Staphylococcus Aureus and Atopic Dermatitis

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Summary

This study discovered that numbers of S.aureus and coagulase negative staph/micro-cocci (CNS/M) correlated between contact plate sampling and the surface wash technique of Williamson and Kligman, 1965. No correlation was found for the diphtheroids. One way analysis of variance across atopic dermatitis (A.D.) scores showed a trend of increasing S.aureus counts with increasing A.D. score for the contact plate method ($p << 0.001$) and to a lesser extent for the surface wash technique ($p<0.001$).

It is suggested that defective delta-6-desaturase (an enzyme of unsaturated fatty acid metabolism) function could be the cause of the abnormal bacterial flora of A.D.

Introduction

Atopic dermatitis (A.D.) or eczema is a common yet poorly understood skin complaint. It has a prevalence of 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. Persistence of approximately 60%, with the worst afflicted most likely to retain the disease, have been shown in follow-up surveys of fifteen to twenty years duration. (The committee, 1979).

Patients suffer from pruritus and conco-mitant lack of sleep as well as the social consequences of the skin's appearance (The committee, 1979). Skin changes other than xerosis include sweat retention, low sebum output and high pH(Abe et al, 1978). The elevated pH is a direct result of diminished sweat delivery and the higher pH values are to be found in the sites of predilection for the disease i.e. 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 (Noble, 1981). Patients frequently display the other atopic diseases of asthma and/or hayfever.

Other differences from healthy unaffected persons are those of the bacterial skin flora and the immune system of A.D. sufferers.

Bacterial Skin Flora

The normal bacterial flora of skin consists of the coagulase negative staphylococci and micrococci (CNS/M) as well as the diphtheroids (sometimes termed Coryneforms). These bacteria are predominant though much smaller numbers of Streptococci and Gram negative bacilli may be found. *Staphylococcus aureus* may be carried in the nose, 10-40% (Williams, 1963), or perineum of healthy subjects (Hay, 1985) but it is rarely found (approximately 5%) in other clinically normal sites of the 'normal' population.

People with A.D. display abnormal carriage of S.aureus. In one study 79% of those examined had S.aureus nasal carriage. More significantly S.aureus carriage was shown to be 76% and 93% for uninvolved and lesional skin respectively (Aly, Maibach & Shinefield, 1977). In the lesions S.aureus constituted 91% of the aerobic flora, coagulase negative staphylococci 9%. Uninvolved skin had flora that was 63% coagulase negative staphylococci, 30% S.aureus and 2% lipophilic diphtheroids. It was suggested that preponderance of lipophilic diphtheroids, as seen on the skin of healthy subjects, was indicative of normal healthy skin.

The stratum corneum of the A.D. patient may be unusually receptive to S.aureus colonisation on the basis of binding, nutrient factors or deranged normal flora (Dahl, 1983) Respiratory atopics without dermatitis were shown not to have S.aureus colonisation of their skin (Morton, Lever et al, 1988)

In a study by Cole and Silverberg, 1986 S.aureus was the only bacterial species that was able to adhere avidly to A.D. cor-neocytes. The Cowan 1 S.aureus strain, which was rich in protein A, was found to adhere much more readily than did the protein A-deficient Wood strain. The percentages of cor-neocytes examined with twenty plus S.aureus adhered (PAI) was 40% and 10% respectively. Cole and Silverberg also found after an addition of protein A to an adherence mixture of S.aureus and cor-neocytes the PAI was reduced from 56% to 27%. Both these results suggest a role for protein A in adherence though Bibel et al, 1982 believed that the degree of epithelial cell keratinisation as well as a specific receptor for S.aureus was responsible.

Deranged normal flora is another possible explanation. Xerosis of A.D. skin is a likely explanation for the paucity of lipophilic diphtheroid numbers mentioned earlier (Aly et al, 1977). The absolute requirement of the lipophilic diphtheroids for unsaturated fatty acids, and the fact that these strains are by far the most numerous aerobic diphtheroids in the normal flora

(Marples, 1969), might explain the reduced numbers observed. S.aureus colonisers of the skin would then have less competative interference from pre-existing skin flora.

The Immune Systems

Immune system abnormalities are present in both the humoral and cell-mediated immunities. Immunoglobulin E(IgE) antibody levels have been found to be elevated in more than 80% of A.D. patients. Much has been written about the role of IgE in A.D. but this has tended to be inconclusive. Elevated IgE levels roughly correspond with the disease severity, yet its significance is questionable as every study shows there are A.D. patients with normal IgE levels. It has been shown though that skin IgE levels may be elevated irrespective of serum levels (Jansen et al, 1973). Raised serum IgE levels also occur in non-A.D. associated conditions and IgE levels may remain elevated after clinical remission of A.D.

One study suggested that serum IgE levels are highest in those A.D. patients also with allergic respiratory disease (A.R.D.), e.g. asthma and hayfever, whereas they are relatively low in those with A.D. but not A.R.D. Less noticeable differences have also been reported for the other immunoglobulin classes i.e. increased IgG and IgM as well as decreased IgD levels in a group of A.D. patients (Hanifin and Lobnitz, 1977)

Substantially lower numbers of T-lymphocytes (T cells) were found in A.D. patients (Rogge and Hanifin, 1976). Again this might be linked to elevated IgE levels as Tada et al, 1973 found abnormally high and prolonged IgE production in T cell depleted rats.

Increased IgE binding to S.aureus but not S.epidermidis has been observed in A.D patients. In hyperimmunoglobulaemia-E-staphylococcal-abscess syndrome (HESA), a condition with chronic eczematous dermatitis resembling that of A.D., increased IgE binding to S.epidermidis as well as S.aureus was

seen. It was suggested that interaction of staphylococcal antigens from the bacteria, with anti-staphylococcal IgE antibodies on mast cells could induce the release of mast cells' contents e.g. histamine causing pruritus and aggravation of A.D. This is partly supported by the fact that A.D. frequently improves when treated with topical or systemic antibodies (Abramson et al, 1982). Studies by Schopfer et al, 1980 has suggested that the IgE binding is related to the peptidoglycan interpeptide bridge or an unknown antigenic structure within the S.aureus cell wall.

Many cell-mediated immune system abnormalities also exist. Decreased delayed hypersensitivity is seen in A.D. patients. Cell-mediated immunity depression has been found to fluctuate with disease severity by many laboratories. Plasma from A.D. patients has been found to inhibit normal chemotaxis of neutrophils. This might be an explanation for the frequent cutaneous staphylococcal infections seen in A.D. patients (The committee, 1979).

There is evidence that cell-mediated immunity may be involved in A.D. pathogenesis. Demonstration of considerable numbers of IRC (Interdigitating Reticular Cells), Langerhan cells and Macrophage cells within the dermal infiltrate and the proximity of these APC (Antigen Presenting Cells) to helper T cells support the theory of cell-mediated immunity being an intrinsic part of the eczematous process. The relative abundance of IRC suggests that they may be involved in persistence of cell-mediated immune mechanisms in A.D. pathogenesis as IRC are not normally found in healthy skin and human lymphocyte antigen (HLA) group DR positivity, almost universally found on the infiltrating cells, suggests lymphocyte activation. This is further evidence of immunostimulation (Zachary et al, 1985).

Natural killer cell responses in A.D. patients have also been observed to be significantly impaired compared to controls (Larsen et al, 1985). The multiple cell functional defects seen during acute A.D. flares could be due to

an inhibitory plasma factor though the 'factor' would have to have long term affects on cells as was observed in various in vitro experiments. If such a factor was introduced by an infecting organism S.aureus is a good candidate. S.aureus produces a plethora of enzymatic and other substances e.g.α -toxin(dermonecrotic)β, γ and δ toxins, leucocidins F and S (dermonecrotic and toxic to human polymorphonucleocytes), enterotoxins A,B,C_1,C_2 E and F, lipase, neuraminidase, hyaluronidase, DNAse, staphylokinase (activates plasminogen, dissolves clots), staphylocoagulase as well as cell-bound coagulase and others (Stephen and Pietrowski, 1986).

A.D. Management

The usual treatment of A.D. is with topical corticosteroid formulations and daily attention, by the patient, of general management methods e.g. avoidance of exertion, overheating and avoidance of skin irritants (The committee, 1979). A case has been made for steroid-antibiotic combination treatment. Leyden and Kligman, 1977 demonstrated that combination treatment produced superior clinical and bacteriological results in one week's time than did either the antibiotic or steroid alone. They noted that an appreciable clinical improvement occurred only in those patients whose lesions initially contained more than 10^6 S.aureus/cm^2.

Study Aims

We wanted to demonstrate correlation of bacterial numbers between two skin bacterial sampling techniques-contact plates and the surface wash technique of Williamson and Kligman, 1965. Correlation would allow the faster more convenient contact plates to be used for extensive sampling studies. The quantitative sampling performed also meant the numbers of the various bacteria could be examined in relation to the clinical severity of the

skin. Quantitative examination of bacterial numbers was also made when treatment of the patient's skin by mupirocin (a novel anti-staphylococcal antibiotic), a steroid or both in combination was performed. The treatment that gave the best clinical results could then be related to bacterial numbers on the skin. The antibiotic sensitivities of S.aureus isolated was also examined to check if mupirocin resistance was developing among S.aureus populations. Multiply-resistant S.aureus are frequently found in hospitals now. Bacteriophage-typing was also undertaken to see if any of the classified groups were more prevalent than others on A.D. patients.

2.Materials and Methods

Two quantitative and one qualitative techniques were used to examine bacterial skin flora. The qualitative technique was used in body sites inaccessible to the quantitative methods of examination.

2.1 Agar Plate Impression Method

Sterile 55mm contact plates (Rodac dishes) were used. Molten agar, having been autoclaved at 15lbs. $sq.in^{-1}$ for 15 minutes, when poured into the contact plate forms a raised convex meniscus which remains as the agar sets. The agar surface area is 24 sq.cm. Plates were poured in the sterile conditions of laminar flow cabinets. In this study the medium used in the plates was a Cystine-Lactose-Electrolyte-Deficient (C.L.E.D.) medium (Oxoid Ltd, U.K). This medium was chosen as all the main skin bacteria of A.D. sufferers, staphylococci (both S.aureus and S.epidermidis) as well as corynebacterial species (also called 'diptheroids') grew on it. S.aureus, being the bacterium of main interest, also frequently exhibits its characteristic golden-yellow pigmentation on the C.L.E.D. medium facilitating identification.

During sampling the raised curved surface area of the agar in the plate is placed on the skin of the site to be examined. The plates were subsequently incubated at 37°C for 24 hours. All bacterial colonies were then examined and the different types, as determined by colony size, morphology and pigmentation, were enumerated and their numbers noted. One representative colony of each of the colony types was then Gram stained (Appendix 1) Corynebacteria were morphologically identified upon examination, under a x 100 oil immersion microscope lens, being Gram positive rods or clubs in shape, while ranging from long bacillary to short cocco-bacillary forms.

Bacterial cocci, as viewed by the x100 magnification, were further tested by the 'Staphaurex Test' (Wellcome Diagnostics, U.K.). This involved emulsifying a bacterial colony in sterile distilled water followed by addition of a latex bead suspension from the test-kit. If the enzyme coagulase, specific to S.aureus, is present the sensitised latex beads aggregate rapidly forming clumps identifying a S.aureus isolate. No clumps form when the colony is coagulase negative and as such is either coagulase negative staphylococci or micrococci. These two classifications may consist of various species but it has not been thought they are important enough factors in A.D. to warrant the time-consuming biochemical tests necessary to differentiate the various species in the grouping. The same is true for the corynebacterial grouping.

Any cocci colony deemed coagulase negative whose Gram stain exhibited cocci in chains, charateristic of streptococci bacteria, rather than the bunches, characteristic of staphylococci/micrococci bacteria, was inoculated onto 7% defi-brinated horse blood agar (Appendix 2). If haemolysis was demonstrable after 24 hours incubation at 37°C the bacteria were 'Streptex Test' (Wellcome Diagnostics, U.K.) tested. This is a similar kit to the Staphaurex kit mentioned previously, using latex beads sensitized with immunoglobulins specific for bacterial cell wall antigens of the Streptococci Lancefield groupings, A, B, C, D, F and G.

Using the above procedure all the bacteria on contact plates were classified into the following groupings:-1.S.aureus, 2.Coagulase negative staphylococci and micrococci, 3. Coryneforms (diphtheroids) and 4. other bacteria.

Each subject had a minimum of eighteen contact plate sites. These sites included the flexure sites frequently affected by A.D. as well as other sites which in total gave an even spread mapping of the skin surface. The eighteen common sites are detailed in Appendices 3 and 4.

Occasionally severely eczematous skin was found at other sites and extra contact plates were taken of these areas.

2.2. Surface Wash Technique (Williamson P. and Kligman A.M., 1965)

The surface wash procedure utilises a teflon ring, diameter 2.Ø37cm to circumscribe skin area of 4.15sq.cm. Pressed firmly against the skin at the sample site the ring seals off an area of skin. One millilitre of sterile Ø.Ø75M phosphate buffer, Ø.1% triton X-1ØØ, pH7.9 was poured into the ring. The fluid was agitated by evenly rubbing the skin inside the ring by a blunted teflon rod, diameter Ø.5cm. After one minute the fluid inside the ring was aspirated by pipette and returned to the sterile bijou from which it came. The method was then repeated using fresh wash fluid. 97-98% of the total bacteria were removed from the skin by the combined "washes".

The "best" fluid, as determined by Williamson and Kligman has an added attraction in this study. Triton X-1ØØ, a non-ionic detergent was chosen for its low irritancy to the skin, low bacterial toxicity, as well as its effectiveness as a detergent and dispersant. Low irritancy is an especially important factor in this study due to the fact that atopic dermatitis sufferers skin is frequently rough and broken due to their condition. Low irritancy of the wash fluid thus helps to keep the patient's discomfort to a minimum during the taking of samples.

At each sampling a minimum of twelve sites were taken (Appendices 3 and 4). A thirteenth site was frequently taken when the patient's worst eczematous site did not occur in one of the designated sample sites.

One difference in our technique and that of its originators existed. Four teflon rings were in our possession meaning that each ring was used a minimum of three times at each sampling. As it was not practical to sterilise the rings between use at different sample sites, the rings were instead disinfected in Presept disinfectant (Surgikos, U.K.) strength 23Ø ppm,

along with the rods for 30 seconds before being rinsed in sterile distilled water. The rings and rods were then left to air dry on absorbant tissue before reuse. An aliquot of the "sterile" distilled water fluid was plated onto 7% horse defibrinated blood agar (Appendix 2) after sampling. This was followed by a 24 hour incubation at 37°C and showed that no carry over of bacteria from one site to the next occurred as no bacterial growth on the plate was observed.

Subsequent to sampling the two aliquots recovered from a single site were pooled. The pooled samples were measured using 2ml pipettes, and the total fluid recovered from each site was noted. Appropriate dilutions were made in 0.0325M phosphate buffer, 0.05% triton X-100, pH7.9 diluent. The inclusion of some triton X-100 in the diluent prevents reaggregation of organisms.

The dilutions made for each sample site were 1/2, 1/10, 1/50 and 1/100 for those people without atopic dermatitis and 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} for those with atopic dermatitis. The difference was due to the fact that people with atopic dermatitis tend to have greater numbers of bacteria, by a factor of about 10^{2}-10^{4}, on their skin than on the skin of "normal" people. 50 µl aliquots of the dilutions were then finnpipetted onto 7% defibrinated horse blood agar. A replica for each sample site was made for anaerobic incubation. 25 µl aliquots were dispensed onto MacConkey Agar (Oxoid Ltd, U.K.). The blood agar is a good general purpose growth medium suitable for the growth of most organisms.

MacConkey agar was chosen for its good colour differentiation between bacterial species and its ability to grow Gram negative bacteria.

C.L.E.D. agar perhaps could have been chosen instead of MacConkey agar to have reduced the variables affecting bacterial numbers in the contact plate/SWT comparison part of the study.

The plates inoculated with the dilutions were incubated for 24 hours at 37°C. One of the pair of each blood agar plate for each site along with the MacConkey agar plates were incubated aerobically. The remaining blood agar plates were incubated anaerobically using anaerobic jars and gas generating kits (Oxoid Ltd., U.K.)

Bacterial colonies were identified using the same microscopic and diagnostic techniques as was previously described in the "Agar Plate Impression Method" action. Counts of the bacterial numbers of each grouping (S.aureus, coagulase negative Staph/Micro-cocci and Coryneforms) at each sample site were obtained as follows. An average of the counts for one bacterial group from the various dilutions-y- were taken and substituted in the equation below.

Bacterial nos/sqcm(2 sig. fig) = $\frac{2/z \times y \times}{40\text{(Blood agar) or } 80\text{(MacConkey agar)}/4.15}$

Explanation of terms:-

z = the wash fluid, in ml, recovered at the site in question

2/z = wash fluid applied (ml)/wash fluid recovered (ml) is therefore the term which accounts for different wash fluid recoveries. This makes numbers obtained from different sites comparable by compensating y for the fact that lost fluid would have meant lost bacteria.

40 (blood agar) or 80 (MacConkey agar) - accounts for the fact that only 50 µl aliquots of dilutions were plated on blood agar (25 on MacConkey agar) and thus converts y to the number of bacteria that would have been found had the whole 2ml wash fluid applied been recovered and plated.

4.15 sqcm is the area of skin that was surface washed.

2.3 Swabbing Bacterial Sampling Swabs (Albuswabs, Exogen, U.K.). were taken at the following sites:-

anterior nares	umbilicus
groin	interdigital(toes)

These were chosen because they were inaccessible to the previously described methods. The swabs were streaked onto 7% defibrinated horse blood agar, twice, as well as onto a MacConkey agar plate. One of the blood agar plates for each site sampled was incubated anaerobically while the remaining blood agar and MacConkey agar plates were aerobically incubated, all at 37°C for 24 hours. Subsequently the previously mentioned identification procedures were performed.

2.4 Eczema Severity Each time sampling took place a subjective score, on a 4 point scale, was given to each contact plate/surface wash technique sample site by the physician:-

0 - no eczema

1 - mild eczema

2 - moderate eczema

3 - severe eczema

Two physicians estimated these scores and good correlation between their estimates was observed.

2.5 Bacteriophage Typing of S.aureus isolates

The S.aureus isolate to be tested was initially inoculated into a small amount of nutrient broth (Oxoid Ltd, U.K.) and incubated overnight. This was then used to flood 'Phage agar plates (Appendix 2) which were left open to dry in a hot room (37°C) for 30-45 minutes. Once dry, the Phage (Colindale, U.K.) are dropped onto the lawn of bacteria by applicator loops. The plate was then incubated overnight. Results were read the following day. A positive reaction was where the bacteriophage had formed a plaque by the lysis of S.aureus.

2.6 Antibiotic Sensitivity Testing

60 S.aureus isolates were tested for methicillin and/or mupirocin resistance by the single-disc method of Bauer (Bauer et al, 1966) by Beecham Pharmaceuticals.

2.7 Treatment Studies

Prior sampling of the patients examined in these further studies had revealed high S.aureus counts in A.D. sufferers. Three basic treatments therefore were examined:- (1) Mupirocin immersions, (2) Diprosone immersions and (3) a combination of (1) and (2) treatments. Both (1) and (2) were provided in topical ointment preparations. Bactroban (Beechams, U.K.), i.e. mupirocin 2% w/w in a polyethylene glycol base, was applied to the skin of two patients and mupirocin 2% w/w in a white soft paraffin base was given to four patients in the mupirocin immersion studies. Diprosone (Kirby-Warrick Pharmaceuticals Ltd-U.K.) was given to two patients and the mupirocin/diprosone combination to one. The combination treatment, only given to one patient, involved Bactroban treatment twice daily with a diprosone application between. Treatments (1) and (2) had applications twice daily. Each application of ointment was total involving scalp, hair and anterior nares. No other treatment or baths were allowed. Treatment was for three days after which patients were allowed home with emulsifying ointment BP. Sampling was performed on admission and before being released. A final sampling was undertaken when the patient returned on the fourth day from being released. Aly et al, 1970 found lowest bacterial numbers were obtained three days after cessation of antibiotic treatment. Appropriate strength corticosteroid treatment was resumed after the final samples had been taken.

These studies were initiated with the intention of having equal numbers of patients sampled in each of the treatment groups. This was not possible due to MRSA staphylococci arising after combination treatment (see discussion - p47). It was not thought ethical to put the patients in the position of being colonised with an opportunistic pathogen which would be difficult to eliminate due to its multiple antibiotic resistance. The comparability of the three treatments is thus reduced in view of the small and disparate sample sizes in each group.

2.8 Subjects A diagnosis of atopic dermatitits was established if the patient had a charcteristic clinical presentation and either a personal history of asthma or allergic rhinitis, or a first degree relative with atopic dermatitis. Subjects were considered eligible as controls if they had no personal history of skin disease.

20 A.D. patients and 19 control subjects took part in the study. The two groups were comparable for age and sex (atopics 11 male: 9 female, median age 29 and controls 12 male: 7 female, median age 27). Ethical committee approval was obtained and all subjects gave informed consent. Any individual who had received either systemic or topical antibiotics in the preceding four weeks was excluded from the study. All participants were asked not to wash or apply topical treatment during the six hours prior to sampling.

Each subject had 14-18 contact plate, 12 SWT and 4 swab samples taken from the sites indicated in Appendix 3. Occasionally a badly eczematous skin area fell outwith the designated sites and an extra contact plate or SWT sample was taken, as decided by the physician. Six of the above mentioned A.D. patients were sampled in the manner described at six weekly intervals to examine bacterial numbers and eczema severity over time.

2.9 Statistics Tables 1 and 2 - The Chi-squared (X^2) test was used as this is a suitable statistical test to examine large-group proportion data, as in table 1, or small-group data, as in table 2, providing none of the proportions is less than 25%. Unfortunately this was the case for 3/12 of the comparisons made in table 2 though in each of the three cases (umbilical, groin and interdigital S.aureus carriage) a clear difference in bacterial carriage rates between A.D. patients and controls was observed.

The remaining statistics were provided courtesy of Mr. T.C. Aitchison, Dept. of Statistics, Glasgow University. Figure 1 is a direct linear correlation which determines the degree of association between contact plate and SWT bacterial numbers for each patient and then expresses this on a scale of -1 (no relationship through Ø to +1 (definite relationship). Figure 2 is also a linear correlation determining the degree of association between each patient's correlation (as displayed in figure 1). This was again expressed on a scale of -1 to +1. There were different numbers of patients in each analysis in figures 1 and 2 due to the fact that within each A.D. patient's sample, correlation of bacterial numbers between contact plates and SWT sampling was only undertaken where both were positive for the particular bacterial group being examined. Figures 3-14 - One-way analysis of variance was used to examine if there were significant differences between the means of the bacterial numbers in each of the eczema severity level groups. Sample sizes between contact plates and SWT varied in each bacterial group due to 18 contact plates being taken per patient as opposed to 12 SWT samples.

SWT sample size (in one bacterial group) varied due to the different recovery rates for that group by the various SWT media/incubation requirements.

Figures 15, 16 and 17 were a comparison of the standard deviations (or change in contact plate bacterial numbers) over time for each body site of a patient (the numerator of the ratio) and comparing that to the standard deviation for

that side (the denominator of the ratio) calculated from standard deviatic
obtained for that body site over time for each of patients A, C and D. T
denominator represents the general population variability of the site f
S.aureus numbers.

RESULTS

TABLE 1

Qualitative bacterial carriage of patients and controls

Number of body sites sampled with positive bacterial carriage

		Eczema patients (%)	Controls (%)	Statistical significa ^{2}-p (%)
S. aureus				
- Contact plates		257/369 (69.6)	35/341 (10.3)	<0.1
	medium 1	132/243 (54.3)	14/226 (6.20)	<0.1
- SWT	medium 2	129/238 (54.2)	9/213 (4.20)	<0.1
	medium 3	115/243 (47.3)	4/226 (1.80)	<0.1
CNS/M				
- Contact plates		306/369 (82.9)	293/341 (85.9)	>10
	medium 1	124/243 (51.0)	137/226 (60.6)	>10
- SWT	medium 2	111/238 (46.6)	133/213 (62.4)	5<p<10
	medium 3	120/243 (49.4)	113/226 (50.0)	>10
Diphtheroids				
- Contact plates		271/369 (73.4)	298/341 (87.4)	1<p<5
	medium 1	117/243 (48.2)	155/226 (68.6)	0.1<p<1
- SWT	medium 2	75/238 (31.5)	107/213 (50.2)	0.1<p<1
	medium 3	86/243 (35.4)	106/226 (46.9)	>10

Key - SWT - Surface wash technique
- medium 1 - blood agar incubated aerobically
- medium 2 - blood agar incubated anaerobically
- medium 3 - MacConkey agar
- ^{2} - Chi-squared statistical test

TABLE 2

Qualitative bacterial carriage of swabbed sites of patients and controls

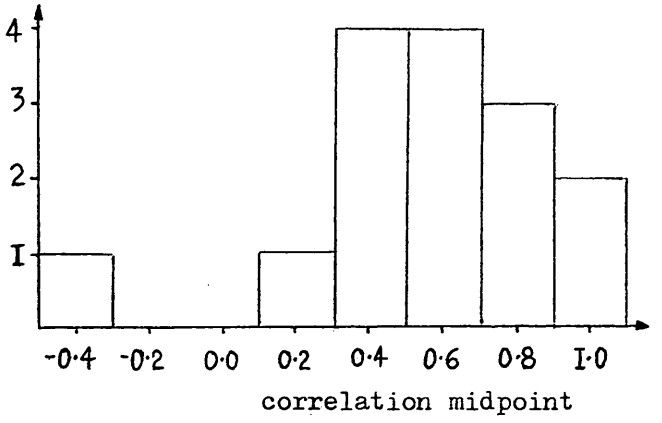
	Bacterial carriage +		Statistical significanc
	Eczema patients (%)	Controls (%)	^{2}-p (%)
S. aureus			
- Nares	18/20 (90.0)	10/19 (52.6)	<0.1
- Umbilicus	12/20 (60.0)	1/19 (5.30)	<0.1
- Groin	12/20 (60.0)	1/19 (5.30)	<0.1
- Interdigital	9/20 (45.0)	1/19 (5.30)	<0.1
CNS/M			
- Nares	17/20 (85.0)	16/19 (84.2)	>10
- Umbilicus	16/20 (80.0)	16/19 (84.2)	>10
- Groin	16/20 (80.0)	13/19 (68.4)	>10
- Interdigital	18/20 (90.0)	15/19 (78.9)	5<p<10
Diphtheroids			
- Nares	9/20 (45.0)	16/20 (84.2)	<0.1
- Umbilicus	11/20 (55.0)	16/20 (84.2)	<0.1
- Groin	9/20 (45.0)	16/20 (84.2)	<0.1
- Interdigital	9/20 (45.0)	16/20 (84.2)	<0.1

- positive bacterial carriage was scored for bacterial appearance on any of the three media
- ^{2} - Chi-squared statistical test

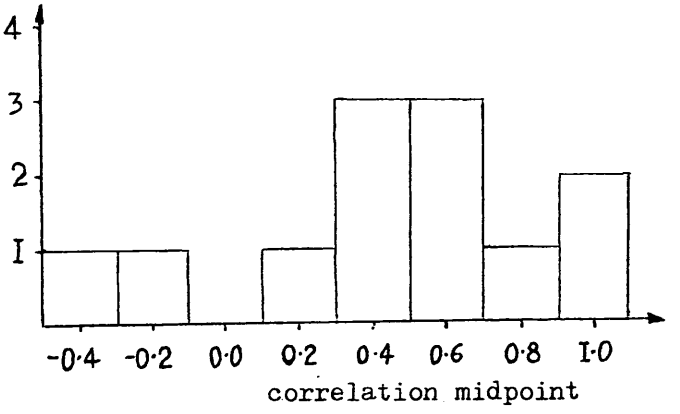
FIGURE I.

Correlation of bacterial numbers within each eczema patients' sample by the two quantitative sampling techniques.

S.aureus (n=I5 patients), median correlation=0·589 (quartiles 0·4I and 0·74).



CNS/M (n=I2 patients), median correlation=0·49I (quartiles 0·32 and 0·83).



Diphtheroids (n=I3 patients), median correlation=0·085 (quartiles -0·30 and 0·52).

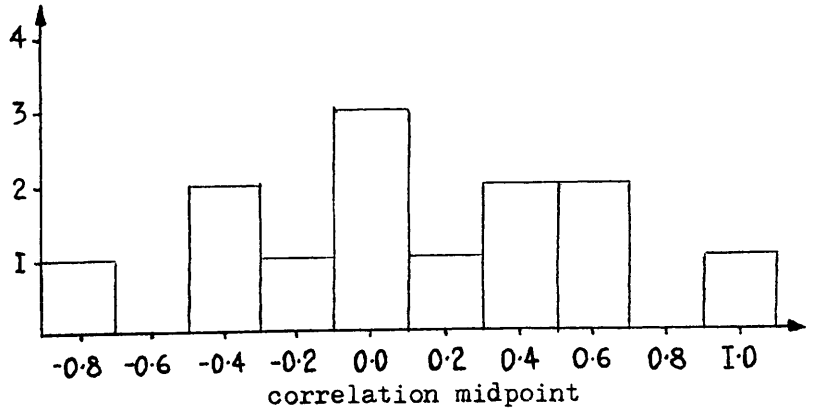
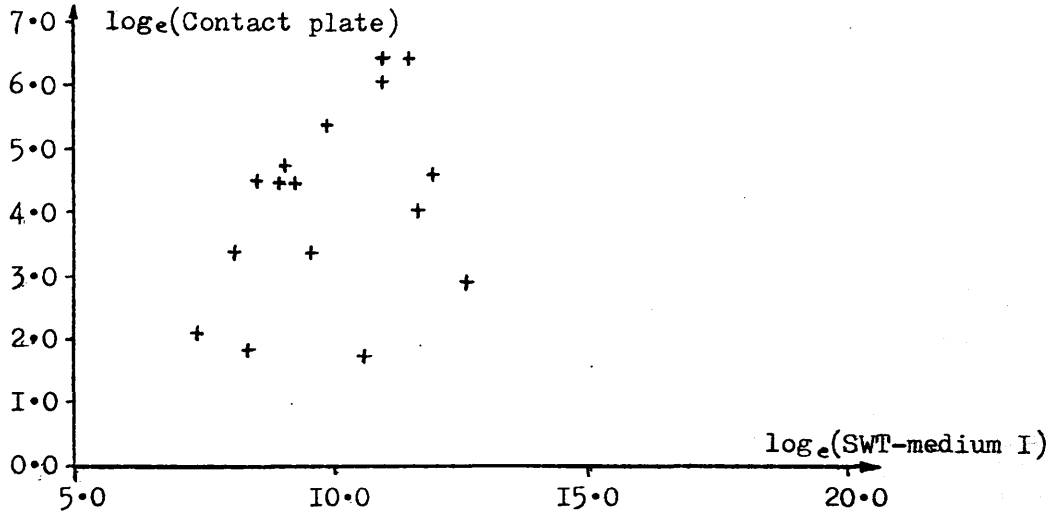


FIGURE 2.

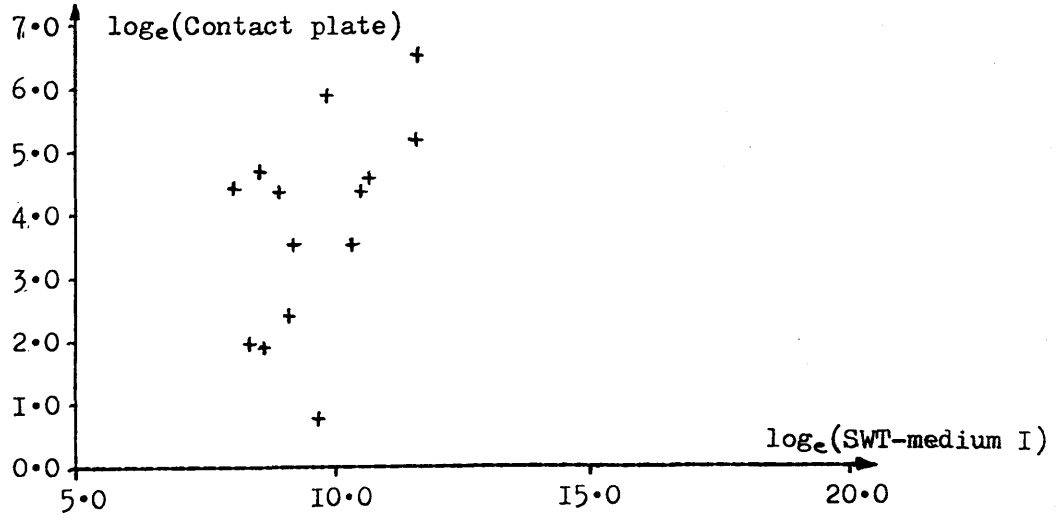
Correlation of bacterial numbers between A.D. patients by the two quantitative sampling techniques.

Correlation was performed on the mean correlations (+) obtained from each A.D. patient's body sites.

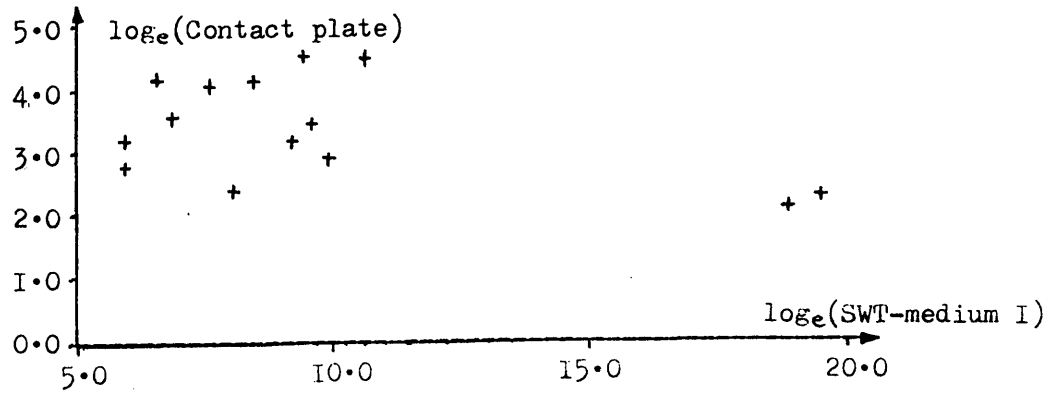
S.aureus (n=I6 patients), correlation =0•4I4



CNS/M (n=I4 patients), correlation =0•552



Diphtheroids (n=I4 patients), correlation = -0•55



Key:- DF (Degrees of freedom) Level (Eczema level) SS (Sum of squares) N (Sample size)

MS (Mean square) St.Dev (Standard deviation) F (Variance ratio) 95 Pct CI's (95% Confidence limits based on pooled standard deviation)

Log_e S.aureus contact plate numbers- eczema level correlation ($p \ll 0{\cdot}001$)

ANALYSIS OF VARIANCE ON C70

SOURCE	DF	SS	MS	F
C23	3	319.05	106.35	31.53
ERROR	219	738.73	3.37	
TOTAL	222	1057.78		

LEVEL	N	MEAN	STDEV
0	56	2.908	2.082
1	97	4.062	1.966
2	48	5.893	1.420
3	22	6.262	1.287

POOLED STDEV = 1.837

INDIVIDUAL 95 PCT CI'S FOR MEAN
BASED ON POOLED STDEV

```
----+---------+---------+---------+--
(--*---)
          (-*--)
                    (--*---)
                     (----*----)
----+---------+---------+---------+--
  3.0       4.5       6.0       7.5
```

Log_e S.aureus SWT medium I numbers- eczema level correlation ($p < 0{\cdot}001$)

ANALYSIS OF VARIANCE ON C70

SOURCE	DF	SS	MS	F
C23	3	198.57	66.19	7.28
ERROR	116	1054.23	9.09	
TOTAL	119	1252.80		

LEVEL	N	MEAN	STDEV
0	26	8.852	2.984
1	52	9.374	2.672
2	27	11.544	2.855
3	15	12.327	4.274

POOLED STDEV = 3.015

INDIVIDUAL 95 PCT CI'S FOR MEAN
BASED ON POOLED STDEV

```
--+---------+---------+---------+----
(-----*-----)
     (---*---)
               (-----*-----)
                 (-------*------)
--+---------+---------+---------+----
 8.0       10.0      12.0      14.0
```

FIGURES 3-14 Correlation of log_e bacterial numbers to eczema level.

Key:- DF (Degrees of freedom) Level (Eczema level) SS (Sum of squares) N (Sample size)

MS (Mean square) St.Dev (Standard deviation) F (Variance ratio) 95 Pct CI's (95% Confidence limits based on pooled standard deviation)

Log_e S.aureus SWT medium 2 numbers- eczema level correlation ($p<0.001$)

ANALYSIS OF VARIANCE ON C70

SOURCE	DF	SS	MS	F
C23	3	227.93	75.98	8.87
ERROR	112	959.12	8.56	
TOTAL	115	1187.05		

LEVEL	N	MEAN	STDEV
0	26	9.001	3.228
1	50	9.791	2.749
2	26	12.051	2.563
3	14	12.918	3.553

POOLED STDEV = 2.926

INDIVIDUAL 95 PCT CI'S FOR MEAN
BASED ON POOLED STDEV

```
----+---------+---------+---------+-----
(-----*-----)
      (---*---)
                (----*-----)
                  (-------*------)
----+---------+---------+---------+-----
   8.0       10.0      12.0      14.0
```

Log_e S.aureus SWT medium 3 numbers- eczema level correlation ($p>0.05$)

ANALYSIS OF VARIANCE ON C70

SOURCE	DF	SS	MS	F
C23	3	70.58	23.53	2.51
ERROR	98	919.34	9.38	
TOTAL	101	989.92		

LEVEL	N	MEAN	STDEV
0	21	9.767	2.238
1	44	10.356	3.258
2	23	11.746	2.174
3	14	11.965	4.462

POOLED STDEV = 3.063

INDIVIDUAL 95 PCT CI'S FOR MEAN
BASED ON POOLED STDEV

```
----+---------+---------+---------+---
(---------*---------)
      (------*------)
              (--------*---------)
            (----------*----------)
----+---------+---------+---------+---
   9.0       10.5      12.0      13.5
```

Key:- DF (Degrees of freedom) Level (Eczema level) SS (Sum of squares) N (Sample size)

MS (Mean square) St.Dev (Standard deviation) F (Variance ratio) 95 Pct CI's (95 % Confidence limits based on pooled standard deviation)

Log_e CNS/M contact plate numbers- eczema level correlation (p>0·05)

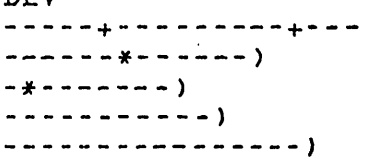
ANALYSIS OF VARIANCE ON C70

SOURCE	DF	SS	MS	F
C23	3	4,34	1,45	0,41
ERROR	235	826,25	3,52	
TOTAL	238	830,59		

LEVEL	N	MEAN	STDEV
0	92	4,525	1,819
1	89	4,307	2,018
2	39	4,192	1,912
3	19	4,202	1,247

POOLED STDEV = 1,875

INDIVIDUAL 95 PCT CI'S FOR MEAN
BASED ON POOLED STDEV



Log_e CNS/M SWT medium I numbers- eczema level correlations (0·01<p<0·05)

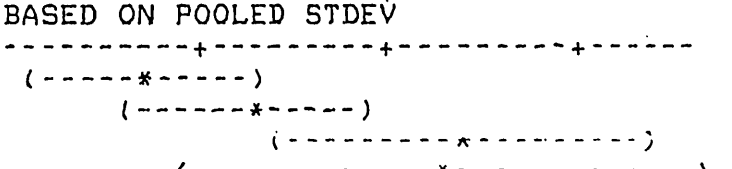
ANALYSIS OF VARIANCE ON C70

SOURCE	DF	SS	MS	F
C23	3	73,29	24,43	3,92
ERROR	111	692,33	6,24	
TOTAL	114	765,62		

LEVEL	N	MEAN	STDEV
0	49	9,198	2,704
1	40	9,914	2,373
2	17	11,319	2,346
3	9	11,188	2,054

POOLED STDEV = 2,497

INDIVIDUAL 95 PCT CI'S FOR MEAN
BASED ON POOLED STDEV



Key:- DF (Degrees of freedom) Level (Eczema level) SS (Sum of squares) N (Sample size)

MS (Mean square) St.Dev (Standard deviation) F (Variance ratio) 95 Pct CI's (95% Confidence limits based on pooled standard deviation)

Log$_e$ CNS/M SWT medium 2 numbers- eczema level correlations (0·01<p<0·05)

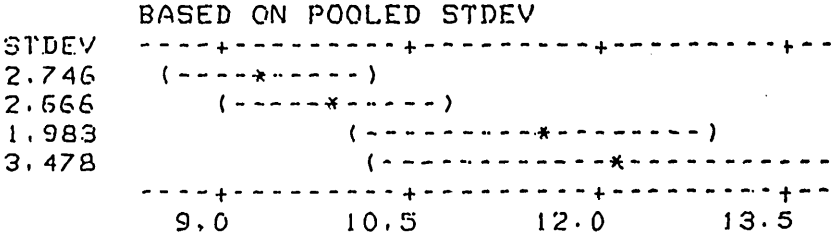
ANALYSIS OF VARIANCE ON C70

SOURCE	DF	SS	MS	F
C23	3	84.35	28.12	3.89
ERROR	93	672.29	7.23	
TOTAL	96	756.64		

LEVEL	N	MEAN	STDEV
0	42	9.363	2.746
1	33	9.879	2.666
2	14	11.523	1.983
3	8	12.088	3.478

POOLED STDEV = 2.689

INDIVIDUAL 95 PCT CI'S FOR MEAN BASED ON POOLED STDEV



Log$_e$ CNS/M SWT medium 3 numbers- eczema level correlation (p>0·05)

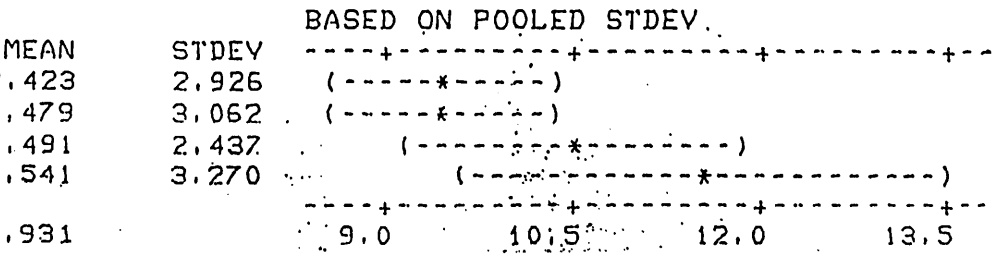
ANALYSIS OF VARIANCE ON C70

SOURCE	DF	SS	MS	F
C23	3	46.91	15.64	1.82
ERROR	110	945.08	8.59	
TOTAL	113	992.00		

LEVEL	N	MEAN	STDEV
0	44	9.423	2.926
1	42	9.479	3.062
2	19	10.491	2.437
3	9	11.541	3.270

POOLED STDEV = 2.931

INDIVIDUAL 95 PCT CI'S FOR MEAN BASED ON POOLED STDEV



Key:- DF (Degrees of freedom) Level (Eczema level) SS (Sum of squares) N (Sample size)

MS (Mean square) St.Dev (Standard deviation) F (Variance ratio) 95 Pct CI's (95% Confidence limits based on pooled standard deviation)

Log$_e$ Diphtheroid contact plate numbers- eczema level correlation (0·001<p<0·01)

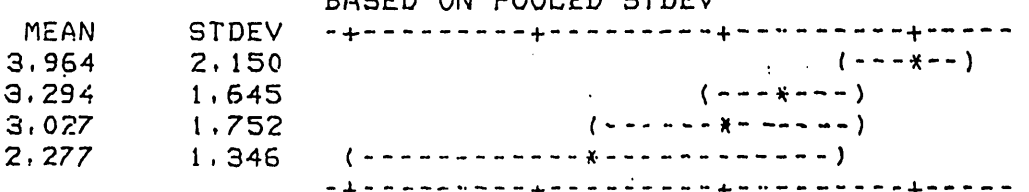
ANALYSIS OF VARIANCE ON C70

SOURCE	DF	SS	MS	F
C23	3	41.87	13.96	3.88
ERROR	204	734.04	3.60	
TOTAL	207	775.91		

LEVEL	N	MEAN	STDEV
0	94	3.964	2.150
1	77	3.294	1.645
2	29	3.027	1.752
3	8	2.277	1.346

POOLED STDEV = 1.897

INDIVIDUAL 95 PCT CI'S FOR MEAN
BASED ON POOLED STDEV



Log$_e$ Diphtheroid SWT medium 1 numbers- eczema level correlation (0·001<p<0·01)

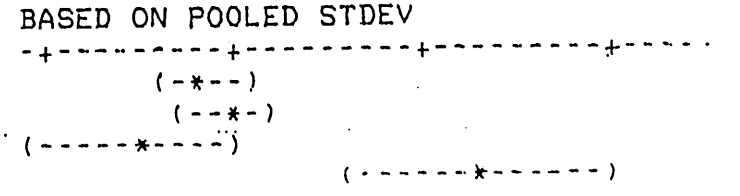
ANALYSIS OF VARIANCE ON C70

SOURCE	DF	SS	MS	F
C23	3	281.3	93.8	5.86
ERROR	85	1360.4	16.0	
TOTAL	88	1641.7		

LEVEL	N	MEAN	STDEV
0	42	9.166	4.134
1	34	9.786	4.084
2	8	7.413	1.589
3	5	16.444	4.782

POOLED STDEV = 4.001

INDIVIDUAL 95 PCT CI'S FOR MEAN
BASED ON POOLED STDEV



Key:- DF (Degrees of freedom) Level (Eczema level) SS (Sum of squares) N (Sample size)

MS (Mean square) St.Dev (Standard deviation) F (Variance ratio) 95 Pct CI's (95% Confidence limits based on pooled standard deviation)

Log$_e$ Diphtheroid SWT medium 2 numbers- eczema level correlation (p>0·05)

ANALYSIS OF VARIANCE ON C70

SOURCE	DF	SS	MS	F
C23	3	42,37	14,12	1,83
ERROR	54	415,91	7,70	
TOTAL	57	458,27		

LEVEL	N	MEAN	STDEV
0	28	8,338	2,506
1	25	8,750	3,076
2	3	7,559	2,161
3	2	12,883	3,131

POOLED STDEV = 2,775

INDIVIDUAL 95 PCT CI'S FOR MEAN
BASED ON POOLED STDEV

```
--------+---------+---------+---------
        (--*--)
         (--*--)
(---------*---------)
                (----------*----------)
--------+---------+---------+---------
      7,0       10,5      14,0
```

Log$_e$ Diphtheroid SWT medium 3 numbers- eczema level correlation (p>0·05)

ANALYSIS OF VARIANCE ON C70

SOURCE	DF	SS	MS	F
C23	3	42,92	14,31	2,72
ERROR	62	326,50	5,27	
TOTAL	65	369,41		

LEVEL	N	MEAN	STDEV
0	38	8,688	2,502
1	20	8,720	2,002
2	5	8,144	1,854
3	3	12,462	1,578

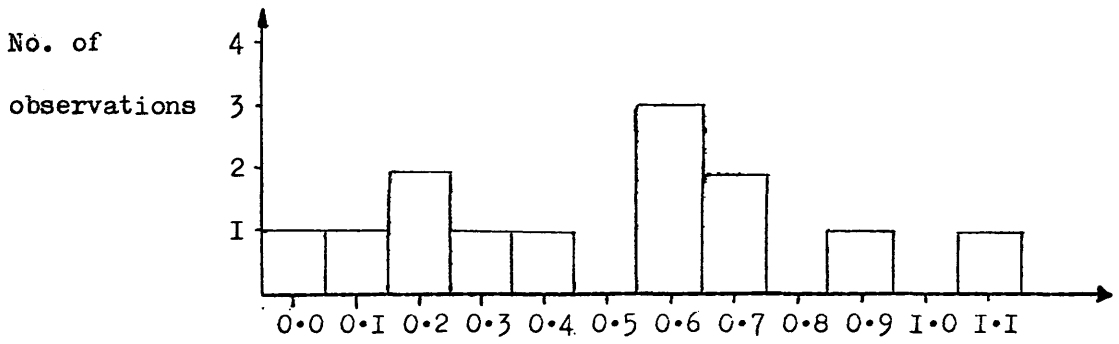
POOLED STDEV = 2,295

INDIVIDUAL 95 PCT CI'S FOR MEAN
BASED ON POOLED STDEV

```
------+---------+---------+---------+
      (--*--)
     (---*---)
(--------*--------)
                (----------*---------)
------+---------+---------+---------+
    7,5       10,0      12,5      15,0
```

FIGURE I5.

Patient A	S.aureus contact plate count on visit						Eczema level					
Body site no.	I	2	3	4	5	6	I	2	3	4	5	6
I	-	-	-	-	-	-	-	0	0	0	I	0
2	933	57	766	28I	25I	328	-	I	I	2	0	2
3	994	520	564	276	265	9	-	2	I	2	I	2
4	-	-	-	-	-	-	-	0	0	0	0	0
5	-	-	-	-	-	-	-	0	0	0	0	0
6	942	29	I3	I73	347	I63	-	0	0	I	0	0
7	I5	0	7	8	45	9	-	0	I	0	0	0
8	279	7	93	80	I6	73	-	0	0	0	0	2
9	799	0	9	7	46	35	-	I	0	0	0	I
I0	48I	7	I26	80	4I	I0	-	0	0	0	0	2
II	442	6	33	40	I8	I0	-	0	0	0	I	I
I2	II5	I6	8	26	37	5	-	0	0	0	0	0
I3	2I	2	I0	8	63	I7	-	0	0	0	0	I
I4	I3	2	I4	5	6	6	-	0	0	0	0	I
I5	I400	30	32	II39	42	506	-	I	2	3	2	3
I6	I400	5	55	800	I8	983	-	I	I	3	3	3
I7	9	0	24	6	9	32	-	0	0	0	0	0
I8	32	4	I7	6	5	5	-	0	0	0	0	0
I9	3I0	200	98	46	43	I2	-	2	2	2	2	2
22	-	-	-	-	-	-	-	2	3	2	-	-



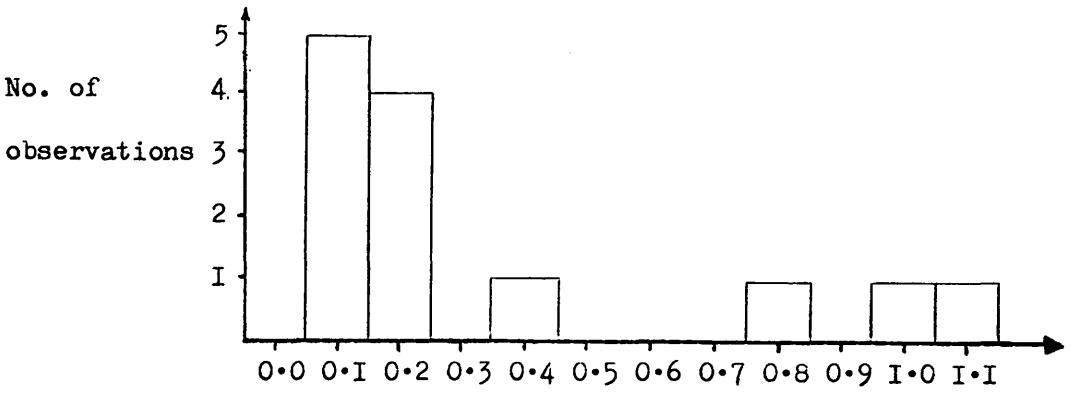
Ratio of within site, across time, standard deviation/ between person standard deviation for that site.

Median ratio = 0·57

Results for patients B, E and F are not given as they were colonised by few if any S.aureus, had no severe eczema flares over the nine month study period and therefore reveal little to connect changing S.aureus numbers with changing eczema severity.

FIGURE 16.

Patient C	S.aureus contact plate count on visit						Eczema level					
Body site no.	I	2	3	4	5	6	I	2	3	4	5	6
I	-	-	-	-	-	-	I	0	0	0	-	0
2	300	240	I400	I23	730	II95	3	2	2	2	-	2
3	I000	I74	I400	85	3II	I242	3	2	2	2	-	2
4	-	-	-	-	-	-	0	0	0	-	-	0
5	-	-	-	-	-	-	0	0	0	-	-	0
6	57	34	300	34	62	I06	I	0	0	0	-	0
7	4	8	34	9	I8	32	I	I	I	I	-	I
8	58	I8	92	3	6	75	0	0	0	I	-	0
9	II	I3	84	2	4	86	0	0	0	I	-	0
I0	I3	286	I03	I2	II	I52	I	2	I	I	-	0
II	34	20	62	7	5	2II	I	I	I	0	-	0
I2	I9	I3	59	I	28	25	I	I	0	I	-	I
I5	28	I97	238	5	25	0	I	I	I	I	-	0
I6	42	29	404	I3	24	I6	0	I	I	I	-	0
I7	4	29	95	I0	49	45	0	0	0	0	-	0
I8	5	9	80	2	2I	I20	0	0	0	0	-	0
I9	244	66	350	46	736	722	3	2	2	2	-	I
23	-	-	-	-	-	-	3	2	2	2	-	I
24	-	-	-	-	-	-	3	2	2	2	-	I
25	-	-	-	-	-	-	3	I	I	I	-	I
26	-	-	-	-	-	-	3	I	I	2	-	2

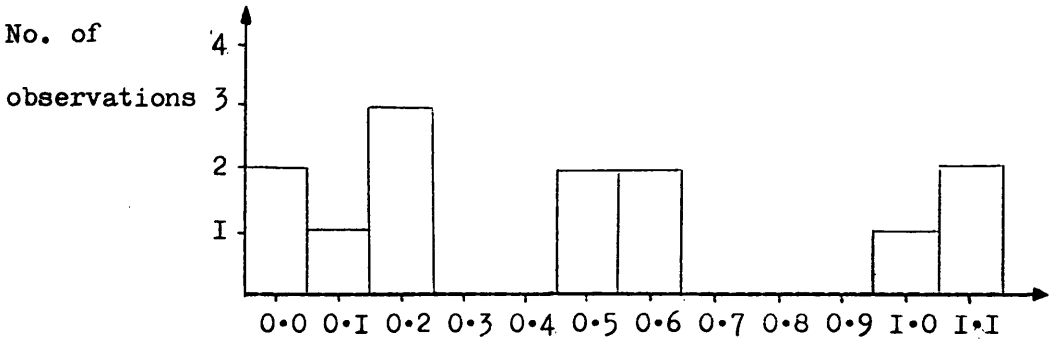


Ratio of within site, across time, standard deviation/ between person standard deviation for that site.

Median ratio = 0·I9

FIGURE 17.

Patient D Body site no.	S.aureus contact plate count on visit 1	2	3	4	5	6	Eczema level 1	2	3	4	5	6
1	-	-	-	-	-	-	1	1	0	0	1	0
2	285	376	50	709	13	60	1	1	1	3	3	2
3	891	124	232	191	37	81	1	1	2	3	3	1
4	37	504	116	45	2	0	0	1	1	1	1	1
5	233	376	8	124	96	0	0	1	1	1	1	1
6	0	1400	51	32	6	8	0	1	2	1	2	1
7	112	38	37	0	193	4	1	1	2	2	2	1
8	28	0	1400	0	1	66	0	0	1	0	1	0
9	70	-	1400	0	1	9	0	1	1	0	0	1
10	25	49	23	169	9	52	2	1	0	2	3	2
11	110	-	19	162	3	15	2	1	2	2	3	2
12	8	21	10	19	4	22	0	0	1	2	2	1
13	10	23	19	51	552	62	0	1	1	2	3	2
14	-	235	6	270	790	378	0	1	1	2	3	3
15	135	45	18	249	28	330	1	2	2	3	1	2
16	0	0	16	34	9	45	1	2	1	1	1	2
17	-	-	-	-	-	-	-	1	1	1	1	-
18	5	-	6	1	156	13	1	1	1	0	2	2
19	357	66	-	407	31	7	3	2	2	3	3	2
27	-	-	-	-	-	-	2	-	-	-	-	-



Ratio of within site, across time, standard deviation/ between person standard deviation for that site.

Median ratio = 0·46

TABLE 3.

Investigation of whether large S.aureus numbers or dermatitis occurs first.

	Observations ↑↑	↑↓
S.aureus numbers rise over 6 week period and eczema level change (up or down) over the following 6 weeks.	34 (63%)	20 (37%)
Eczema level rise over 6 week period and S.aureus numbers change (up or down) over the following 6 weeks.	22 (42%)	31 (58%)

'Observations' was the number of body sites that had the described ocurrence. The above table examines body sites of A.D. patients included in the sequential study. Sampling took place 6 times at 6 weekly intervals for each of 6 patients. It demonstrates that where S.aureus numbers have risen at a body site from one sampling to the second sample taken at that site 6 weeks later, then the eczema severity level is more likely to have risen (65%) than fallen (37%) over the 6 weeks following the second sample date (i.e. from the second to third sampling). The converse was true for S.aureus numbers following a 6 weekly rise in eczema severity at a body site.

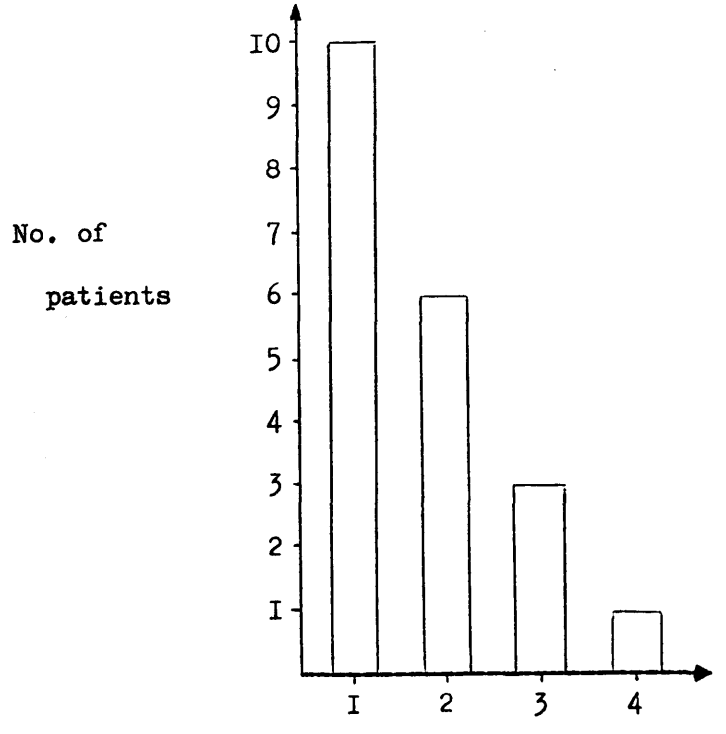
TABLE 4.

Bacteriophage typing of 348 S.aureus isolates from A.D. patients.

Phage type	No. of isolates	(%)
Group I	9	(3)
Group 2	67	(I9)
Group 3	I59	(46)
Other	57	(I6)
Non-typable	56	(I6)

FIGURE I8.

A.D. patients with one or more phage type groups.



TABLES 5-10.

Bacteriophage typing of S.aureus isolates from the body sites of sequentially studied A.D.patients.

Patient A

phage type	Visit 1	2	3	4	5	6
	No. of body sites at which phage type observed.					
group I	-	-	-	-	-	-
group 2	-	-	-	-	-	-
group 3	23+	I4	20+	I8+	22+	I8+
other	-	-	-	2	-	-
non-typable	I	-	I	I	-	-

Patient B

phage type	Visit 1	2	3	4	5	6
	No. of body sites at which phage type observed.					
group I	-	-	-	-	5	-
group 2	-	-	-	-	-	-
group 3	8+	I2+	-	-	7	2
other	-	2	I	-	-	-
non-typable	-	3	I	-	2	2

Patient C

phage type	Visit 1	2	3	4	5	6
	No. of body sites at which phage type observed.					
group I	-	-	-	2	-	-
group 2	-	-	-	I3+	I0	2I
group 3	-	-	-	I	-	-
other	2I+	2I+	24+	I5+	I7+	20+
non-typable	-	-	I	I	-	3+

Key:- + -number includes the nasal isolate phage type where a nasal isolate was recovered.

Patient D	Visit					
	I	2	3	4	5	6
phage type	No. of body sites at which phage type observed.					
group I	-	-	-	-	-	-
group 2	-	-	-	-	I	-
group 3	I5+	I6+	I7+	4	20+	2I+
other	3+	I	I	I5+	-	-
non-typable	-	-	I	-	-	-

Patient E	Visit					
	I	2	3	4	5	6
phage type	No. of body sites at which phage type observed.					
group I	-	-	-	-	-	-
group 2	-	-	-	-	-	-
group 3	II+	I4+	2	I+	2+	4+
other	-	-	-	8	-	-
non-typable	-	-	-	I	-	-

Patient F	Visit					
	I	2	3	4	5	6
phage type	No. of body sites at which phage type observed.					
group I	-	-	I	-	-	-
group 2	2	-	I	-	I3	-
group 3	-	6+	I	3	I	-
other	-	-	-	-	-	-
non-typable	I	I	I	3	-	-

TABLE II. Comparison of nasal S.aureus type with the predominant type found on the skin of A.D. patients.

S.aureus nasal and skin types	No. of observations	(%)
match	I4	(70)
different	2	(I0)
no nasal isolate	4	(20)

TABLE I2.

Frequency of nasal S.aureus isolation.

Patient	S.aureus nasal isolation.	
	yes	no
A	5/6	I/6
B	3/6	3/6
C	6/6	0/6
D	6/6	0/6
E	5/6	I/6
F	I/6	5/6

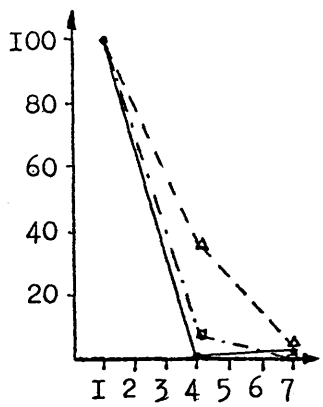
FIGURE I9.)

Comparison of mupirocin, diprosone and "combined" treatments on the bacterial skin flora of A.D. patients.

Key:- mupirocin ———, diprosone - - - -, "combined" - · - · -

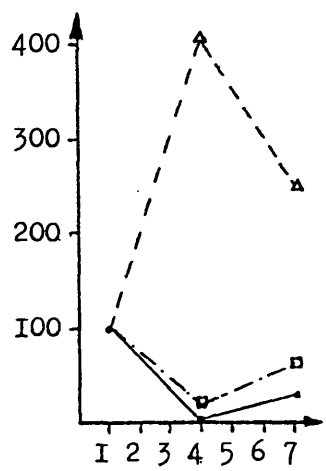
The value of I00% was given to the average bacterial number for each grouping on day I.

S.aureus numbers as a percentage of day I average values.



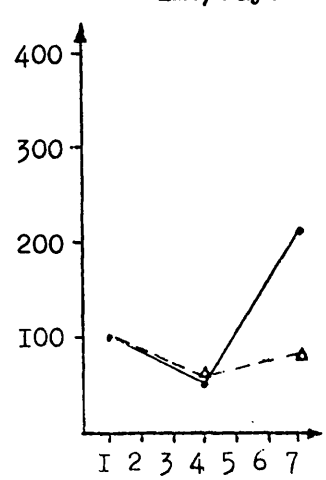
	Day I	4	7
———	I00	0·0	2·4
- -△-	I00	37·9	3·I
- · -□	I00	5·3	0·4

CNS/M numbers as a percentage of day I average values.



	Day I	4	7
———	I00	4·	23·
- -△-	I00	399	25I
- · -□	I00	I6	60

Diphtheroid numbers as a percentage of day I average values.



	Day I	4	7
———	I00	53	209
- -△-	I00	55	82
- · -□	0	-	-

TABLES I3 and I4.

Treatment and clinical condition.

All eczema level scores read in the treatment studies had their initial (day I) and final (day 7) values compared to check for improved, static or worsened clinical condition at each of the body sites.

	No. of body sites.		
Treatment	improved	static	worse
mupirocin	I5,(29%)	29,(56%)	8,(I5%)
diprosone	I2,(52%)	II,(48%)	0,(0%)
"combined"	8,(62%)	5,(38%)	0,(0%)

One patient given mupirocin treatment was followed up, sampling weekly (days I4, 2I and 28), and the clinical condition of the body sites at days 7 and 28 were compared, to that at day I.

	No. of body sites.			average body site eczema
Day	improved	static	worse	level.
7	2,(I8%)	6,(55%)	3,(27%)	I·I25
I4	8,(73%)	3,(27%)	0,(0%)	0·5
2I	7,(64%)	2,(I8%)	2,(I8%)	0·75
28	5,(45%)	6,(55%)	0,(0%)	0·75

TABLE I5.

Antibiotic resistance of 60 S.aureus isolates.

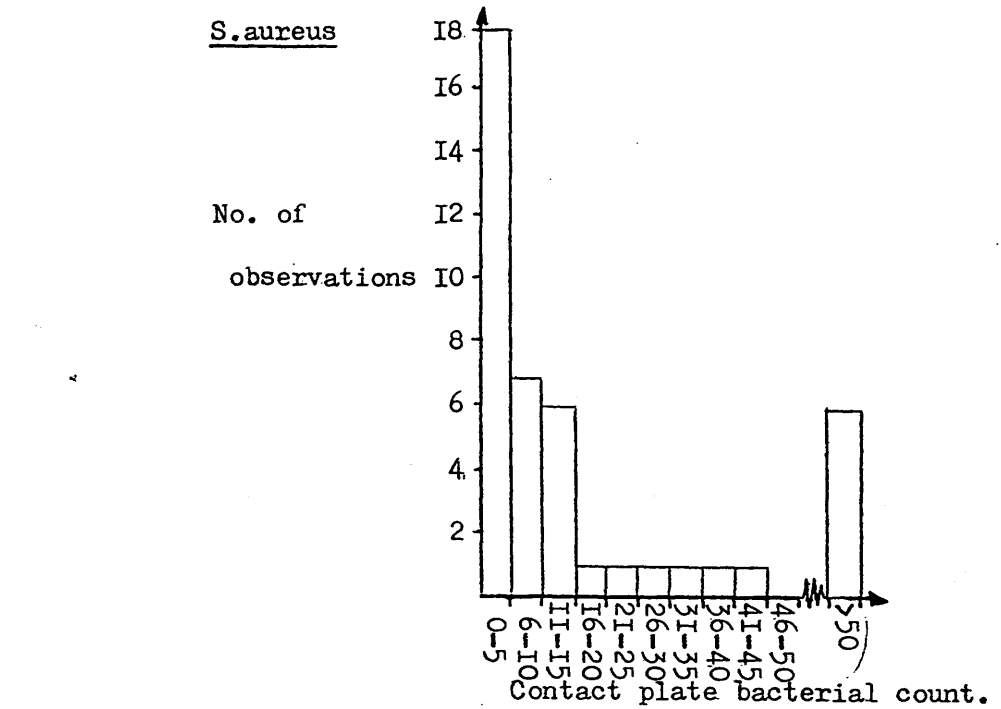
Mupirocin resistance of	0·I25μg/ml - I7 isolates.
	0·25 μg/ml - 30 isolates.
	0·50 μg/ml - I isolate.
	I·00 μg/ml - I2 isolates.
Methicillin resistance of	I·00 μg/ml - 7 isolates.
	2·00 μg/ml - 53 isolates.

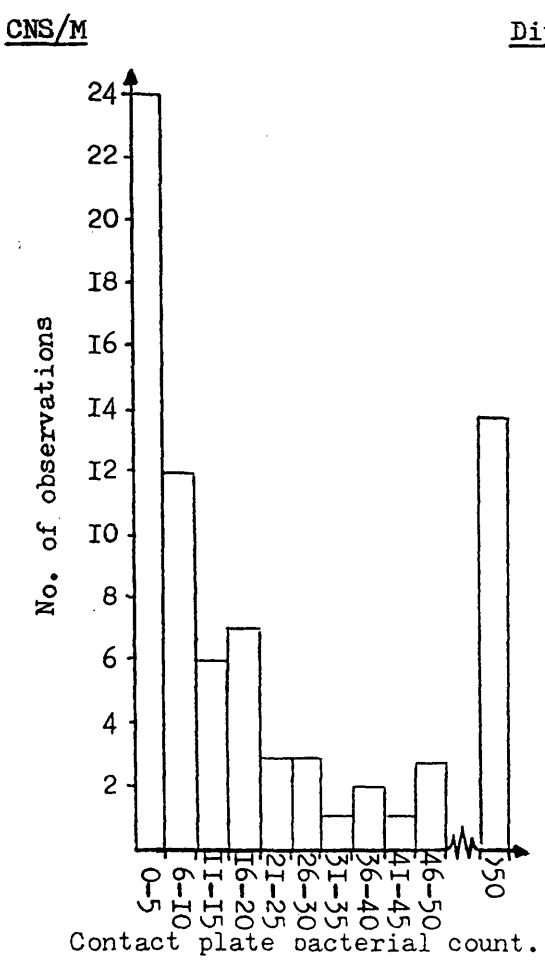
Mupirocin MIC of

Methicillin MIC of

MIC - Minimal inhibitary concentration

FIGURE 20. Bacterial contact plate counts when the SWT count is zero.





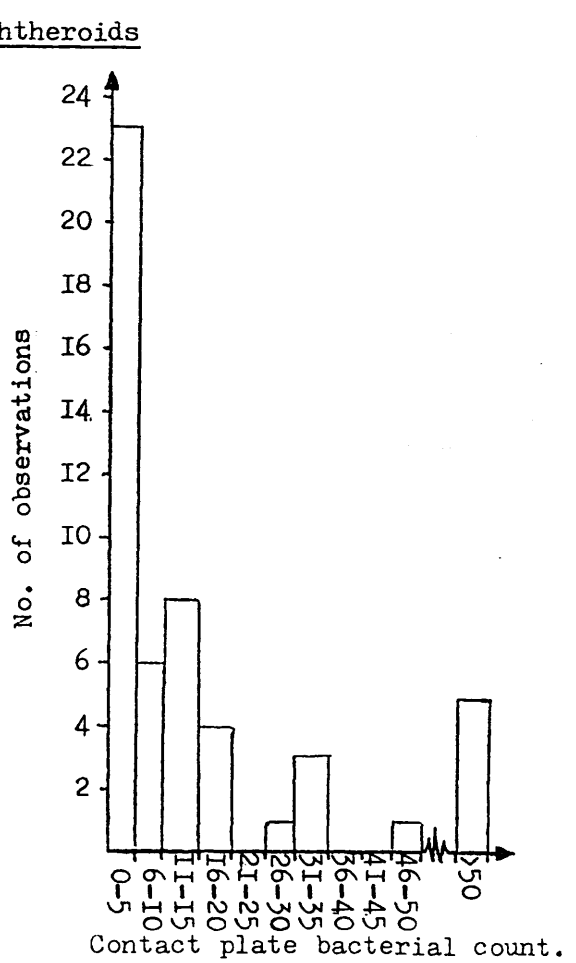
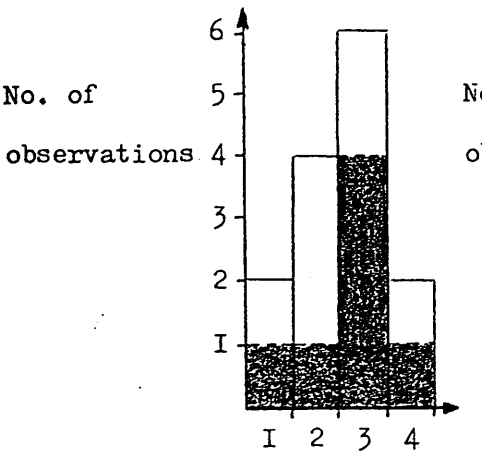


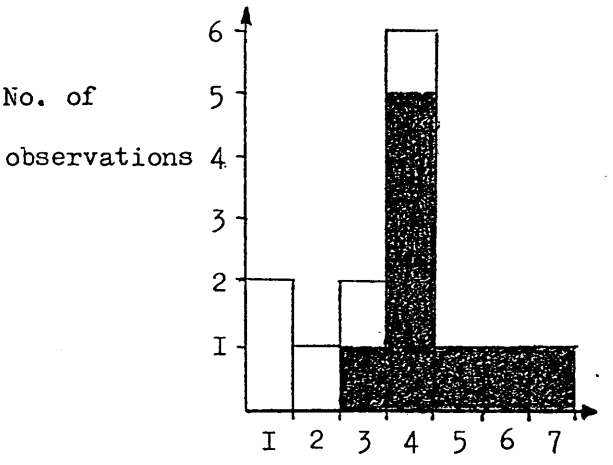
FIGURE 2I.

SWT bacterial counts when the contact plate count is zero.

S.aureus



CNS/M



The coloured areas denote the number of total observations where one or more of the other groups of bacteria numbered over 500 (over 100 below) on the contact plate.

Diphtheroids.

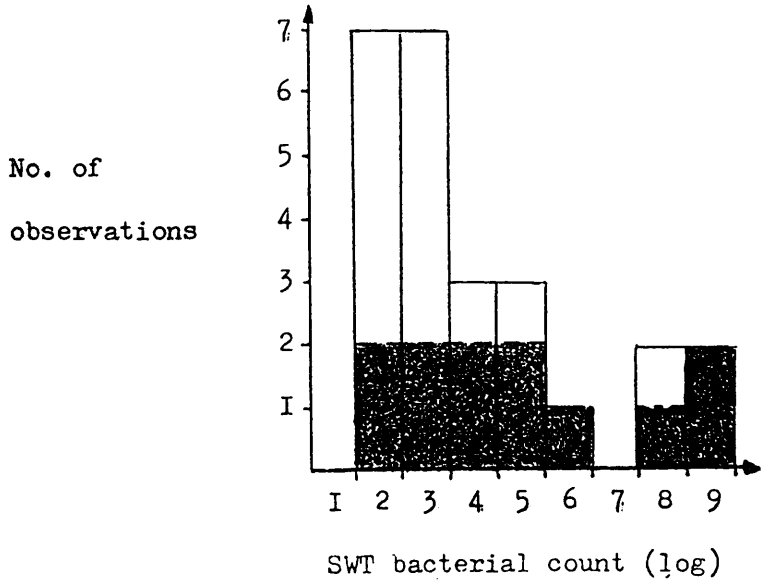
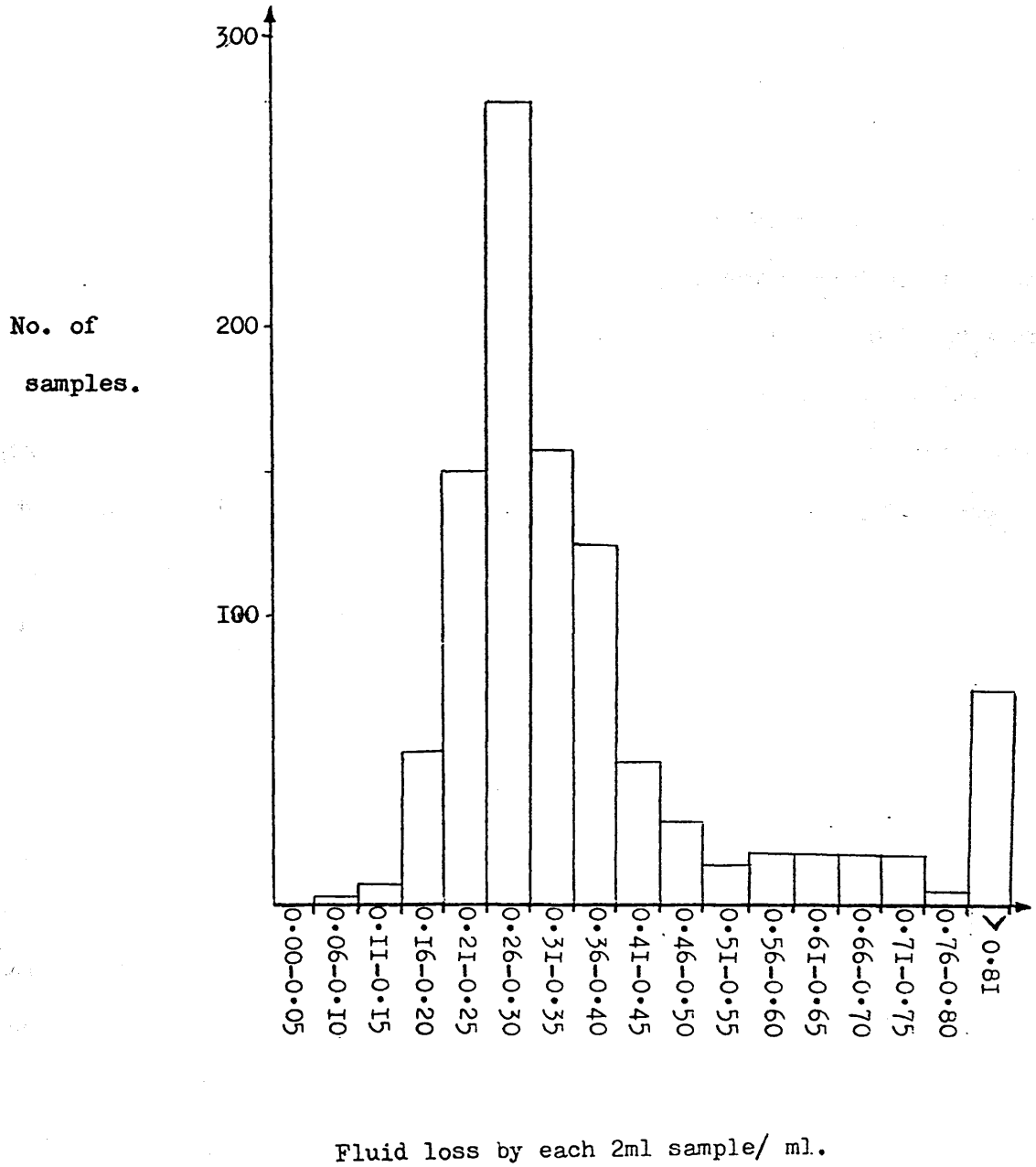


FIGURE 22.

Fluid loss during SWT sampling.



Fluid loss by each 2ml sample/ ml.

4. Discussion and Conclusions

4.1 Bacterial Flora of A.D. patients and controls

Tables 1 and 2 show the qualitative bacterial carriage of the various bacteria for both A.D. patients and controls as was determined by the three sampling techniques - contact plates, surface wash technique (SWT) and swabs.

As has been found previously (Aly et al. 1977, White and Noble, 1986) S.aureus carriage rates were higher for A.D. patients than for the normal, healthy population. This was found to be true for all body site examined. The differences found for CNS/M carriage between the two groups were not statistically significant. Diphtheroid carriage interpretation was less clear with the SWT-medium 3 carriage rates not being significantly different for A.D. patients and controls. The differences for the contact plate and SWT-media 1 and 2 rates were statistically significant though and highly so for all the swabbed body sites.

Carriage rates for S.aureus SWT blood agar (incubated aerobically and anaerobically) are very similar. This is also true for CNS/M and demonstrates the facultative anaerobic ability of staphylococci. Micrococci are obligate aerobes (Morse, 1980) and as the CNS/M anaerobic carriage rate is not greatly less (if less at all) than the aerobic rate, it suggests that the micrococci formed a small part of the CNS/M grouping.

Conversely the diphtheroid carriage rate differences for the SWT aerobic and anaerobically incubated blood agar, might suggest that obligate aerobic diphtheroids constitute a quarter to a third of the total diphtheroid skin flora.

MacConkey agar (SWT-Medium 3) was initially chosen for detection of Gram negative bacterial species. The number of Gram negatives found did not justify this medium's use.It was also generally less good at S.aureus / CNS/M and diphtheroid detection than was blood agar incubated aerobically. In any future studies it might be preferable to use C.L.E.D. medium to see if this medium really is superior to SWT medium 1, as could be construed from their relative carriage rates detected (Table 1), or whether the better detection rate of C.L.E.D. medium is in fact due to using contact plates.

In Table 2 nasal carriage for A.D. patients and controls were found to be 90% and 53% respectively. Aly et al, 1977 found the anterior nares of A.D. patients' S.aureus carriage rate to be 79%. The normal healthy population has shown great variability in studies on S.aureus nasal carriage rates though 19-40% is common (Noble et al. 1967). This study's higher value of 53% may be slightly positively biased due to the inclusion of hospital staff as 6/20 of our controls, 4 of which were S.aureus positive.

4.2 Correlation of bacterial numbers between contact plate sampling SWT-Medium 1

This comparison was undertaken to see if bacterial numbers obtained by contact plate sampling were comparable to those from the SWT. If correlation was found contact plates offer a faster more convenient method for extensive sampling of A.D. patients.

Figure 1 is a comparison of the mean body sites correlation obtained from each A.D. patient. Correlations for S.aureus and CNS/M were found to be fairly consistant and reasonable (Median correlations of 0.59 and 0.49 respectively). As each person's correlation was based on 8-10 observations (i.e. body sites), the consistency is good. There was no correlation for the diphtheroids (0.08 median).

Similar findings were obtained (Figure 2) when the mean correlations for each body site between A.D. patients were compared. S.aureus and CNS/M gave correlations between the various body sites of Ø.41 and Ø.55 respectively whereas that for the diphtheroids was -Ø.55.

The conclusions drawn from these facts are that S.aureus and CNS/M numbers on A.D. patients are comparable between patients or body sites and between contact plate and SWT sampling. This is not true for the diphtheroids. These findings suggest it is possible to use contact plates for extensive monitoring of S.aureus and CNS/M in clinical studies of A.D.

Possible reasons as to why the diphtheroids did not correlate better are as follows:- (1) Williamson and Kligman, 1965 suggest a 36-48 hour incubation for bacteria recovered by the SWT. In this study an incubation of 24 hours was preferred for faster results. In preliminary studies, similar counts of diphtheroids were obtained from examination of a plate after both 24 and 48 hour incubation. Colonies could be extremely small at 24 hours though and therefore it is possible that incorrect counts were taken. (2) An alternative explanation is that triton X-1Ø0 is to some extent bactericidal, particularly so for the diphtheroids. Bloom et al, 1979 recommend no more than 3Ø minutes should elapse between sampling and inoculation of the bacteria onto agar.

This immediately poses a problem in this study as the extensive sampling of a person took 45 minutes to an hour after which samples were measured and diluted before being transferred to agar. Lower SWT $\log_e$ counts for the diphtheroids are seen in Figure 2 with most in the range 6-1Ø compared to S.aureus and CNS/M, range of 8-12. This suggests that the second explanation is the correct one as diphtheroid contact plate numbers tended to come in the middle of the range of S.aureus and CNS/M contact plate numbers. If this is the case then it is likely that contact plates can be used (even preferred) instead of the SWT for extensive clinical sampling.

At higher contact plate bacterial numbers, aggregation of colonies was sometimes observed. A value of 1400 was given to those plates showing semi-confluent bacterial growth. Extreme care was taken counting the higher numbers, though due to the occasional localised aggregation of bacteria colonies seen on some plates, these counts must be considered less accurate than lower bacterial counts. In this aspect contact plates are less accurate at determining bacterial numbers than the SWT.

4.3 Correlation of bacterial numbers and clinical state

All three bacterial groupings were examined for the degree of correlation between the clinical state of the skin (eczema level) and bacterial numbers for the contact plates and SWT media 1, 2 and 3. The results are to be seen in Figures 3-14. Considering first the correlations between eczema level and contact plate bacterial numbers. S.aureus shows an increasing trend of bacterial numbers with increasing severity of the eczema with the high probability ($p \ll 0.001$) this was not a chance observation. CNS/M showed no change ($p > 0.05$) and the diphtheroids decreased in numbers as eczema become worse ($0.001 < p < 0.01$).

The SWT media 1, 2 and 3/eczema level correlation also showed an increase in S.aureus numbers with eczema level though this was less distinct (examining the 95% confidence limits) than for the contact plates. CNS/M showed a small increasing trend of SWT numbers with eczema severity though increases were less overall than for S.aureus. No trend was noticed for the diphtheroids though,as mentioned previously, this might be due to the effects of triton X-100 on the diphtheroid numbers.

In view of the higher sample numbers in the contact plate/eczema level correlations giving higher probabilities than for the SWT/eczema level correlations that the observations were not due to chance alone and the effects of triton X-100 on SWT diphtheroid numbers, the contact plate relationship is most likely to show the "true" relationship.

Although high S.aureus numbers have previously been recovered from eczematous lesions a direct relationship had not been established. CNS/M numbers did not appear to be so significantly related to eczema severity though all three SWT results indicated increasing CNS/M numbers with eczema level. Significant inverse correlation between diphtheroid numbers and eczema level was found.

Two questions arising from these results are do S.aureus (and less so CNS/M) and diphtheroids compete for binding sites on skin that on A.D. patients are more favourable to S.aureus or less favourable to diphtheroids and does S.aureus cause or aggrevate lesions on eczematous skin? These questions are considered more fully in 4.11 'Speculations on A.D. pathogenesis'.

4.4. Longitudinal study of A.D. Patients

Of the 6 patients examined 6 times at 6 weekly intervals only 3 showed S.aureus carriage at all body sites each time they were examined. Patients A, C and D results are shown in Figures 15, 16 and 17. The first point of note is that the S.aureus numbers vary greatly from site to site and visit to visit. Histogram plots of the ratios of within body site, across time standard deviation (a measure of a patient's body sites' S.aureus numbers variation over time)/between person standard deviation for that site (a measure of the variation of different patients' S.aureus numbers at a particular body site) are given to demonstrate the variability of S.aureus colonisation between the sites across time. Patients A and D show great variability whereas patient C's body sites are less variable in relation to S.aureus colonisation.

Eczema levels, as recorded, usually change by only 1 over 6 weeks, occasionally 2 and only once 3 suggesting that the mechanisms or factors involved in skin damage or healing takes longer than 6 weeks to change healthy skin to severely eczematous skin or vice versa.

4.5 Large S.aureus numbers precede dermatitis or vice versa?

The above question was considered in view of the direct relationship between S.aureus numbers and eczema severity found in this study. Change in S.aureus numbers (up or down) and in eczema level (up or down if not

static) over the 6 weekly intervals between visits of the longitudinal study were examined (Table 3). It may be seen that if S.aureus numbers rise, the eczema level is twice as likely to rise than to fall over the subsequent six weeks. There were 75 observations where the eczema level remained static after a rise in S.aureus numbers, though it can also be seen that subsequent to a rise in eczema level then S.aureus numbers are more likely to fall than rise. This last observation does not concur with the direct relationship found between S.aureus numbers and eczema level. Large S.aureus numbers are thus more likely to precede eczema than vice versa.

The table figures are likely affected by the fact that no differentiation was made in +1 or +1000 S.aureus numbers, both being counted as a rise. Greater elucidation may be brought about by changing the time period between visits although greater clarity of the results may not be possible if S.aureus is only one factor which may cause dermatitis (see later in 4.11 'Speculations on A.D. pathogenesis').

4.6 Bacteriophage typing of S.aureus isolates

Table 4 shows the phage types of all S.aureus isolates recovered from eczema patients during the study. As may be seen, the predominant group was that of group 3 (46% of isolates) whilst least observed was group 1 (3%) These results may be compared to those of Lever et al, 1988 from which it may be seen that fewer of our isolates were non-typable and of the typable groups, group 3 was again the most often observed - also found by Aly, 1980. Table 5 demonstrates that 50% of the patients examined had two or more groups isolated from their skin.

An examination of the phage types over time is shown for the sequentially studied patients (Tables 5-10) These show that those patients with the greater numbers of S.aureus, patients A, C and D, tended to have a dominant group although other transient groups may be found. Patient E also

seems to show this, while patients B & F have more variety of S.aureus groups found. An explanation of these findings may be given if skin S.aureus is thought to occur through constant dissemination from the nares (White and Smith, 1964). Table 11 shows that 14/16 observations have nasal and predominant cutaneous S.aureus of the same group wheras the 2 observations that did not still had the nasal isolate group on the skin in quantity. Indeed those patients with dominant groups A, C, D and E almost always have a nasal S.aureus isolate (Table 12) while those that did not, B and F, were those where more cutaneous S.aureus group variety was found (Tables 5-10).

4.7 Skin treatment affect on bacterial numbers

Comparison of the three treatments was made by considering the % change of bacterial numbers giving the day 1 number a value of 100% (Figure 19) Mupirocin treatment was performed on five patients, initially 6 but one patient was withdrawn due to an eczema flare between days 4 and 7 and was given appropriate topical steroid treatment. Averages were made of the bacterial numbers for each day. Two patients had diprosone treatment and one 'combined' treatment. Further treatment studies were not made as methicillin resistant staphylococci (MRS) arose after combination treatment although no MRS arose subsequent to mupirocin or steroid treatment on their own.

S.aureus numbers declined with all the treatments. The antibiotic mupirocin's direct effect on S.aureus cleared the skin totally by the second sampling though some recolonisation was observed by day 7. Diprosone was also highly effective in reducing S.aureus over the week though at no time was S.aureus eradicated. Reduction was also slower than by use of mupirocin with only approximately 60% S.aureus reduction by day 4. The combination treatment by day 4 gave S.aureus reduction intermediate to either of the other treatments but was closer to mupirocin treatment alone than to diprosone alone. By day 7 the combined treatment produced the greatest S.aureus reduction, though again S.aureus was not totally eliminated.

CNS/M were initially greatly reduced by mupirocin and combination treatments though mupirocin was less effective at reducing diphtheroids. This confirms mupirocin as chiefly an antistaphylococcal agent (Mellows, 1985)

It is interesting that mupirocin and combination treatments had less of an effect on CNS/M numbers than S.aureus numbers with 5.7% and 15.8% CNS/M survival rates and 0.0% and 5.3% S.aureus survival rates respectively. After mupirocin treatment the CNS/M recover to 23.9% by day 7 compared with 2.4% for S.aureus though S.aureus eradication (as detected by the samples) would have a bearing on its recovery. After combined treatment CNS/M recover to 49.7% at day 7, compared to a continued decrease to 0.4% for S.aureus. At day 7 it is also interesting that diprosone has increased CNS/M numbers and only slightly reduced the diphtheroids while having greatly reduced S.aureus from the day one numbers.

4.8 Clinical effects of the treatments

Comparison of the treatment affects was made by examining the percentage of body sites examined that improved, were static or grew worse clinically over the week's treatment (Table 13).

While mupirocin does remove S.aureus the best, it proved to be the least clinically effective of the three treatments with 15% of sites deteriorating. No clinical deterioration of sites was seen with steroid or combination treatments the latter of which achieved the largest percentage of improved sites. The anti-inflammatory effect of the topical steroid was most beneficial in addition to the antibiotic affect. The results therefore concur with those of Leyden and Kligman, 1977.

Table 14 demonstrates that maximal improvement with mupirocin treatment occurred after 14 days. Perhaps S.aureus (an aggrevating factor?) removal allows the skin to heal, though less rapidly than with diprosone.

4.9 S.aureus isolate antibiotic sensitivities

Methicillin resistant S.aureus (MRSA) strains are spreading causing significant amounts of hospital acquired infection (MacDonald, 1982). These epidemic strains possess various mechanisms for resisting antibiotic action. For this reason we examined the antibiotic sensitivities of 60 of our isolates for methicillin and mupirocin resistance. Mupirocin offers a potential method of control for multiple resistant strains.

None of our 60 isolates (from the initial pre-mupirocin/diprosone treatment part of the study) showed MRSA or mupirocin resistance though MRSA found during the treatment studies (by the Western Infirmary Bacteriology Dept.) caused the termination of the treatment studies on ethical grounds.

4.10 Contact plate and SWT comparison

The three histograms in Figure 20 demonstrate that no bacteria obtained by the SWT, when at the same site a positive contact plate result for that bacterium grouping was obtained, occurred overwhelmingly when the contact plate numbers were 20 or less. This was the case for 73% of S.aureus, 64% of CNS/M and 80% of diphtheroid contact plate positive, SWT zero observations. The other 20-36% discrepancy may be as a result of the fact that the two sampling techniues were performed immediately adjacent to each other, at the same body site, to avoid one technique affecting the bacterial numbers removed by the other technique as would occur when performed on the exact same site.

Conversely the number of observations obtained when SWT gave positive bacterial counts and contact plats none are shown in Figure 21. When the coloured area observations are removed it may be seen, especially for the CNS/M and diphtheroids, that higher SWT counts are less common. The total

number of all observations for all the bacteria are smaller than the observations on the previous page implying that contact plates are more likely to detect the three bacterial groups than the SWT. It should be noted however that both sets of observations are small compared to the total number of observations with both positive or both negative.

The diagram in Figure 22 shows another factor affecting calculation of bacterial numbers by the SWT. Fluid loss during sampling has an affect on number calculation as numbers calculated are corrected for each sample's fluid loss. Some allowance for this has been made though this assumed that fluid was not recovered on final aspiration of the fluid whereas occasional leakage around the edge of the teflon ring during sampling was observed. If leakage only occurred at the end of each minute's sampling the "correction factor" would accurately allow for the bacterial numbers lost. If leakage on the other hand occurred during sampling the final concentration of the bacteria in the wash fluid will have been altered lessening the accuracy of the "correction factor". Figure 22 suggests that the calculated bacterial numbers from each sample were generally comparable as most observations were normally distributed about Ø.26-Ø.3Øml of non-recoverable wash fluid.

4.11 Speculations on atopic dermatitis pathology

In this thesis I have shown that as clinical severity of eczema rose, the numbers of S.aureus bacteria also rose whereas those of the diphtheroids declined. Paucity of diphtheroids on eczematous skin had been previously noted-especially for the lipophilic diphtheroids (Aly et al, 197Ø). The study showed there was an inverse relationship for these two bacterial types on eczematous skin.

CNS/M numbers appeared unaffected by eczema severity, as determined by contact plate, though the SWT did show some increase in CNS/M numbers with eczema severity. This was less than was seen for S.aureus. An inverse relationship for CNS/M and diphtheroids had previously been noted in the nares

of "normal" individuals (Aly et al, 1970) and on the skin (Marples and Williamson, 1969). Marples et al, 1969 proposed that inhibitor strains exist among the lipophilic diphtheroids which control the ecological status of both cocci and enterobacter species as the removal of lipophilic diphtheroids by antibiotics allows the emergence of S.aureus or enterbacteria as well as growth of CNS/M. Indeed Aly et al, 1977 wrote "the preponderance of lipophilic diphtheroids is indicative of normal healthy skin". So why does both uninvolved and dermatitic skin of eczema patients have relatively few lipophilic diphtheroids?

Nutrient factors available to skin bacteria are important. Many diphtheroid bacteria have an absolute requirement for unsaturated fatty acids. Such strains are by far the most numerous aerobic diphtheroids of the normal skin flora (Marples, 1969). Pollock et al, 1949 more specifically found this to be absolute dependance on oleic acid (c18:1). The powerful self disinfecting ability of the skin for potential pathogens, such as streptococci and staphylococci has also been shown by Burtenshaw, 1942 and Ricketts et al (personal communication in Pollock et al, 1949) to be due to the presence of oleic acid derived from sebaceous secretions. Oleic acid was also found to be S.aureus inhibitive by Wynne and Foster, 1950 though contrary to all these findings were those of Fritsche and Zitz, 1973 whose study suggested oleic acid "stimulated" S.aureus growth. These variable results are probably due to test procedures with different concentrations of oleic acid and other growth medium substances. The attentuation of fatty acid bactericidal effect by calcium and magnesium ions has been observed (Galbraith et al, 1971) as has the mutual detoxifying effect of cholesterol and oleic acid, both inhibitory on their own, may be some of the factors that explain the range of results. Cholesterol forms a greater proportion of atopic dermatitis sebaceous lipids than that of a healthy person and atopic dermatitis sebum has a relative deficiency of unsaturated fatty acids (Abe et al, 1978 and Ead et al, 1977).

The requirement of diphtheroids for oleic acid and its inhibitory affects on other bacteria could explain the dearth of diphtheroids and increase in cocci if oleic acid is less readily available on atopic dermatitic skin. This is quite likely (see below). It is interesting to note that the C.acnes bacterium, responsible for acne at puberty, is stimulated by oleic acid (Puhvel and Reisner, 1970 and Pillsbury and Rebell, 1952). Unsaturated fatty acids in the skin are derived largely from the sebum which has increased output at puberty (Wheatly, 1965). In addition, acne patients have a lower incidence of atopic dermatitis (Liddell, 1976).

Removal of fatty acid from the skin by acetone enhances survival of staphylococci though replacement of the acetone soluble material will restore the inhibitory action of normal skin (Aly et al, 1972). Aly et al, 1975 showed that the skin surface lipid of people who possess high normal skin populations and tolerate large numebrs of S.aureus and C.albicans on the skin was less toxic in vitro to these organisms than lipids from the skin of people on which these organisms do not persist.

Prose, 1965 noted the sebaceous glands in the scalp of atopic dermatitic infants are reduced in number and show retarded development. In addition their basal cells are smaller than normal. Low sebum output was recorded in atopic dermatitic individuals and the skin surface lipids present were deficient in constituents derived from sebaceous glands (Wheatly, 1965).

Pollock et al, 1949 found that on dry, glaborous skin while non-lipophilic diphtheroids occur, they are few in numbers. Thus the skin of atopic dermatitis patients has lost its sebum bactericidal protection and diphtheroid interference. It is likely this is the reason for the increased numbers of other bacteria seen on atopic dermatitic skin as well as the increased susceptibility of A.D. skin to skin infection by other microorganisms (Noble, 1981).

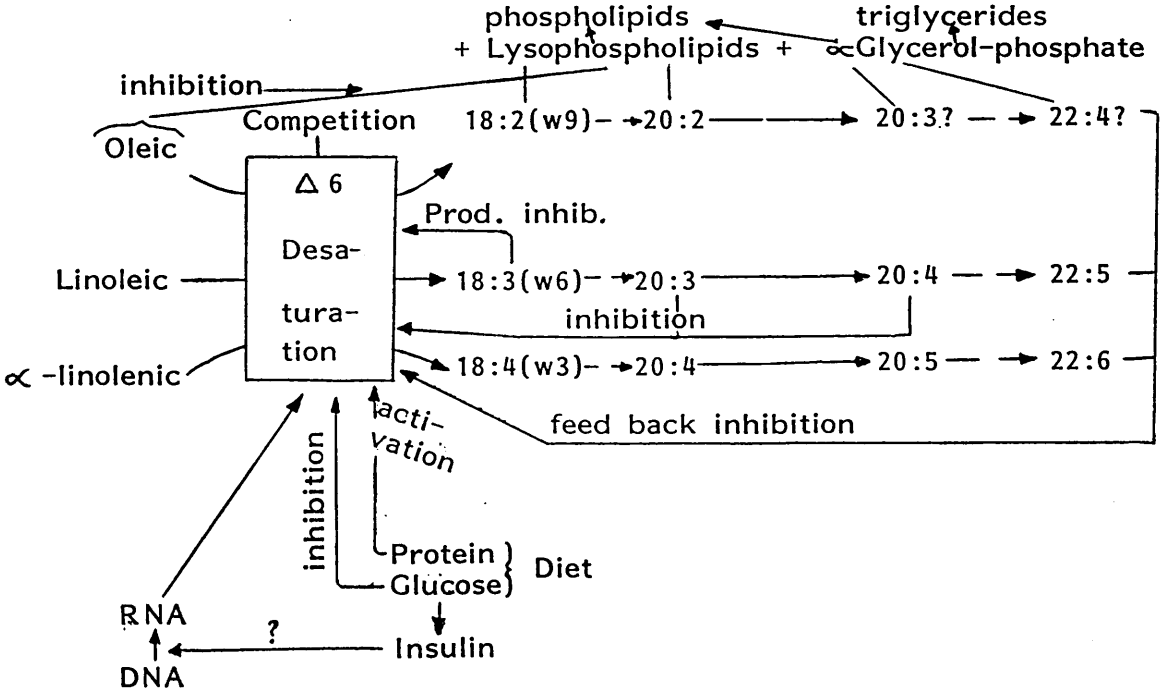
The question arises as to why the skin lipids should be lacking in unsaturated fatty acid. Unsaturated fatty acid metabolism of both atopic dermatitis and healthy individuals was examined through the literature available. Hansen, 1937 regarded eczema as an expression of unsaturated fatty acid deficiency. On treatment of patients with unsaturated fats he found that coincident with a serum level increase of unsaturated fats was a clinical improvement. His research was based on that of Burr, 1929 who found that rats placed on completely fat free diets (but otherwise adequate) soon develop a definite syndrome which began with skin scaliness. This disappeared on readdition of fat to the diet.

Cornbleet, 1935 using maize oil (an unsaturated fatty acid source) orally administered also found eczema improvement. Other findings, such as those of Taub and Zakon, 1935 using linseed oil, did not confirm the original reports and the treatment was brought into disrepute.

Recently the subject has resurfaced with evening primrose oil (Efamol) being advocated for treatment. Evening primrose oil (EPO) is a rich source of unsaturated fatty acids. The composition of EPO (Manku, 1983) is:- palmitic acid (c16:0) - 6%, stearic (c18:0) - 2%, oleic (c18:1) - 11.3%, linoleic (c18:2 w6) - 72.3% and gamma-linolenic (c18:3w6) - 8.9%. Sunflower-seed oil compositon:- palmitic - 9.3%, stearic - 3.7%, oleic - 24.3%, linoleic - 61.5% and linolenic -1.0% was also found beneficial to eczema (Press et al, 1974). Note the high % contents of oleic, linoleic and less so linolenic acids.

Nine papers support the value of unsaturated fatty acid/EPO treatment (Hansen, 1933; Cornbleet,1935; Press et al, 1974; Wright and Burton, 1982; Wright et al, 1983; Galland, 1986; Schalin-Karrila et al, 1987; Bordoni et al, 1988 and Biagi et al, 1988) and three do not confirm these findings (Pettit, 1954; Bamford et al, 1985 and Greaves and Corbett, 1988).

Manku et al, 1982 found reduced levels of prostaglandin precursors in the blood of atopic patients and proposed defective delta-6-desaturase function as a biochemical basis for atopy. In a further study, Manku found that of plasma phospholipids, linoleic acid was significantly elevated (Manku et al, 1984) but all its metabolites were significantly reduced. The delta-6-desaturase enzyme catalyses the first step of conversion of linoleic acid to its metabolites (Brenner, 1971). Wright, 1985 also suggested defective delta-6-desaturase function as a basis for atopy. The delta-6-desaturase is a central enzyme in unsaturated fatty acid synthesis (see below - from Brenner, 1971).



Delta-6-desaturase metabolises the acids preferentially (i.e. alpha-linolenic before linoleic before oleic). The diagram shows the linoleic metabolite (dihomo-gamma-linolenic acid etc) inhibition of the delta-6-desaturase enzyme. Delta-5, 8, 11 eicosatrienoic acid (w-9) accumulates in essential fatty acid deficient diet skin (Prottey, 1976). Usually it is minimal in normal skin (Wilkinson, 1972) except when the amounts of linoleic acid falls (as in the onset of essential fatty acid deficiency) in which case the enzymes that normally convert linoleic acid via dihomo-gamma-linolenic acid to arachidonic acid instead converts oleic acid to w-9-eicosatrienoic acid (Fulco and Mead, 1950)

In view of Manku's findings that the plasma phospholipid level of linoleic acid was significantly higher for eczema patients than normal while that of its metabolites were significantly lower (Manku et al, 1984) it seems possible that defective delta-6-desaturase function is to blame for atopic dermatitis. I propose that this defect is in the concentration of linoleic acid needed for it to be metabolised preferentially to oleic acid, so that normal dietary intake of linoleic and alpha-linolenic acids does not prevent delta-6-desaturation of oleic acid. This is consistant with the only significant finding in pre-treatment plasma fatty acid levels of Schalin-Karrila et al, 1987 who found that in the EPO treatment group oleic acid serum levels were significantly lower than that of the normal controls. In the placebo group, oleic acid serum levels were also lower than that of the controls, although not significantly so. It was stated though that the EPO treatment groups' initial eczema severity was worse than that of the placebo group. As the study continued the boosted dietary concentration of linoleic acid was enough for it to be metabolised to dihomo-gamma-linolenic acid (which increased significantly). The metabolism of linoleic and the inhibition of further delta-6-desaturation by dihomo-gamma-linolenic would prevent oleic

acid metabolism. Thus freed from delta-6-desaturase use, oleic acid may be diverted to the skin by some other means (possibly in triglyceride form). The six week further drop in plasma oleic acids levels, compared to initial values, might be due to its use by metabolic systems other than delta-6-desaturase. By twelve weeks the level has risen again, as has the skin improved, suggesting that as the skin continues to improve the oleic acid serum levels will rise as the skin needs less to bring its sebum back to normal composition. This trend should continue until the skin is clinically normal.

The model thus predicts increased sebum output, less xerotic skin, fewer bacteria and fewer S.aureus as oleic acid promotes diphtheroid growth and has coccal bactericidal action.

In 4.5 an examination of whether S.aureus numbers rises or eczema severity rises first is discussed. As is shown (Table 3) the answer from 6 weekly intervals is not clear but this may be explained. The lack of skin sebum (with oleic acid) and diphtheroids leaves the skin of eczematous individuals dry and disposed to S.aureus colonisation which on reaching sufficient numbers initiate a flare. S.aureus is not the sole irritant though as a dry sebum-barrier-less skin is more irritable. As Hill in comments in Wheatley, 1965 stated:- "infants where skin is normal apart from eczematous patches are more likely to resolve the condition than those with totally abnormal skin which is more likely to be unusually susceptible to irritation of any kind regardless of demonstrable allergy". Probably skin inflammation is set in action when skin mast cells degranulate after cell-bound IgE is cross-linked by S.aureus or other antigen/irritants (Roitt, 1984). S.aureus would be more irritant than other substances as it is self-replicating and produces various toxins (Stephen and Pietrowski, 1986).

Abnormal fatty acid metabolism may have consequences on related metabolic processes e.g. prostaglandin production. The fatty acids previously mentioned are the precursors of arachidonic acid and various prostaglandins.

The reason why all investigations have not supported EPO/unsaturated fatty acid treatment may be that they did not include oleic acid in their reckoning which I suggest is at least initially needed to make up for its deficiency in sebum. Greaves and Corbetts' letter commented on the paper by Schalin-Karrila et al, 1987. A reply by one of the Schalin-Karrila co-authors is also given. This corrects their first paper and disagrees with the remaining points questioned by Greaves and Corbett. Bamford's study was the most comparable to the supportive studies (Bamford et al, 1985). It did not consider that irritants less affected by increased sebum production due to EPO treatment than S.aureus may give rise to skin lesions. The irritants other than S.aureus were perhaps more significant in Bamford's patient group.

Therefore unless the age of the sufferer (see below), patient contact with non-S.aureus irritants and the relative ratios of dietary oleic, linoleic (and thus dihomo-gamma-linolenic) and alpha-linolenic acids are considered the clinical improvement or otherwise seen in these studies is not really just a measure of EPO treatment.

The following points also suggest the hypothesis:- (1) Improvement of A.D. is seen at puberty, especially for males (Rajka, 1984). Perhaps increased sebum stimulates diphtheroid growth on A.D. skin (as happens on the skin of acne sufferers) also reducing S.aureus by increased sebum bactericidal effect. More sebum also creates a better skin barrier to other irritants. Testosterone increases both the sebaceous gland size and the sebum output of pre-pubertal boys (Wood and Bladon, 1985) which may explain the improvement in males. (2) On approach to puberty until at about fifteen yers of age the sebum composition gradually changes until it resembles that of an adult (Wood

and Bladon, 1985). Even the amount of sebum produced by a healthy child proportionally is less than that of healthy adults. This might explain why atopic dermatitis has a tendency to clear with age (Rajka, 1974). The enzyme delta-6-desturase is also used less with age (Brenner, 1971). It was suggested that younger animals desaturase more fatty acids, perhaps due to the building of new tissues. (3) Circadian rhythm studies of sebum secretion (Burton et al, 1970) showed secretion peaked in the morning while Rajka, 1974 showed atopic dermatitis sufferers found their skin was most itchy during evening and night (i.e. non-synchronous by half a day). Obviously it is unlikely the increase in sebum has too great an effect on the skin flora in this time, however the increased sebum could increase the skin's irritant barrier sufficiently for a qualitative difference.(4) Contra-indicated prophylaxis is avoidance of strong defatting substances (Rajka, 1974). Soaps etc. would remove diphtheroid nutrients and sebum bactericidal effect. (5)Steroid treatment is the preferred treatment for atopic dermatitis. Gurr, 1974 states that steroids inhibit desaturase enzymes. This would stop delta-6-desaturase metabolism of oleic acid freeing it for use on the skin. (6)Epidermal thickening occurs in essential fatty acid deficient rats (Prottey, 1976). Perhaps this is comparable or related to plaque development on atopic dermatitis skin. (7) The sites of prevalence of atopic dermatitis are where lipophilic diphtheroids (and by my model sebum oleic acid) are least likely to be found (McGinley et al, 1985).

Thus it may be seen that Manku's suggestion of defective delta-6-desaturase metabolism as a cause of atopy is also a likely explanation for the abnormal bacterial flora of atopic dermatitis (Manku et al, 1982) Further research into unsaturated fatty acid metabolism and the processes it affects is needed.

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6.appendix 1 - The Gram Stain

The Gram stain is a procedure for the division of bacterial groups and is used as a standard identification procedure.

1. Emulsify bacteria in droplet of water on a microscope slide.

2. Heat-fix the bacteria by passing the slide through a bunsen burner flame.

3. Using a dropper 'spot' a droplet of Crystal Violet dye onto the heat-fixed bacteria. Leave for one minute then wash off using water.

4. Using a dropper 'spot' a droplet of Iodine Solution onto the heat-fixed bacteria. Leave for one minute then wash off using water.

5. Similarly apply acetone to the heat-fixed bacteria. Leave for only two seconds before washing with water.

6. Finally apply dilute Carbol Fuchsin dye. Leave for thirty seconds before washing with water.

7. After air-drying the slide may be examined microscopically using x100 oil immersion magnification. Gram positive bacteria retain the purple colouring obtained from the third and fourth steps whereas Gram negative bacteria lose this colour by step five and appear the pink colour of Carbol Fuchsin from step six.

Appendix 2 - Media

Horse blood agar

---

		g/l
nutrient agar	(Oxoid)	28
+7% sterile defi-brinated horse blood (Gibco)		w/w

The nutrient agar after sterilisation at 15 p.s.i. for 15 minutes should be cooled to 50°C before the blood is added.

Bacteriophage agar

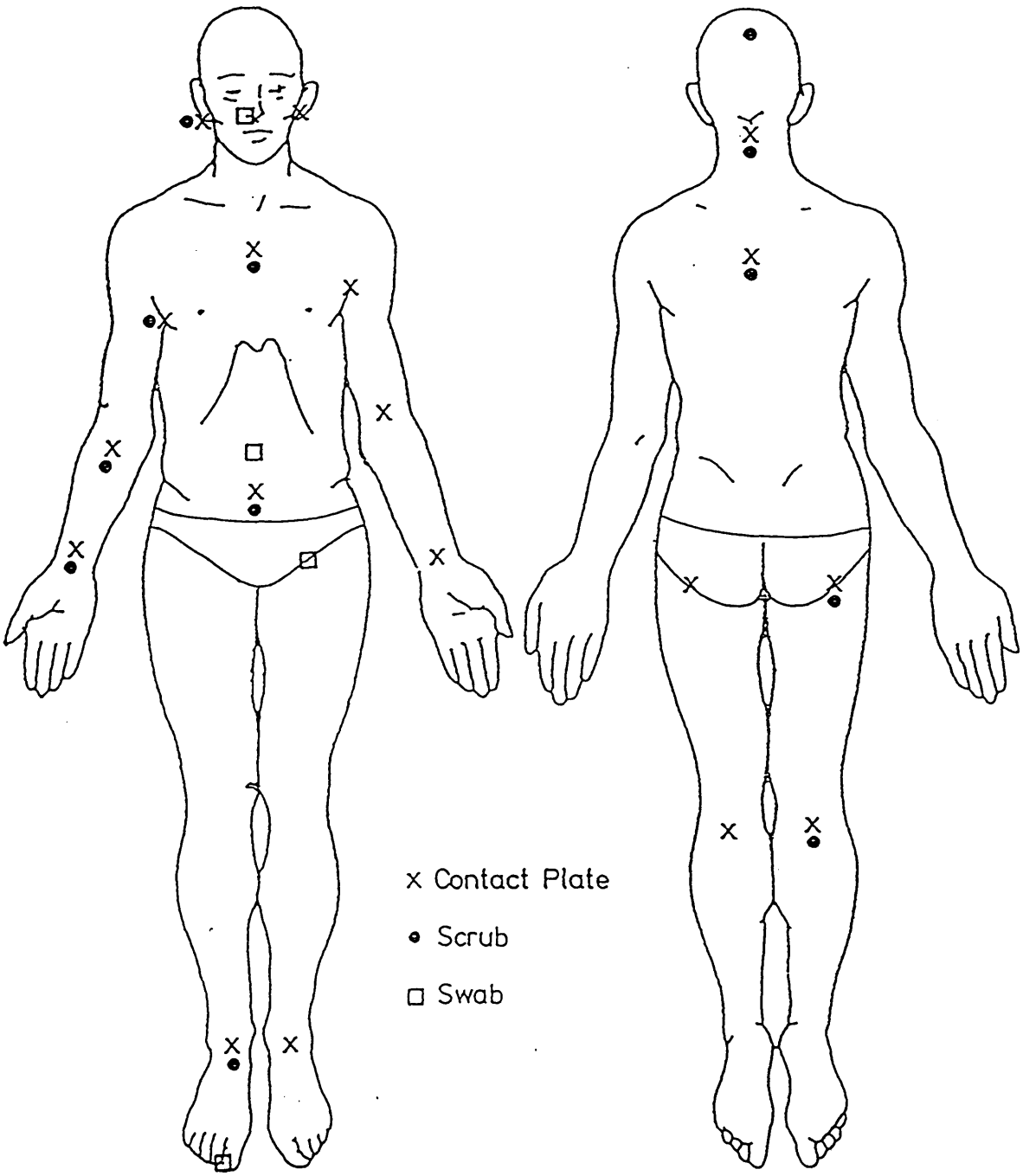
---

		g/l
nutrient broth No. 2	(Oxoid)	25
agar No.1	(Lab M)	12
NaCl		5
$CaCl_2$		0.5

$CaCl_2$ at this concentration does not make agar cloudy. It should be added to the already sterile liquid agar to ensure the amount of free calcium needed for some of the phages' action.

APPENDIX 3.

SITES AND TECHNIQUES OF BACTERIOLOGICAL ASSESSMENT



APPENDIX 4

Body site number identification

1.	Scalp	2.	Left Post Auricular
3.	Right Post Auricular	4.	Left Axilla
5.	Right axilla	6.	Sternum
7.	Back	8.	Left Antecubital Fossa
9.	Right Antecubital Fossa	10	Left Wrist
11.	Right Wrist	12	Suprapubic
13.	Left Gluteal	14.	Right Gluteal
15.	Left Popliteal Fossa	16.	Right Popliteal Fossa
17.	Left Ankle	18.	Right Ankle
19.	Nuchal	20.	Forehead Centre
21.	Right Cheek	22.	Dorsal Right Hand
23.	Left Forehead	24.	Right Forehead
25.	Left Cape	26.	Right Cape
27.	Cape Centre		