Treatment of dermatitis of the head and neck with ketoconazole in patients with type 1 hypersensitivity to *Pityrosporum orbiculare*. Seminars in Dermatol 1983; 2: 26-29.

96. Bergstresser PR. Sensitization and elicitation of inflammation in contact dermatitis. Immunol Ser 1989; 46: 219-245.

97. Baadsgaard O, Wang T. Immune regulation in allergic and irritant skin reactions. Int J Dermatol 1991; 30: 161-172.

98. Sauder DN. Allergic contact dermatitis. In: Thiers BH, Dobson RL, eds. Pathogenesis of skin disease. Churchill Livingstone, 1986: 3-12.

99. Barker JNWN. Role of keratinocytes in allergic contact dermatitis. Contact Dermatitis 1992; 26: 145-148.

100. Kalish RS. Recent developments in the pathogenesis of allergic contact dermatitis. Arch Dermatol 1991; 127: 1558-1563.

6. APPENDICES

SITE	VALID N	SCRUB WASH MEAN PERCENTAGE				VALID N	CONTACT PLATE MEAN PERCENTAGE			
		FORM1	FORM2	FORM3	P.ORB		FORM1	FORM2	FORM3	P.ORB
1-SCALP	8	12.5	0.0	28.2	59.3	5	40.0	0.0	21.7	38.3
2-EAR	11	9.8	9.1	19.7	61.4	11	10.3	27.3	39.2	23.3
3-NECK	16	17.8	12.5	44.7	25.0	17	6.0	19.5	44.6	29.9
4-CHEST	15	13.8	6.9	61.9	17.3	15	7.2	10.0	62.7	20.0
5-ELBOW	10	10.0	10.0	50.0	30.0	2	0.0	0.0	100.0	0.0
6-HAND	7	14.3	28.6	57.1	0.0	4	0.0	25.0	75.0	0.0
7-FOOT	4	0.0	75.0	25.0	0.0	3	22.2	66.7	11.1	0.0
8-KNEE	1	0.0	0.0	80.0	20.0	3	0.0	0.0	66.7	33.3
9-INV.	10	25.0	10.0	55.0	10.0	17	14.7	17.6	40.6	27.1
10-UNINV.	16	7.3	14.3	53.5	24.9	14	14.3	21.4	47.6	16.7
11-FACE	5	30.9	0.0	57.7	11.4	8	2.3	0.0	60.2	37.5

APPENDIX 1: RELATIVE PERCENTAGES OF *Pityrosporum ovale* FORMS 1, 2 OR 3 AND *Pityrosporum orbiculare* OBTAINED FROM THOSE SEBORRHOEIC DERMATITIS PATIENTS WITH POSITIVE *Pityrosporum* CARRIAGE.

SITE	VALID N	SCRUB WASH MEAN PERCENTAGE				VALID N	CONTACT PLATE MEAN PERCENTAGE			
		FORM1	FORM2	FORM3	P.ORB		FORM1	FORM2	FORM3	P.ORB
1-SCALP	11	9.1	0.0	15.3	75.6	11	9.1	0.0	27.3	63.6
2-EAR	7	14.3	0.0	14.3	71.4	8	25.0	0.0	37.5	37.5
3-NECK	5	20.0	0.0	60.0	20.0	6	0.0	0.0	90.3	9.7
4-CHEST	10	10.0	0.0	66.4	23.6	9	11.1	0.0	63.1	25.8
5-ELBOW	3	0.0	0.0	100.0	0.0	4	25.0	0.0	75.0	0.0
6-HAND	5	20.0	0.0	60.0	20.0	4	25.0	0.0	75.0	0.0
7-FOOT	1	100.0	0.0	0.0	0.0	2	50.0	0.0	50.0	0.0
8-KNEE	5	20.0	0.0	60.0	20.0	3	33.3	0.0	66.7	0.0
9-INV.	2	50.0	0.0	50.0	0.0	3	33.3	0.0	57.8	8.9
10-UNINV.	5	20.0	0.0	60.0	20.0	3	33.3	0.0	66.7	0.0
11-FACE	6	16.7	0.0	52.6	30.7	9	0.0	0.0	58.7	41.3

APPENDIX 2: RELATIVE PERCENTAGES OF *Pityrosporum ovale* FORMS 1, 2 OR 3 AND *Pityrosporum orbiculare* OBTAINED FROM THOSE ATOPIC DERMATITIS PATIENTS WITH POSITIVE *Pityrosporum* CARRIAGE.

SITE	VALID N	SCRUB WASH MEAN PERCENTAGE				VALID N	CONTACT PLATE MEAN PERCENTAGE			
		FORM1	FORM2	FORM3	P.ORB		FORM1	FORM2	FORM3	P.ORB
1-SCALP	5	11.4	1.3	0.0	87.3	8	0.0	0.0	37.5	62.5
2-EAR	3	0.0	0.0	33.3	66.7	6	0.0	0.0	33.3	66.7
3-NECK	5	0.0	0.0	40.0	60.0	6	0.0	0.0	50.0	50.0
4-CHEST	6	0.0	0.0	17.3	82.7	7	0.0	0.0	50.6	49.4
5-ELBOW	1	0.0	0.0	0.0	100.0	0	-	-	-	-
6-HAND	1	0.0	0.0	100.0	0.0	1	0.0	0.0	0.0	100.0
7-FOOT	0	-	-	-	-	1	0.0	0.0	100.0	0.0
8-KNEE	0	-	-	-	-	0	-	-	-	-
9-INV.	1	0.0	0.0	100.0	0.0	1	0.0	0.0	100.0	0.0
10-UNINV.	0	-	-	-	-	0	-	-	-	-
11-FACE	2	0.0	0.0	0.0	100.0	6	0.0	0.0	50.0	50.0

APPENDIX 3: RELATIVE PERCENTAGES OF *Pityrosporum ovale* FORMS 1, 2 OR 3 AND *Pityrosporum orbiculare* OBTAINED FROM THOSE CONTACT DERMATITIS PATIENTS WITH POSITIVE *Pityrosporum* CARRIAGE.

SITE	VALID N	SCRUB WASH MEAN PERCENTAGE				VALID N	CONTACT PLATE MEAN PERCENTAGE			
		FORM1	FORM2	FORM3	P.ORB		FORM1	FORM2	FORM3	P.ORB
1-SCALP	11	18.2	0.0	22.4	59.5	12	0.0	0.0	51.1	48.9
2-EAR	12	0.0	0.0	47.2	52.8	16	6.3	0.0	39.3	54.5
3-NECK	13	0.0	0.0	73.3	26.7	16	6.3	0.0	63.5	30.2
4-CHEST	15	0.0	0.0	69.6	30.4	15	0.0	0.0	77.6	22.4
5-ELBOW	5	0.0	0.0	66.4	33.6	8	0.0	0.0	91.7	8.3
6-HAND	1	0.0	0.0	100.0	0.0	5	0.0	0.0	80.0	20.0
7-FOOT	2	0.0	0.0	100.0	0.0	0	-	-	-	-
8-KNEE	1	0.0	0.0	100.0	0.0	1	0.0	0.0	100.0	0.0
9-INV.	0	-	-	-	-	0	-	-	-	-
10-UNINV.	0	-	-	-	-	0	-	-	-	-
11-FACE	5	0.0	0.0	80.0	20.0	10	0.0	0.0	59.0	41.0

APPENDIX 4: RELATIVE PERCENTAGES OF *Pityrosporum ovale* FORMS 1, 2 OR 3 AND *Pityrosporum orbiculare* OBTAINED FROM THOSE CONTROLS WITH POSITIVE *Pityrosporum* CARRIAGE.

SITE	Staph. aureus		CNS/MIC		Diptheroids		Pityrosporum from Scrub		Pityrosporum from Plate	
	Mean	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max
1-SCALP	$1.0x10^3$	$1.7x10^4$	$6.5x10^5$	$1.1x10^7$	$9.2x10^4$	$9.6x10^5$	$1.8x10^2$	$3.3x10^3$	<1.0	2.0
2-EAR	2.3x10	$4.5x10^2$	$3.1x10^5$	$3.7x10^6$	$2.0x10^4$	$1.8x10^5$	$2.2x10^2$	$2.0x10^3$	4.0	5.5x10
3-NECK	0.0	0.0	$4.6x10^5$	$4.7x10^6$	$4.0x10^3$	$3.5x10^4$	$9.3x10^2$	$8.2x10^3$	4.0	5.6x10
4-CHEST	$6.5x10^2$	$1.3x10^4$	$1.5x10^6$	$2.1x10^7$	$3.1x10^5$	$3.7x10^6$	$8.3x10^2$	$4.0x10^3$	2.3x10	$1.1x10^2$
5-ELBOW	0.0	0.0	$6.5x10^4$	$5.2x10^5$	$5.7x10^4$	$5.8x10^5$	9.0	7.5x10	<1.0	<1.0
6-HAND	0.0	0.0	$2.5x10^4$	$3.7x10^5$	$1.0x10^3$	$1.8x10^4$	2.0	1.7x10	<1.0	<1.0
7-FOOT	0.0	0.0	$1.9x10^5$	$1.7x10^6$	$7.6x10^3$	$1.5x10^5$	<1.0	3.0	<1.0	1.0
8-KNEE	$2.4x10^3$	$4.7x10^4$	$8.9x10^2$	$8.9x10^3$	0.0	0.0	1.0	1.1x10	<1.0	<1.0
9-INV.	0.0	0.0	$1.2x10^6$	$1.0x10^7$	$2.1x10^6$	$4.1x10^7$	$8.6x10^2$	$7.0x10^3$	9.0	$1.1x10^2$
10-UNINV.	0.0	0.0	$1.7x10^5$	$2.2x10^6$	$2.5x10^4$	$3.7x10^5$	$2.1x10^2$	$2.0x10^3$	3.0	5.5x10
11-FACE	$2.4x10^2$	$4.7x10^3$	$7.4x10^5$	$8.2x10^6$	$5.7x10^5$	$7.0x10^6$	3.0	1.9x10	<1.0	2.0

All minimum values were zero.

APPENDIX 5: NUMBER OF COLONY FORMING UNITS OF EACH ORGANISM PER CM^2 OF SKIN OF EACH SITE FROM SEBORRHOEIC DERMATITIS PATIENTS.

SITE	Staph. aureus		CNS/MIC		Diptheroids		Pityrosporum from Scrub		Pityrosporum from Plate	
	Mean	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max
1-SCALP	$5.4x10^7$	$6.8x10^8$	$1.6x10^7$	$2.3x10^8$	$1.8x10^5$	$1.8x10^6$	$4.0x10^3$	$6.7x10^4$	<1.0	2.0
2-EAR	$1.6x10^7$	$2.6x10^8$	$8.4x10^6$	$1.2x10^8$	$6.0x10^5$	$9.4x10^6$	1.1x10	9.6x10	<1.0	4.0
3-NECK	$4.0x10^6$	$5.2x10^7$	$4.1x10^6$	$4.2x10^7$	$1.5x10^4$	$2.5x10^5$	5.7x10	$4.2x10^2$	<1.0	1.0
4-CHEST	$2.0x10^5$	$1.8x10^6$	$4.2x10^6$	$6.3x10^7$	$7.9x10^4$	$1.3x10^6$	$1.0x10^2$	$5.6x10^2$	1.0	4.0
5-ELBOW	$1.6x10^8$	$2.4x10^9$	$8.1x10^6$	$8.5x10^7$	$3.4x10^4$	$5.8x10^5$	8.7x10	$1.4x10^3$	<1.0	1.0
6-HAND	$8.3x10^5$	$1.4x10^7$	$1.1x10^6$	$7.6x10^6$	0.0	0.0	2.0	2.1x10	<1.0	1.0
7-FOOT	$4.1x10^6$	$6.9x10^7$	$1.1x10^6$	$1.8x10^7$	$3.5x10^4$	$5.5x10^5$	$2.8x10^2$	$4.8x10^3$	<1.0	1.0
8-KNEE	$1.1x10^7$	$7.6x10^7$	$1.8x10^6$	$1.6x10^7$	$2.0x10^5$	$3.3x10^6$	3.6x10	$6.0x10^2$	<1.0	2.0
9-INV.	$2.3x10^8$	$2.3x10^9$	$2.9x10^7$	$4.9x10^8$	$6.8x10^4$	$7.1x10^5$	7.0	$1.1x10^2$	<1.0	4.0
10-UNINV.	$8.4x10^4$	$1.0x10^6$	$6.1x10^5$	$6.7x10^6$	$8.2x10^3$	$1.0x10^5$	1.3x10	$2.0x10^2$	<1.0	<1.0
11-FACE	$3.4x10^7$	$5.8x10^8$	$4.1x10^7$	$6.2x10^8$	$3.9x10^5$	$5.0x10^6$	9.7x10	$1.6x10^3$	<1.0	3.0

All minimum values were zero.

APPENDIX 6: NUMBER OF COLONY FORMING UNITS OF EACH ORGANISM PER CM^2 OF SKIN OF EACH SITE FROM ATOPIC DERMATITIS PATIENTS.

SITE	Staph. aureus		CNS/MIC		Diptheroids		Pityrosporum from Scrub		Pityrosporum from Plate	
	Mean	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max
1-SCALP	$2.1x10^6$	$1.5x10^7$	$4.1x10^5$	$3.6x10^6$	$1.1x10^3$	$1.1x10^4$	2.8x10	$1.7x10^2$	1.0	2.0
2-EAR	$1.4x10^3$	$1.3x10^4$	$9.8x10^4$	$5.3x10^5$	$2.1x10^5$	$2.1x10^6$	4.0	2.2x10	1.0	4.0
3-NECK	$4.3x10^2$	$4.3x10^3$	$1.8x10^4$	$7.1x10^4$	$9.2x10^3$	$6.1x10^4$	4.6x10	$2.7x10^2$	1.0	4.0
4-CHEST	0.0	0.0	$2.9x10^4$	$9.5x10^4$	8.4x10	$8.4x10^2$	3.8x10	$1.3x10^2$	1.0	3.0
5-ELBOW	0.0	0.0	$5.0x10^4$	$4.2x10^5$	$2.5x10^2$	$2.5x10^3$	<1.0	2.0	0.0	0.0
6-HAND	$2.5x10^6$	$2.0x10^7$	$5.8x10^5$	$5.4x10^6$	0.0	0.0	<1.0	2.0	<1.0	<1.0
7-FOOT	$2.3x10^6$	$2.3x10^7$	$1.5x10^6$	$1.4x10^7$	$2.0x10^5$	$2.0x10^6$	0.0	0.0	<1.0	<1.0
8-KNEE	$2.1x10^2$	$1.3x10^3$	$1.9x10^4$	$1.8x10^5$	4.5x10	$4.5x10^2$	0.0	0.0	0.0	0.0
9-INV.	$4.9x10^6$	$2.9x10^7$	$3.2x10^5$	$3.2x10^6$	0.0	0.0	1.0	6.0	<1.0	<1.0
10-UNINV.	0.0	0.0	$3.4x10^3$	$1.6x10^4$	$1.3x10^2$	$1.3x10^3$	0.0	0.0	0.0	0.0
11-FACE*	$4.0x10^2$	$4.0x10^3$	$4.9x10^5$	$2.6x10^6$	0.0	0.0	1.0	5.0	1.0	4.0

All minimum values were zero, except * CNS/MIC obtained from the face (minimum value $2.8x10^3$).

APPENDIX 7: NUMBER OF COLONY FORMING UNITS OF EACH ORGANISM PER CM^2 OF SKIN OF EACH SITE FROM CONTACT DERMATITIS PATIENTS.

SITE	Staph. aureus		CNS/MIC		Diptheroids		Pityrosporum from Scrub		Pityrosporum from Plate	
	Mean	Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max
1-SCALP	0.0	0.0	$7.6x10^4$	$4.9x10^5$	$1.6x10^5$	$1.1x10^6$	$1.2x10^4$	$6.7x10^4$	4.0	5.5x10
2-EAR	$3.7x10^3$	$6.3x10^4$	$9.9x10^4$	$7.6x10^5$	$2.7x10^4$	$1.6x10^5$	2.8x10	$2.2x10^2$	1.0	3.0
3-NECK	$1.1x10^2$	$1.8x10^3$	$3.4x10^4$	$4.1x10^5$	$1.6x10^3$	$1.5x10^4$	$6.2x10^2$	$3.0x10^3$	1.0	4.0
4-CHEST	0.0	0.0	$1.4x10^4$	$1.4x10^5$	$3.9x10^2$	$1.9x10^3$	$5.3x10^2$	$4.6x10^3$	2.0	9.0
5-ELBOW	0.0	0.0	$1.3x10^4$	$1.1x10^5$	$1.8x10^3$	$2.0x10^4$	1.0x10	$1.2x10^2$	<1.0	1.0
6-HAND	2.0	4.0x10	$2.7x10^2$	$1.3x10^3$	$1.1x10^2$	$9.0x10^2$	<1.0	5.0	<1.0	<1.0
7-FOOT	0.0	0.0	$9.9x10^4$	$1.2x10^6$	$1.9x10^4$	$2.5x10^5$	<1.0	5.0	0.0	0.0
8-KNEE	0.0	0.0	$4.0x10^2$	$1.4x10^3$	9.0x10	$1.3x10^3$	<1.0	2.0	<1.0	<1.0
9-INV.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10-UNINV.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11-FACE	0.0	0.0	$9.4x10^4$	$4.9x10^5$	$4.0x10^4$	$4.6x10^5$	2.0	1.1x10	<1.0	2.0

All minimum values were zero.

APPENDIX 8: NUMBER OF COLONY FORMING UNITS OF EACH ORGANISM PER CM^2 OF SKIN OF EACH SITE FROM CONTROLS.