A STUDY OF SCALP RINGWORM

IN THE WESTERN CAPE.


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I declare that this thesis is my own work.

It is being submitted for the Masters Diploma

in Technology (Medical Technology),

to the Cape Technikon, Cape Town.

It has not previously been submitted for any diploma,

degree or examination at any other institution.

This work was carried out in the Microbiology Department,

Red Cross War Memorial Children's Hospital.

The opinions and conclusions drawn are not necessarily

those of the Cape Technikon.

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Gail Neil                        Date

27. 11. 91

I dedicate this work to my husband and son,

Alan and David Neil.

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SUMMARY


Scalp ringworm, a potentially disfiguring and
emotionally traumatic disease of childhood, appears to
have attained an unaceptably high incidence in the
southern and western Cape.  This study examined aspects
of tinea capitis and was undertaken in four distinct
sections, each evolving from the prior study.  Attempts
were made to establish the local incidence, the
clinical presentation and the causative dermatophytes;
to ascertain the effects of geographical influences on
this spectrum of organisms; to determine the number of
fungal carriers within certain communities and to
examine both in-vivo and in-vitro effects of
easy-to-use, topical preparations on this group.


On examining the incidence, it was shown that
approximately 3% of children seen at the Red Cross
Children's Hospital in Rondebosch attend specifically
for scalp ringworm but many more, particularly of low
socio-economic backgrounds remain untreated (Neil,
1987).  Random visits to primary schools along the West
Coast and to childcare institutions in the Cape
Peninsula revealed an infection rate as high as 53% and
29% respectively.


Following examination of the varied clinical

presentations evoked by infection with T.violaceum, the predominant manifestation of disease was shown to be various degrees of scalp scaling. The findings highlighted the often subtle and easily overlooked clinical presentations which presumably contributed to the high prevalence of this disease amongst the community. Laboratory investigation revealed the major aetiological agent to be the anthropophilic fungus, T.violaceum (92%). This was followed by M.canis and M.audouinii constituting only 2,8% and 1,7% of isolates respectively. Small numbers of seven other dermatophytes, including T.verrucosum, T.yaoundei, T.mentagrophytes, T.schoenleinii, T.tonsurans, M.gypseum and E.floccosum, were also isolated. Examination of geographical influences showed that none of the associated factors, with particular reference to altitude and climate, appeared to exert any discernable effect on the prevailing dermatophyte species in any particular region. T.violaceum was isolated with overwhelming frequency from both the South Western Cape and the Western Cape Coast in spite of vastly different climates and altitudes (Mediterranean and semi-desert, and sea-level and up to 1322m respectively). Other dermatophyte species were similarly isolated from both areas but numbers were too small to allow meaningful analysis.

Research into the possible sources of tinea capitis

revealed that in addition to the high incidence of clinical infection approximately 24% of children in 8 local childcare institutions were fungal carriers with no discernable clinical symptoms. All were colonised with T.violaceum and served as reservoirs for the spread and persistence of tinea capitis. Following studies spanning various time periods (6 weeks to 8 months), it was shown that only 14% of carriers were likely to develop clinical disease while the chance of remaining a carrier or becoming "negative" was approximately equal in the remaining patient sample. Thus a significant number of carriers remained a constant threat to susceptible individuals.

In-vivo trials on the effects of three antifungal preparations on the carrier state showed that all produced some degree of beneficial effect by reducing the fungal load of carriers as well as infected children. The shampoos used in the trial included econazole nitrate (Pevaryl; Fison's Laboratories), selenium-sulphide (2,5% Selsun; Abbott Laboratories), povidone-iodine (Betadine; Adcock-Ingram Laboratories) and Johnson's Baby Shampoo (Johnson & Johnson), a bland preparation, as a control. Although small improvement was noted following the use of each preparation, the povidone-iodine shampoo significantly reduced the fungal load of infected children (p<0,001) and completely eradicated the organisms from fungal

carriers. The comparatively moderate benefits of econazole nitrate, selenium-sulphide and the control shampoo were considered to be due largely to the physical removal of fungal spores by improved hair hygiene.

Cultural and ultrustructural examination of the effects of the preparations on the spores of T.violaceum supported these in-vivo findings. Mild to moderate effects on growth patterns and cellular changes were noted following the use of econazole nitrate, selenium-sulphide and the control shampoos but total suppression of germination and gross degeneration of cells by the povidone-iodine preparation was clearly demonstrated.

These studies have served to highlight the incidence of tinea capitis and the role of fungal carriers in our community. Greater knowledge and awareness of the many clinical manifestations of the disease as well as the eradication or control of the carrier state should be considered vital in our efforts to effectively reduce the numbers of clinically infected children. Regular use of a suitable shampoo and improved hair hygiene practice in local institutions and the community at large would be a positive contribution towards achieving these aims.

Although further studies are clearly necessary, this study has revealed that the availability, ease of application and minimal side-effects of the povidone-iodine preparation make Betadine Shampoo an ideal product for regular use as a prophylactic measure.

Minimising opportunities for contracting this potentially disfiguring and psychologically traumatic disease would be a valuable contribution towards improving the quality of life in our third world society.

OPSOMMING

Omlope van die kopvel is 'n potensiele skendende en
emosioneel troumatiese siekte in kinders en het 'n
onaanvaarbaar hoe insidens in die Suid-Wes Kaap
bereik. In hierdie studie is aspekte van tinea capitis
aantasting paslik ondersoek in vier onderafdelings na
gelang van die bevindings van 'n vorige proef. Daar is
gepoog om die lokale insidensie en die kliniese beeld
vas te stel en oorsaaklike dermatofiete te
identifiseer. Die invloede van geografiese ligging op
die spektrum van organismes wat voorkom asook die getal
draer individue in sekere gemeenskappe in die
verskillende gebiede is ook bestudeer. Die _in_ _vivo_ en
_in_ _vitro_ effekte van maklike aanwendbare topikale
formulerings op organismes vanuit hierdie groepe is
voorts ook geevalueer.

Na ondersoeke rakende insidensie is getoon dat ongeveer
3% van alle kinders wat by die Rooi Kruis Hospitaal in
Rondebosch ondersoek word, die hospitaal spesifiek
besoek vir klagtes wat verband hou met omlope van die
kopvel. Daar word egter aanvaar dat 'n veel groter
persentasie van kinders onbehandel bly (Neil, 1987).
Ewekansige besoeke aan primere skole in die Weskus
gebied en aan inrigtings in die skiereiland het getoon

dat die infestasiekoers so hoog as 53% en 29%, respektiewelik mag wees.

Na ondersoeke na die verskillende maniere waarvolgens infeksie met T.violaceum kan voordoen is vasgestel dat verskillende grade van kopvel afskilfering die predominante simptoom of teken was. Die bevindings het die dikwels subtiele en maklik misgekykte kliniese presentasies beklemtoon wat blykbaar bydra tot die hoe voorkoms van die siekte in die gemeenskap.

Laboratorium ondersoeke het aangetoon dat die hoof etiologiese agens die antropofiliese swam T.violaceum (92%) was. Hierdie is gevolg deur M.canis en M.audouinii wat 2,8% en 1,7% van isolate, respektiewelik, verteenwoordig het. Klein persentasies van sewe ander swamme is ook geisoleer en sluit in T.verrucosum, T.yaoundei, T.mentagrophytes, T.schoenleinii, T.tonsurans, M.gypseum en E.floccosum.

Ondersoeke na geografiese invloede het getoon dat geeneen van die geassosieerde faktore, met spesiale verwysing na hoogte bo seespieel en klimaat, 'n groot rol gespeel het in die voorkoms van die verskillende gebiede. T.violaceum is geisoleer teen 'n hoe frekwensie vanuit beide die Suidwes Kaap en die Weskaap nieteenstande die groot verskille in die klimate en die

hoogtes bo seespieel (Meditereens en half-woestyn en seevlak tot 1322m bo seespieel, respektiewelik). Ander dermatofiet spesies is op 'n soortgelyke wyse geisoleer van beide areas maar die getalle was te klein om sinvolle analise moontlikte te maak.

Ondersoeke na die moontlike herkoms van tinea capitis het getoon dat, bykomend tot die hoe insidens van kliniese infeksie, ongeveer 24% van kinders in 8 plaaslike kindersorg inrigtings draers van swamme was met geen opmerkbare kliniese simptome. Alle inwoners was met T.violaceum gekoloniseer en het as reservoirs vir verspreiding en behoud van tinea capitis gedien. Opvolg studies oor periodes van verskillende lengtes (6 weke tot 8 maande) het getoon dat slegs 14% van draers kliniese siekte sou ontwikkel terwyl daar 'n 50% kans bestaan het dat 'n individu in die groep van die oorblywendes van die infeksie ontslae sou raak. Dus het 'n konstante bron van infeksie vir vatbare individue bly voortbestaan.

In vivo proewe op die effekte van 3 anti-fungale preparate op die draerstaat het getoon dat almal 'n mate van voordelige effek uitgeoefen het deurdat dit die swam lading in draers asook in geinfekteerde kinders verminder het. Die sjampoe formulerings wat in die proewe beproef is het ingesluit: ekonasoolnitraat,

(Pevaryl: Fison Laboratoria), Selenium-sulfied (Selsun: Abbott Laboratoria), povidoonjodium (Betadine: Adcock-Ingram Laboratoria) en Johnson se babasjampoe (Johnson & Johnson). Laasgenoemde is 'n sjampoe sonder aktiewe bestanddele wat as kontrole gedien het. Alhoewel 'n klein verbetering waargeneem is na gebruik van elke preparaat, het die povidoonjodium bevattende sjampoe die swamlading betekenisvol verminder in geinfekteerde kinders (p<0.001) en die swam heeltemal verwyder in draers.

Die relatief beperkte voordele van ekonasoolnitraat, selenium-sulfied en die kontrole sjampoe is toegeskryf aan die gepaardgaande higiene eerder as die anti-swam aktiwiteit van die bestanddele.

Ondersoek na die effekte van hierdie formulerings op swam spore, strukture en ultra-strukture van T.violaceum in vitro het die in vivo bevindings bevestig. Minimale tot matige effekte op groei patrone en sellulere veranderings is waargeneem na gebruik van ekonasoolnitraat sjampoe, selenium-sulfied sjampoe en die kontrole sjampoe maar totale onderdrukking van ontkieming van spore en uitgesproke degenerasie van selle is waargeneem na blootstelling aan die povidoonjodium preparaat.

Hierdie studies het gedien om die hoe insidens van tinea capitis asook die rol van draers van aansteeklike swamme in die gemeenskap te beklemtoon. Meer kennis en bewustheid van die vele kliniese manifestasties van die siekte deur die professie is nodig asook die mees doeltreffende metodes om die infektiewe draer staat uit te wis, alles in 'n poging om die getalle van geinfekteerde kinders drasties te verminder. Gereelde gebruik van 'n geskikte sjampoe en toepassing van verbeterde haar higiene in plaaslike inrigtings en die gemeenskap in die algemeen sou 'n groot bydra maak tot bereiking van hierdie doelstellings.

Alhoewel verdere studies nodig is, het hierdie werk aangetoon dat die beskikbaarheid, gemak van aanwending en minimale newe-effekte van die povidoonjodium formulering Betadine sjampoe die ideale produk maak vir gereelde gebruik as 'n profilaktiese maatreel teen swam infeksie van die kopvel.

Indien die kanse dat 'n kind hierdie onooglike en potesieel sielkundig troumatiese siekte kry drasties verminder kan word sou dit 'n waardevolle bydra lewer tot die verbetering van die kwaliteit van lewe van kinders, veral in ons Derde Wereld bevolking.

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CHAPTER 1.

# 1. INTRODUCTION

"Tinea capitis is still with us.  The hair has been
beaten into submission by irradiation in past years;
the organisms have been beaten into submission by
griseofulvin and other agents;
yet the disease lives on."

Lawrence Solomon (1985).

Following work at local outpatient clinics and a
Community Health field trip along the West Coast, the
overwhelming incidence of tinea capitis in these
regions prompted further investigation into this
insidious disease.

In spite of valuable continuing research throughout the
ages, scalp ringworm has remained a public health
problem.  The overwhelming prevalence and the
potentially disfiguring severity of the disease has
ensured continued interest and investigation into the
aetiology of the dermatophytes, their distribution
patterns and also more effective medical treatment.
This study was undertaken to enlighten associated
medical and community workers of many aspects of the
disease, with particular reference to our own local
findings.

Tinea capitis has reached epidemic proportions in the southern and western Cape. Often overlooked as a trivial disease, this condition frequently results in severe psychological trauma and permanent scarring. Only about 3% of children attending the Dermatology Outpatients Department at the Red Cross Children's Hospital attend specifically for tinea capitis, but recent studies have shown that many more, particularly of low socio-economic background, remain undetected and untreated (Neil, 1987). In recent years this infection has become a major public health problem locally, exacerbated by overcrowding and poor hygiene encountered in the severely affected lower income groups. Scant information on the aetiology of this infection in the southern and western Cape has revealed a definite need for further investigation into this potentially intractable disease. Examination of the aetiological agents of scalp ringworm in this area, the possible effects of geographical influences on these organisms, as well as investigation into the many varied clinical presentations of this condition, could contribute greatly to effective management and control of this potentially disfiguring disease.

Some of the earliest reports of ringworm infections in the Cape were made by Brede in 1962 (Brede, 1962). His

first study included over 300 clinically suggestive
fungal cases seen at a white general hospital and
revealed that the predominant causative dermatophyte in
the Cape Peninsula was Trichophyton mentagrophytes.
This was followed in decreasing frequency by
T.violaceum, Epidermophyton floccosum, T.schoenleinii,
and Microsporum canis.  Comparisons were drawn between
the results of this study and those of Lurie who had
undertaken a similar survey, including mixed population
groups, on the Witwatersrand in 1955 (Lurie, 1955).
Lurie's results showed that M.canis (75%) was the
predominant dermatophyte in this area with
T.mentagrophytes accounting for only 8% of isolates and
T.violaceum for only 4%.  T.schoenleinii was concluded
to have equal incidence in both areas (4%).
Brede attributed this vast difference in the prevailing
dermatophytes, isolated from these geographically
contrasting areas, to climatic factors.  This deduction
was enhanced following favourable correlation of his
results with those obtained in Mediterranean countries
experiencing similar climatic influences to the Cape
Peninsula (Neves, 1963).

Further studies undertaken in the Cape by Marshall two
years later showed that T. violaceum was the
predominant cause of tinea capitis amongst black
children in the Cape while M.canis remained the

commonest dermatophyte infecting predominantly white children in the Transvaal (Marshall, 1964). Contrary to the earlier reports suggesting climate as a major influencing factor, Marshall reputedly stated that the microphytoses were generally encountered most frequently in low-lying areas and the trichophytoses in mountainous regions but that the reverse appeared true in South Africa (Daynes, 1974). However, observations made by Marshall on studies in North Africa (1964), suggested that T.violaceum, together with T.schoenleinii, was common all around the coastal lands of the Mediterranean Sea. He concluded too, that infections caused by M.audouinii were common only in the tropics. In summary, Marshall attributed the prevalence of the superficial mycoses and the clinical forms they took principally to environmental factors including way of life, hygienic conditions and clothing types, rather than geographical or climatic influences.

Following the establishment of a mycology unit at the University of Stellenbosch, Brede was later able to update his earlier findings (Brede, 1972). The patient sample included in Brede's original survey appeared to consist predominantly of caucasians, however no reference to the population groups under examination were made in this subsequent study. In 1972, his report on the dermatophyte spectrum of the Cape coastal

belt showed that T.violaceum now constituted 42% of isolates with T.mentagrophytes, originally reported as the predominant organism, the second most common dermatophyte causing only 21% of infections. In contrast, Brede discovered that M.canis was the most important dermatophyte in the elevated, dry regions of the Karoo. T.violaceum was not isolated on the plateau and Brede thus concurred with Marshall that this organism was not encountered in high, dry areas. As this organism had also been isolated in the Transvaal lowveld, the Rhodesian midlands, Botswana and Mocambique, Brede remained convinced that certain geographical influences, particularly altitude, determined the occurrence of this dermatophyte species in geographically similar areas.

His findings for M.audouinii, however, contrasted sharply with this theory. This organism was found along the Cape coastal belt (8,0%), in Natal (12%), Zambia (43%), and southern Mocambique (60,6%), all areas with differing altitudes, latitudes and climatic influences (Brede, 1972). Daynes findings of a 75% isolation rate of M.audouinii in the mountainous Tsolo district of the Transkei further confused the picture (Daynes, 1974).

In 1973, an investigation undertaken by Scott et al in Bloemfontein, O.F.S., revealed that T.violaceum was the

main causative organism of tinea capitis (63,5%) on this elevated plateau (Scott, 1973). These findings were similarly reversed to those of Marshall and Brede, suggesting that their earlier interpretations on the influence of altitude and the associated prevalence of particular dermatophyte species were invalid. Similar findings for the Transvaal were reported by both Findlay in 1974 and Young in 1976 (Findlay, 1974; Young, 1976). T.violaceum was likewise noted as the predominant dermatophyte causing tinea capitis in black children. Young's survey included the northern and eastern reaches of the Transvaal. A more recent survey of the spectrum of dermatophytes encountered in the Pretoria area revealed that T.violaceum to date remained the chief cause of scalp ringworm among black and coloured children (Vismer, 1988). M.canis was reported as responsible for 73,8% of cases in white children.

Although the data collections in Vismer's study were taken only from clinical records, and not from the general population in their natural environments, this survey highlighted the most common prevailing dermatophytes among the various indigenous groups. Similarly, many of the earlier reports were based on clinical records only and thus not always accurately reflecting local infection rates.

The present decade has produced great advances in all
fields of medical mycology worldwide and of particular
note has been the renewed interest in epidemiological
studies (Bronson, 1983; Sinski, 1984; Ozegovic, 1985;
Alteras, 1986; Caprilli, 1987; McClean, 1987;
Greatorex, 1988; Weir, 1988; Attapattu, 1989; Di
Silverio, 1989). However, unlike the previous two
decades, the number of epidemiological surveys
performed in S.A. appears to have decreased during the
1980s. Reports on only three studies appear to have
been published. These included an extensive study on
the prevailing fungi in the Southern and Western Cape,
undertaken by Neil et al (1987) and a similar survey on
superficial mycoses occurring in the Transvaal,
performed by Vismer et al in 1988. Results of an
interesting study on the occurrence of tinea capitis in
adults were also published in Cape Town (Barlow, 1988).


Examination of all the above-mentioned reports
examining the worldwide occurrence and distribution of
the dermatophytes prevailing in the Southern and
Western Cape, suggested that altitude, latitude,
climate and racial influences played a far lesser role
than initially suspected.

Research into the geographical distribution of specific
dermatophytes has revealed that T.violaceum, regarded
locally as the most common dermatophyte, is today
considered the dominant pathogen in North and Eastern
Africa as well as Eastern Europe (Binazzi, 1983).
Although the distribution of numerous dermatophytes has
changed markedly since the turn of the century, the
occurrence of T.violaceum has remained virtually
constant.  Rapid, worldwide travel, emigration and
labour migration are factors which have contributed to
the changing prevalence of many dermatophyte species
and have emphasised the need for continued
epidemiological monitoring.  T.tonsurans was until
recently rarely isolated in the U.S.A..  The migration
of peoples from the Latin American countries, however,
has resulted in this organism supplanting M.audouinii
as the major causative agent of tinea capitis in
certain North American states (Prevost, 1979).
Similarly, it has been suggested that T.soudanense,
also a cause of tinea capitis and endemic in Central
Africa, recently isolated in Brazil and the U.S.A., may
have been introduced as a result of slave trading which
flourished in earlier centuries.  Improved expertise
and laboratory procedures available today may also have
contributed to the more accurate and precise
identification of species, reducing the chance of
misidentification and resulting in a more accurate

definition of geographical trends.

European and Asian countries with a predominance of
T.violaceum include Spain, Portugal, Yugoslavia,
Central Europe, Jordan, India and Russia (Neves, 1963;
Cabrita, 1973; Kamalam, 1973; Binazzi, 1983;
Ali-Shtayeh, 1986).

The appearance of T.tonsurans and T.yaoundei in South
Africa was thought to be due in part to migration of
peoples, emigration and social habits (Philpot, 1973;
Rippon, 1988). Zoophilic fungi, M.canis, T.verrucosum,
and T.mentagrophytes, and the geophile M.gypseum have
been reported in this country.

Their present day, worldwide distribution is sporadic
and largely dependent on the presence and numbers of
their respective hosts. The natural reservoirs of
M.canis and T.mentagrophytes are almost exclusively the
domestic dog and cat resulting in the ubiquitous
distribution of these organisms.

T.verrucosum is primarily a pathogen of cattle and as
such is found predominantly amongst rural, farming
populations worldwide (Kane, 1978; Binazzi, 1983). In

a review undertaken by Philpot in 1978, it was shown that T.verrucosum was the second most common cause of tinea capitis in Great Britain and Ireland (Philpot, 1978).

T.mentagrophytes var granulare has infrequently been reported as a cause of scalp ringworm worldwide but was reported to predominate in regions of Canada, Sri Lanka and Nigeria (Ogbonna, 1986; Sekhon, 1986; Attapattu, 1989).

M.gypseum is geophilic and isolated frequently from rich garden soils worldwide (Rebell, 1974). This organism was reported by Philpot as cosmopolitan in its geographical distribution (Philpot, 1978).

The geographic distribution of the anthropophilic species T.schoenleinii includes predominantly Eurasia and North Africa following a similar pattern to that for T.violaceum. Endemic foci occur worldwide and are fequently restricted to a single tribe or family (Jacyk, 1982).

Reports on the clinical manifestations of tinea capitis have classically described ringworm lesions as a spreading area of inflammation surrounding a central

zone of spontaneous healing (Rippon, 1988). This
typical presentation, however, has rarely been reported
on scalps of infected children in the Cape Peninsula
and along the West Cape Coast. Community Health field
trips undertaken in these areas showed that the
classical textbook presentation was the exception
rather than the rule and that the varied presentations
encountered resulted in many infections remaining
undetected and untreated. Honing et al (1979)
suggested that physicians other than dermatologists are
generally not familiar with the many varied clinical
presentations of tinea capitis. They added that
paediatric literature and textbooks failed to emphasise
these findings in discussion on scalp ringworm. This
appears to be a widespread problem, common also to
clinicians in the Southern and Western Cape.

Scant information on the varied clinical presentations
encountered following infection with T.violaceum is
currently available. The available literature,
however, reports a strong similarity between disease
caused by T.tonsurans and T.violaceum. Both organisms
are anthropophilic in nature and cause endothrix
invasion of hair. Hansen in 1985 clearly summarised
the clinical patterns evoked by T.tonsurans and
suggested that each could be readily misdiagnosed as
other dermatological conditions (Hansen, 1985). His

first category, described localised inflammation with
kerion formation, a hypersensitivity reaction.  Kerion
response has been described as ranging from numerous
superficial pustules to a fluctuant abcess (Rasmussen,
1985).  Kerion with associated hair loss, adenopathy,
fever and leukocytosis often lead to a misdiagnosis of
bacterial pyoderma of the scalp.  In the second
category, described as black dot alopecia with minimal
scalp scaling, Hansen suggested that the broken-off
hairs may be mistaken for empty follicles of alopecia
areata or the stubble and perifollicular haemorrhage of
trichotillomania. The third category described,
included the most common form of presentation - diffuse
or patchy scalp scaling with minimal or no hair loss.
Krowchuk et al in 1983 reported that many patients
infected with T.tonsurans complained of dandruff.
Honing suggested that tinea capitis be included in the
differential diagnosis of diffuse scaling of the scalp
as well as seborrhoeic dermatitis, atopic dermatitis
and impetigo (Honing, 1979).

Examination of medical records at the Red Cross
Children's Hospital indicated that the clinical
manifestations observed for T.violaceum closely
parallelled those of T.tonsurans described above.
Referrals from clinicians other than dermatologists

suggested that the varied clinical forms of tinea capitis were unknown or overlooked. In an effort to remedy this widespread situation, Hansen perhaps wisely suggested that all scalp diseases in childhood should be considered tinea capitis until proved otherwise. Further investigation into Dermatology Outpatient records in Cape Town revealed recurrent tinea capitis infections in a small group of institutionalised children. Spanning a 3 year period, it was noted that 11 pupils in two children's homes were reinfected with T.violaceum following apparently successful treatment with griseofulvin. These children were noted as being clinically and culturally negative following completion of each antifungal therapy. Six children were re-infected more than twice.

It is suggested in the literature that some degree of immunity is produced following primary ringworm infection but that this protection varies in extent and duration (Lepper, 1969; Knight, 1972; Rippon, 1988). This variability is ascribed to various factors including the site of infection, the species of dermatophyte, as well as the response of the host (Georg, 1960). Rippon (1988) interpreted a finding by Friedman et al, that none of a large series of children treated for tinea capitis returned with a second infection, as evidence of acquired resistance

(Friedman, 1960). Rippon noted, too, that Lepper had reported this phenomenon in agricultural workers infected with T.verrucosum, further enhancing this theory (Lepper, 1969). More recent reports, however, indicate that *no protective effect* against future infections is provided (Roig, 1987). In spite of an extensive study including $\pm$ 300 children, Roig et al concurred with Rippon that the field of·immunity and resistance is the most poorly understood subject in the field of Mycology, yielding few satisfying explanations.

Local evidence of re-infection in the afore-mentioned pupils provoked much interest suggesting that acquired resistance was unlikely or alternatively that certain children were significantly more predisposed to ringworm infections.

It has been widely reported that dermatophytes can readily be isolated from fomites, air and various sites of the body e.g. the scalp (Gip, 1966; Midgley, 1972). Midgley and co-workers described scalp fungal carriage as a transient event merely representing contamination by spores from an infected child. She added that once the clinical cases were removed from the institution or

school the carrier state and environmental
contamination decreased or disappeared. However, the
longevity of dermatophyte spores in the environment
would appear to ensure their survival and ability to
cause disease regardless of the continued presence of
the primary infection source (C.N.Young, personal
communication).

Ive reported a 24,5% carriage rate in Nigerian schools
in 1966 (Ive, 1966). To examine the possible
transience of this carrier state he reassessed these
patients following a four-month time period. In this
way, he was able to demonstrate that 42% of the culture
positive, asymptomatic children remained persistent
carriers. Similar investigations were undertaken by
Clayton in primary day schools in five London boroughs
(Clayton, 1968). Scalp carriage rates in these schools
varied considerably ranging from 1% to 30%. Clayton
attributed this difference to the presence or absence
of infected pupils in the classes under study. No
attempt was made to investigate the transience or
persistence of scalp carriage in this study.

In 1982, Polonelli and co-workers performed a survey in
the public and private schools of Rome to determine the
incidence of scalp fungal carriage in this Italian
community (Polonelli, 1982). Their study demonstrated

fungal carriage in only 2 of 754 students (0,3%). In spite of these low figures, Polonelli remained convinced that carriers were a potential source of infection and played an important role in the persistence and spread of scalp ringworm in the community. In a recent study to investigate scalp carriage, undertaken in Texas in 1988, Sharma et al isolated _T.tonsurans_ from 8 of 200 (4%) healthy children (Sharma, 1988). The rationale for this study was the endemic nature of the disease which resulted in constantly occurring incidences among the local black population, suggesting that a state existed that was symptomatically and clinically undetectable to the patient and clinician respectively. Sharma reasoned that, as _T.tonsurans_ had not been demonstrated in soil or animals, a carrier state in humans was a possibility.

Thus in summary, the literature reviewed above strongly suggests that the carrier state of scalp ringworm exists and poses a serious threat to effective and lasting cure of infected persons.

Investigations for possible treatments for use in institutions with high numbers of fungal carriers

revealed that infection, confined to a closed
community, could effectively be controlled by selective
treatment of clinical cases and fungal carriers alone
(Mackenzie, 1963). Mackenzie concluded that an
alternative treatment to full dosage griseofulvin was
necessary, reasoning that the cost of mass treatment
with griseofulvin would be prohibitive. Klokke et al
used these findings as a basis for their trials in an
orphanage in India (Klokke 1966). They determined,
however, that selective treatment was not feasible due
to poorer socio-economic conditions in India and the
likelihood of continued, communal use of infected
brushes, clothing and bedding. Klokke administered a
low-dosage regimen of griseofulvin to all children over
a period of eight weeks with minimal success.
Twenty-two percent of pupils had positive cultures
prior to commencement of this trial and, of these, 80%
remained positive following completion of the regimen.
In 1988, Nyawalo et al continued the search for more
cost-effective treatment of tinea capitis by
undertaking an extensive trial in Kenyan schools in
1988 (Nyawalo, 1988). This study examined the effects
of administering a single dose of griseofulvin,
followed by intermittent treatment regimens. Only
children that were clinically infected were included
in this trial. They reported that a single
dose of griseofulvin of 2 to 3 grammes resulted

in a cure rate of 58,6%, (normal dosage : 0,5 - 1 g
daily). The dose was repeated after four weeks,
increasing the cure rate to 79,1%, and again after
eight weeks, resulting in a cure rate of 91,2%.


In 1982, Allen et al examined the effects of
griseofulvin used in conjunction with selenium-sulphide
shampoo, a sporicidal agent (Allen, 1982). Their study
included infected children and the investigators were
able to demonstrate that cultures became negative
following only two weeks of combined therapy while
cultures of patients treated with griseofulvin alone,
or together with either a bland shampoo or clotrimazole
lotion, frequently showed significant growth up to
eight weeks later. Selenium-sulphide shampoo had been
selected for its sporicidal activity as well as its
property of deposition on the scalp surface. Allen
concluded that this agent was an important adjunct in
the therapy of tinea capitis, suppressing viable spores
on the scalp surface and minimising the chances for
spread to occur.
More recent studies on the effects of topical
treatments in the therapy of tinea capitis were
undertaken by Wright et al in Zimbabwe in 1986 (Wright,
1986). They included 41 infected children in a home
for the physically handicapped in their study. The
prevalence of proven scalp ringworm was 39%. Wright

randomly allocated Whitfield's ointment and miconazole
cream to each of two groups of children. Their results
showed that these preparations were only partially
effective. Reasons proposed by the investigators for
this treatment failure included too short a treatment
period (5 weeks), environmental factors e.g. sharing of
brushes, clothing, towels and dormitory overcrowding,
and poor hygiene practices. They concluded that
improvement in the general hygiene of the environment
would be more cost effective than repeated attempts at
treatment with the tested topical agents.

Objectives of this study were :-

To investigate the prevalence of tinea capitis in the
South Western Cape, to determine the aetiological
agents responsible for the disease and to examine the
influence of geographic location (altitude and climate)
on these dermatophytes. These aims were felt necessary
as little current information is available on these
aspects of scalp ringworm in our region. Not only
would the knowledge above assist with deeper
understanding into the mode of spread of disease but
enlighten one on the possible source of this seemingly
endemic disease.

Further aims were to determine the prevalence of the
carrier state in schools and institutions, to examine
the transience and persistence of fungal carriage as
well as the number of carriers developing overt
clinical ringworm.  The various clinical presentations
of suspected cases of tinea capitis were also
examined.  As few studies have been undertaken on
fungal carriage, these trials were planned to
investigate the precise role, if any, played by the
carrier.  The often subtle and easily overlooked
clinical presentation of this disease prompted further
investigation into this aspect in an effort to document
and highlight the problematical area of diagnosis.

The third section of study was undertaken to determine
the in-vivo effects of selected antifungal shampoos on
the carrier state of scalp ringworm.  The obvious need
for a simple, regularly used prophylactic measure in
child care institutions, to effectively reduce or
eradicate fungal carriage, prompted this project.

The final aim of this thesis was to assess the in-vitro
effects of the selected shampoos on the ultrastructure
of spores of Tricophyton violaceum.  This study was
designed to substantiate the shampoo trial findings and
to examine the physical effects of the antifungal
shampoos on the fungal spores.

This project was undertaken to provide deeper insight and understanding into a disease which appears to be endemic in our community.

CHAPTER 2.

.

LITERATURE REVIEW

Historical and Current Reports on the Epidemiology of
Dermatophytes Causing Tinea Capitis.

The highly visible and superficial nature of the
dermatophytoses has been noted and described from the
earliest historical times.  Both the ancient Greeks and
the Romans contributed to the formulation of the
medical terminology still used today.  The
circular-shaped lesions resulted in the Greeks naming
the disease "herpes", meaning "ring-form", while the
Romans associated the disease with insects and named
the infection "tinea", meaning "small insect larva".

Ringworm, and in particular favus, has long been
recognised.  Records have shown that the Tudors granted
licences to enable loyal sufferers of scalp ringworm to
leave their heads covered in the king's presence
(Ainsworth, 1976).  Ainsworth also suggested that the
first record of a tropical mycosis was made by William
Dampier who, while voyaging around the world wrote in
his journal after a visit to the Phillipines in 1686,
"the Mindanao People are much troubled by a sort of
Leprosie, the same as we observed in Guam.  This
Distemper runs with a dry scurf all over their Bodies,

and causes great itching in those that have it, making them frequently scratch and scrub themselves which raiseth the outer Skin in small whitish flakes, like the scales of little Fish, when they are raised on edge by a Knife. This makes their Skin extraordinary rough". He was thought to be describing "Tokelau Itch" caused by Trichophyton concentricum.

Most early formal research on the dermatophytoses was undertaken in Europe and by 1890, Raymond Sabouraud, a highly renowned dermatologist, began to publish his scientific and systematic studies on the dermatophytes. In 1910 he published his accumulated work in a classic of medical literature, "Les Teignes". His classification of the three genera, Trichophyton, Microsporum and Epidermophyton and his basic methodology and observations on treatment remained virtually unchanged for the next fifty years.

Some of the earliest reports on findings on the African continent began appearing in the 1950's. In 1953, Clarke and Walker published a report on superficial fungal infections in Nigeria and 2 years later Lurie documented his findings on fungal diseases following a similar trial undertaken in South Africa (Clarke, 1953; Lurie, 1955). Both Findlay and Murray undertook work amongst the black populations which was later published

in 1957 (Findlay, 1957; Murray, 1957). A year later,
Vanbreuseghem investigated tinea capitis in the Belgian
Congo and Ruanda Burundi and shortly thereafter Findlay
and Clarke respectively produced papers on ringworm
infection in the Transvaal and skin diseases in the
Bantu (Vanbreuseghem, 1958; Clarke, 1959; Findlay,
1959). During this decade most published work in
Africa took the form of reports documenting the
prevalence of the dermatophytoses in various regions.

The 1960's and 1970's saw numerous investigations,
undertaken predominantly in South Africa, not only
documenting the prevalent dermatophytes but questioning
the influence of geography and race on the distribution
of the ringworm fungi (Marshall, 1963; Dogliotti, 1970;
Bentley-Phillips, 1972; Scott, 1973; Daynes, 1974;
Findlay, 1974). With reference to the above-mentioned
surveys, most authors of these reports speculated not
only on the prevalence but on the possible reasons for
the discovery of certain dermatophytes which appeared
restricted to various geographical, climatic or racial
areas.

In 1974, Verhagen reviewed the literature available on
the causative agents of tinea capitis in the continent
of Africa (Verhagen, 1974). He deduced that
anthropophilic fungi predominated and that their

geographic distribution could be divided into three zones; T.violaceum and T.schoenleinii dominated in the north, T.soudanense and M.audouinii in the west, and that a mixed flora found in the south and east was dominated in most parts by T.violaceum. He found that T.violaceum was virtually restricted to a crescent shaped area stretching from Morocco to Cape Town, covering a vast spectrum of geographic, climatic and racial differences. Following this comprehensive study, he postulated that the many theories explaining the differing geographical distributions on the basis of climate and race were not valid.

During the 1980's extensive reports on dermatophytes in Nigeria were published including a study specifically on tinea capitis and a survey on ringworm infections of the Fulani herdsmen (Jacyk, 1982; Ogbonna, 1985 & 1986)'. Similar work on tinea capitis in Rwanda, Tripoli and Libya was also undertaken (Bugingo, 1983; Bhakhtaviziam, 1984; Kanwar, 1987). A review of dermatophytes prevalent in East Africa by Lubwana was also published in 1986.

In keeping with Verhagen's postulates, research revealed that dermatophytes other than T.violaceum predominated in the East African country of Tanzania and the West African country of Libya. The most common

fungi causing causing tinea capitis in these regions were M.audouinii and T.rubrum respectively (Nsanzumuhire, 1974; Kanwar, 1987).

In Africa, T.tonsurans has been reported only sporadically in the Cape Province as a cause of tinea capitis but has been isolated extensively as the predominant dermatophyte in certain areas of North and South America including the USA, Mexico, Haiti and Peru. This organism is also common along the east Asian coast as well as around the north, west and southern perimeters of Australia (Brede, 1972; Binazzi, 1983; Neil, 1987; Rippon, 1988). A survey of dermatophytes causing tinea capitis in the USA during the period 1979-1981 was undertaken by Sinski et al in 1984. They identified T.tonsurans as the primary cause of this infection and a more recent study, undertaken in 1987 in New York, revealed that this organism has retained its dominant position as the most commonly isolated dermatophyte (McLean, 1987).

T.yaoundei was considered one of the few truly geographically restricted dermatophytes, yet the emergence of this organism in South Africa was noted by Young in 1976 (Young, 1976). This dermatophyte was previously considered exclusively endemic to Central Africa.

M.canis has emerged as the prevailing dermatophyte in
Central Europe, Italy, Japan, Israel, South America
(including the Caribbean area, Cuba and Puerto Rico),
New Zealand and central and eastern Australia (Binazzi,
1983; Rippon, 1988; Weir, 1988).

Caprilli et al (1987) examined 2091 patients in Rome
presenting with differing ringworm lesions.  Unlike
findings for T.violaceum in Africa, which have remained
fairly constant, they reported a changing
epidemiological pattern in southern Italy.
Trichophytoses, particularly those due to T.violaceum
or T.tonsurans, had prevailed until the 1930's.
Gradually dermatomycoses caused by Microsporum species
increased, numerically outnumbering infections from
Trichophyton species by 1952.  Following a burgeoning
population of stray, domestic cats inhabiting the
capital city, Caprilli reported in 1987 that M.canis
had become the predominant dermatophyte in Rome and its
environs.  He failed, however, to separate the
different clinical forms and their causative organisms.

The findings of Caprilli were further enhanced
following results of a 13-year survey in Pavia, Italy,
carried out by Di Silverio et al in 1989.  During the
period from 1974 to 1987, 4100 patients suspected of
fungal infection were included in this study.  Their

findings showed that M.canis had been the causative organism in over 89% of scalp ringworm infections and was also the predominating fungus in cases of tinea corporis and tinea facialis. A similar trend was reported by Alteras et al in Israel in 1986. M.canis was isolated from 60% of paediatric scalp ringworm cases during this study.

A recent study on dermatophyte infection in Somerset, England, revealed that T.verrucosum had eclipsed M.canis as the predominant cause of scalp ringworm in this area (Greatorex, 1988). This organism was similarly reported by Chadegani et al in 1987 as the major fungus causing scalp infection in Esfahan, Iran, in 1987. They were able to demonstrate infection due to this dermatophyte in 64,3% of scalp ringworm cases.

Studies from three geographically remote countries revealed T.mentagrophytes var granulare to be a major causative agent of infection in these regions. Sekhon examined 185 clinically infected Canadian children (all with clinical signs of scalp ringworm) during a 13 year study, 1972-1984 (Sekhon, 1986). All specimens within the province of Alberta, (from clinics, physicians, dermatologists and private hospitals) were submitted to the Provincial Laboratory for diagnostic purposes. His results showed that T.mentagrophytes was responsible

for 54% of all fungal scalp infections. A similar
study was undertaken by Attapattu in Sri Lanka covering
the period 1978-1987 (Attapattu, 1989). A total of 462
patients, referred from clinics in Colombo (a large
town) and its suburbs, as well as the surrounding rural
areas, presenting themselves for examination, were
investigated for scalp ringworm. She was also able to
demonstrate that T.mentagrophytes was the predominant
dermatophyte causing tinea capitis in her country
(33%). She found that the zoophilic fungi,
T.mentagrophytes var granulare and M.canis, and the
geophilic species M.gypseum, together accounted for 81%
of all dermatophytes isolated from scalp infections.
Ogbonna undertook extensive studies in Nigeria,
reporting a similar spectrum of dermatophytes infecting
the nomadic Fulani herdsmen to that infecting the
general population (Ogbonna, 1986). This survey
included all forms of dermatophytic infection with no
specific reference to tinea capitis or its causative
organisms. Both the migrant tribesmen and the
urbanised communities were found to be infected
predominantly with T.mentagrophytes, followed by
T.rubrum. T.verrucosum and E.floccosum respectively
were the third and fourth most common isolates of each
group. T.violaceum was not isolated at all during this
survey. An earlier study undertaken by Ogbonna,
however, revealed that M.audouinii was the predominant

cause of tinea capitis amongst young children attending schools in the small Nigerian town of Jos (Ogbonna, 1985). Although the earlier survey on tribesmen presumably included adults (not as predisposed to tinea capitis), these conflicting reports suggested that tinea capitis was not studied in the follow-up trial or that this form of disease has a low prevalence rate amongst the tribesmen.

Excluding reports on South African findings, T.schoenleinii, the causative agent of favus, has been reported worldwide as causing pockets of infection among small population groupings. In 1982, Jacyk et al reported an ethnically restricted outbreak in Islamic rural Hansa-Fulani tribesmen in Nigeria while Rippon referred to a small focus in Appalachia involving descendants from the same central European village where the disease was endemic (Jacyk, 1982; Rippon, 1988).

To date, with the exclusion of S.African studies referred to in this thesis, the results of few further surveys on the geographic distribution of dermatophytes most commonly associated with tinea capitis appear to have been published.

CHAPTER 3.

3.    MATERIALS AND METHODS

3.1  Epidemiology and Aetiology of Scalp Ringworm in the
     Southern and Western Cape

Patient Group

Group A: Dermatology Clinic Patient Sample

Scalp scrapings were collected from 1073 children
attending the Dermatology Outpatient Department at the
Red Cross War Memorial Children's Hospital.  Specimens
were collected at routine weekly clinics spanning a
period of four years (1984-1987). Ages of the children
were rounded off to the nearest year and the resultant
mean age was 6,3 years with a range of 1 to 16 years.
Sexual distribution revealed that 605 were female,
constituting 56,4% of the patient sample.  All children
were resident in the Cape Peninsula, Cape Flats and
surrounding environs (see Fig.1).  The Red Cross War
Memorial Children's Hospital in Rondebosch serves a
predominantly urban area within a radius of
approximately 25km.  This region experiences a
Mediterranean climate with hot, dry summers
(20,1-22,5'C) and cool, wet winters (12,6-15,0'C).
Average annual rainfall figures for this area are

FIG.1

SOUTH WESTERN CAPE SHOWING RED CROSS HOSPITAL

AND ENVIRONS.

FIG. 1: GEOGRAPHICAL DISTRIBUTION OF
DERMATOLOGY OUTPATIENTS.

CAPE TOWN

BELLVILLE

Rondebosch
RXCH

CAPE FLATS

MITCHELLS PLAIN

FISH HOEK

FALSE BAY

SIMONSTOWN

Densely populated
urban areas.

Abb: RXCH - Red Cross
Children's Hospital.

1 : 500 000
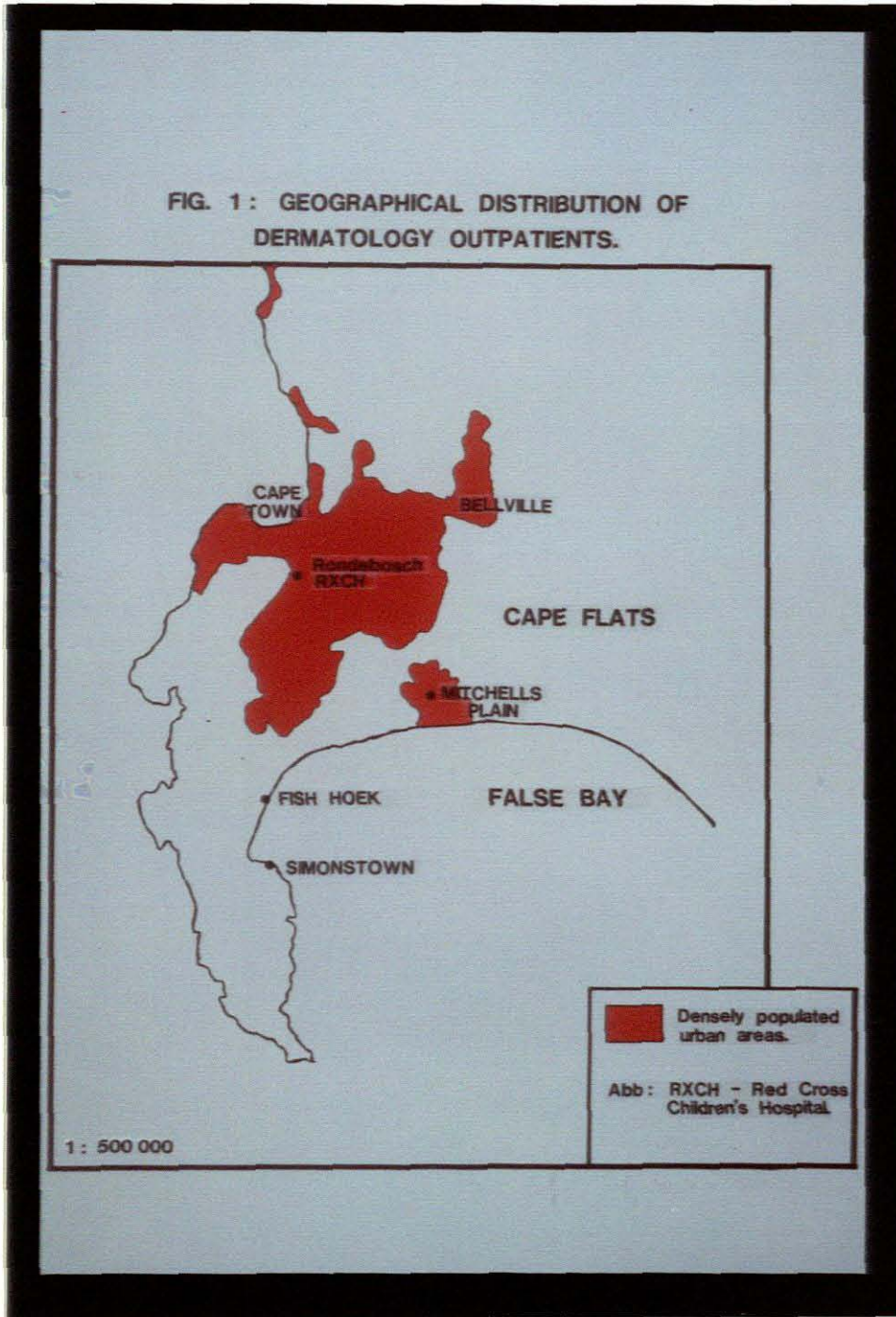
62-750mm. Strong south easterly and north westerly
winds prevail in summer and winter respectively. The
Red Cross Hospital and environs are situated at
sea-level. Coloured patients constituted over 89,9% of
the patient sample, while only 103 (9,6%) were black
and white children numbered only 5(0,5%). Patients
presented with a variety of clinical symptoms
suggestive of tinea capitis.


Group B: Community Health Clinics Patient Sample


Specimens were collected from 48 children attending
Community Health Clinics along the Western Cape Coast.
All samples were collected during a three day field
trip to this region (August-1984). Exact ages of
children in this group were unobtainable due to lack of
medical records and unreliable information from
patients. None of the patients, however, appeared to
have reached puberty. Approximately two-thirds (66,6%)
of the children were female.
All patients in Group B were inhabitants of the West
Cape Coastal Belt. Towns extended from Vredendal in
the southern reaches to Steinkopf in the north. The
majority of patients were resident in the more heavily
populated mining areas of O'Kiep, Concordia, Nababeep
and Springbok. All children appeared to be from low
socio-economic backgrounds.

The West Cape Coast experiences desert climatic
conditions with little rainfall (<62-250mm) and lengthy
hot, dry periods. Terrain is arid with sparse
vegetation. Temperatures vary from 10,0'C in winter to
an average of 25,0'C in summer. Altitudes vary from
town to town with a range of 126m (Vredendal) to 1322m
above sea-level (Springbok area).
All children presented with overt clinical symptoms of
tinea capitis, the most common presentation being
moderate scalp scaling.


Group C: Community Health School Patient Sample


Specimens in this group were collected from 319
children by a nursing sister, a community health
employee, with no formal dermatological experience.
Samples were obtained from pupils in primary schools
which were experiencing apparent tinea capitis
outbreaks.
All schools were situated along the West Cape Coast in
the copper mining belt (see Fig.2). The climatic
conditions for this area have been described
previously. Approximately half (47%) of the children
were female. Patients ages were not submitted to the
laboratory but all the children had not yet reached
Std.3, suggesting that they were predominantly <10
years of age. No details of clinical presentation were

FIG.2

WESTERN CAPE COAST - DISTRIBUTION OF SCHOOLS

AND CLINICS.



FIG. 2. GEOGRAPHIC DISTRIBUTION OF WEST COAST CHILDREN.

submitted to the laboratory. However, as specimens were collected from all pupils in selected classes, and included 52 pupils recorded as having no visible clinical manifestations of scalp ringworm (i.e. asymptomatic children), this would suggest that the remainder presented with discernable symptoms.

Specimen collection

Specimens from patients presenting with diffuse scaling were collected from any representative scaly area on the scalp. Cleaning of the scalp with alcohol prior to specimen collection was generally not undertaken unless ointments or other topical preparations had recently been applied.

Specimens from children presenting with "black dot" ringworm were collected by vigorous scraping of these "spotted" areas. These short, broken-off hair stumps proved easy to extract.

A minority of children presented with the classical ring-shaped lesions. Scrapings from this group were collected by removing scales from the advancing edges of the lesions.

Sterile small forceps were frequently used to pluck out infected hair stumps in children presenting with kerions. Firm pulling with a gentle pressure easily released these hairs from the follicles. All scales

and hairs were collected into sterile petri dishes.
Brushing samples were collected by vigorous brushing
over the entire scalp surface. (Electrostatic charges
formed on the polythene brushes attract particulate
matter from the hair and scalp.) Scalp brushings using
sterile, plastic scalp massage brushes were frequently
collected from unco-operative children (see Fig.3).
Brushes were supplied by the microbiology laboratory in
sterile plastic bags and were readily available.

Group A:- Scalp scrapings were collected weekly from
all children presenting with scalp scaling at the
Dermatology Outpatient Clinic. The instrument of
choice was the blunt end of a "Medipoint" blood
lancet. This was simple to use and minimised
psychological trauma. These lancets were readily
available, pre-sterilized and disposable. Blunted
scalpels were occasionally used when available but
frequently proved hazardous when used on young,
unco-operative patients. Surgical curettes were also
used when available in the clinic and these proved
highly successful. Brushing samples were frequently
collected at this clinic.

Group B:- All specimens were collected from pupils
using the blunt ends of the blood lancets.

FIG.3

SCALP MASSAGE BRUSH AND CULTURE OF

TRICHOPHYTON VIOLACEUM.
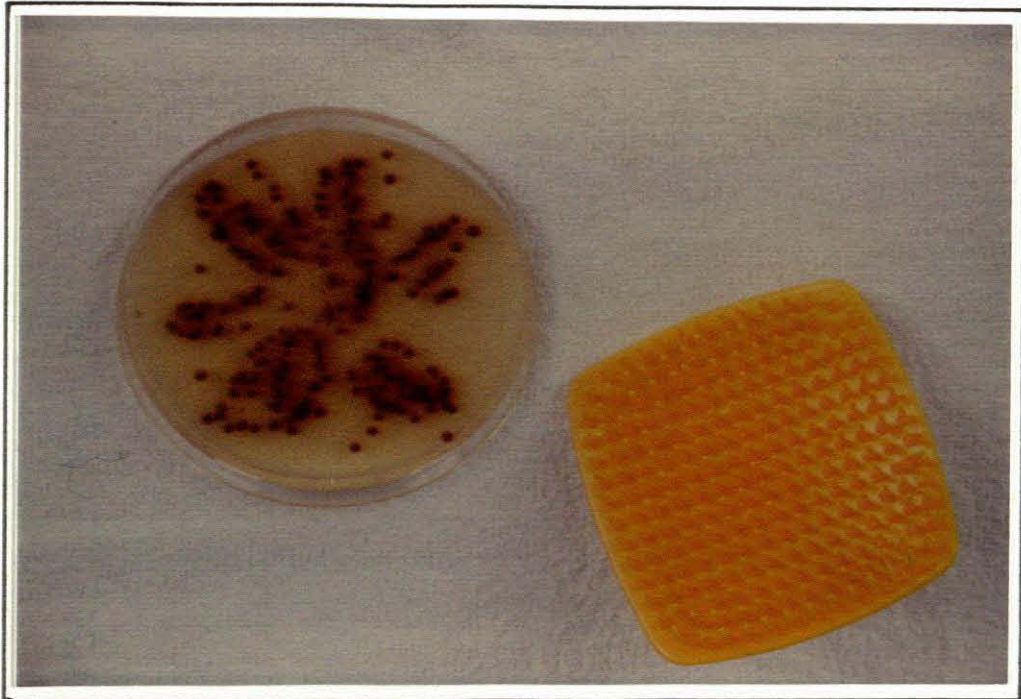


Culture grown on Sabouraud's Dextrose Agar

plus chloramphenicol.

Samples from Groups A and B were collected into sterile petri dishes, clearly labelled, and stored at room temperature prior to processing in the laboratory.

Brush samples were returned to the plastic bags, labelled, and stored as above.

Group C:- Specimens were similarly collected using blood lancets. Scrapings were collected into labelled paper envelopes and submitted to the laboratory by post.

Microscopical Examination

Direct microscopy was performed on 79,3% of all specimens constituting groups A, B and C.
There was insufficient material for direct microscopy in 11,3% of specimens; most samples in this group were submitted by the community health worker and the majority of inadequate scrapings were obtained from asymptomatic pupils. Mycological culture alone was performed on these specimens. The remaining 9,4% of specimens were obtained from brushings, thereby precluding microscopy.

Material for microscopical examination was examined in

15% potassium hydroxide (KOH). All slides were gently heated using a spirits burner to clear the keratin in the sample. Material was examined using light microscopy and a 40X objective. The presence of spores and their position were recorded.

Brushings collected on scalp massagers (9,4%) were unsuitable for microscopical examination and were cultured directly onto media*.

Culture Methods and Media

.

All specimens were inoculated onto commercially available Mycosel agar (BBL 11462). This medium is a selective medium for the isolation of dermatophytes, the antimicrobial agents cycloheximide and chloramphenicol, inhibiting only saprophytic fungi and bacteria respectively.

-------------------------------------------------------

* Recipes for all stains and media are given on pages 60 - 69.

A depth of 4mm of this medium was necessary to allow sufficient degree of penetration of the brush bristles. Adequate inoculation of the adherent fungal spores was thus ensured.

All plates were incubated at 25'C in the dark for two weeks and examined twice weekly.

Morphological and Microscopical Identification of Dermatophytes

All fungal growths were examined in Lacto-Phenol Cotton Blue Stain (LPCB). Sellotape strippings taken from the surface of mould colonies were the most common method utilized in the laboratory. A drop of Lactophenol Cotton Blue stain was positioned centrally on a glass slide and the inoculated sellotape stretched, sticky side down, across it.

Dermatophytes with a waxy consistency e.g. Trichophyton violaceum, T.yaoundei and T.verrucosum, however, did not lend themselves to this technique. Conventional needle mounts were performed on these organisms.

### i.  T.violaceum:

Colonies appeared waxy, wrinkled and heaped up and
produced a deep violet pigment on both the surface and
the undersurface (see Fig.4).  White, downy tufts
appeared in mature cultures.  Atypical, non-pigmented
strains were isolated infrequently.

Microscopically only short, tortuous hyphae were
noted.  Neither macroconidia nor microconidia were
present.  (See "Special Investigations" section for
vitamin requirements - Pg 50).

### ii.  T.verrucosum:

Colonies were very slow growing and appeared white,
glabrous, heaped up and wrinkled on culture.  No
pigment was observed on the undersurface.
Microscopy performed on colonies grown on Sabouraud's
Dextrose Agar (SDA) revealed only tortuous, gnarled
hyphae and chlamydospores.  No macroconidia nor
microconidia were observed.

### iii.  T.yaoundei:

Colonies were morphologically similar to those of
T.violaceum but were brown in colour.  Microscopical
features were identical to those observed with

FIG.4

CULTURAL MORPHOLOGY OF TRICHOPHYTON VIOLACEUM
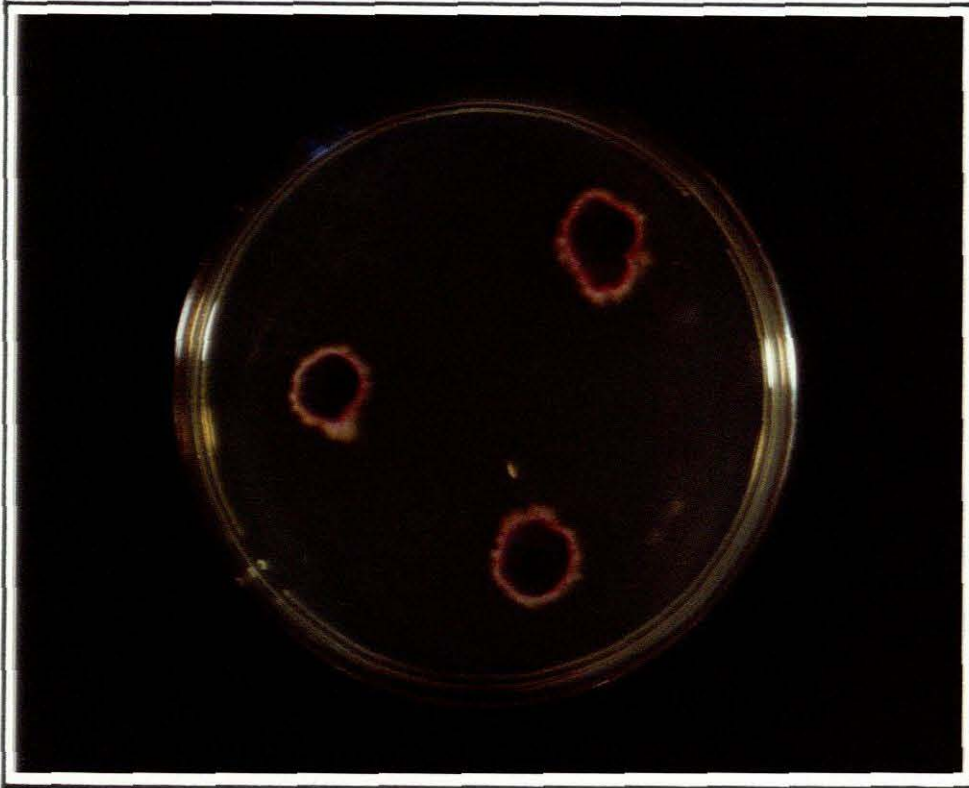


Culture grown on Sabouraud's Dextrose agar

plus chloramphenicol.

__T.violaceum__.

iv.  __T.mentagrophytes__:

A flat, powdery, white to cream thallus was commonly observed.  A tan-brown pigment was noted on the undersurface.
Small, spherical microconidia, often present in clusters, were observed microscopically.  Macroconidia and coiled spirals were occasionally encountered.

v.  __T.schoenleinii__:

Slow growing, waxy, convoluted, off-white colonies were observed.  Deep growth into the agar was noted and spreading subsurface growth was often encountered in mature cultures.
Classical "nail head hyphae" were noted microscopically in most isolates.  Faviform hyphae (antler-like hyphae) were present in all cultures.  No macroconidia nor microconidia were observed.

vi.  __T.tonsurans__:

Mature colonies of __T.tonsurans__ appeared folded with a suede-like surface.  Isolates were brownish-buff with a reddish-brown undersurface.

Microscopically, abundant, large, tear-shaped
microconidia were present. These occurred on thickened
terminal or branching lateral hyphae. Balloon forms
(enlarged microconidia) were noted in most cultures.
Occasional cylindrical macroconidia were present.

vii.  **Microsporum canis**

Colonies produced a coarsely fluffy, white, radially
spreading growth. A deep yellow pigment was produced
on the undersurface (see Fig.5).
Long, spindle-shaped, thick walled macroconidia,
usually with asymmetrical knobs, were noted in all
isolates. Microconidia were usually present.

viii.  **M.audouinii**:

Characteristic flat, radially spreading, tannish-white
colonies were observed. A deep salmon-pink pigment was
present on the undersurface.
No macroconidia nor micrconidia were observed
microscopically. Occasional terminal chlamydospores
were noted.

ix.  **M.gypseum**:

Colonies of **M.gypseum** were characteristically rapidly

49.

FIG.5

FIG.5a : SURFACE MORPHOLOGY OF MICROSPORUM CANIS.
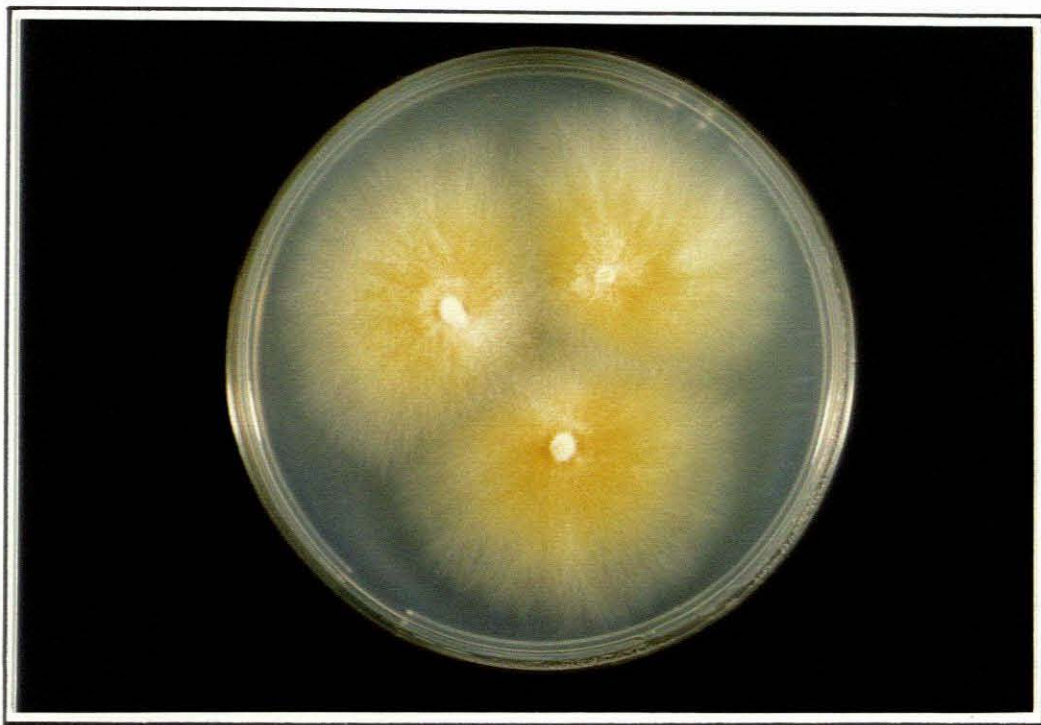


FIG.5b : UNDERSURFACE OF MICROSPORUM CANIS.
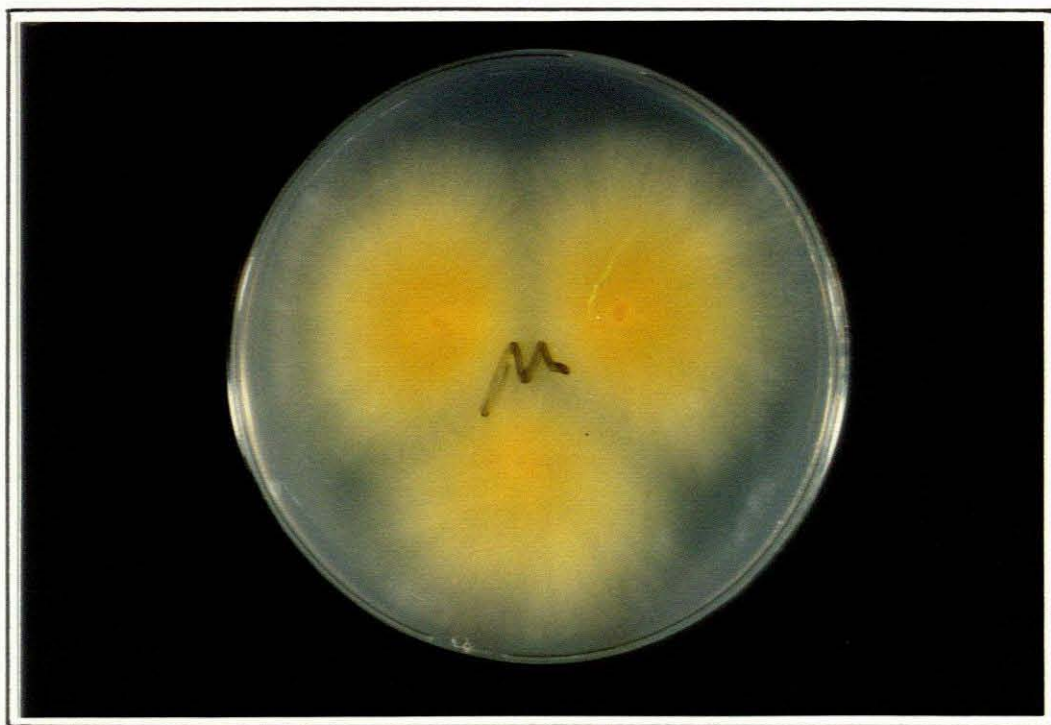
spreading with a profusely powdery texture.  Isolates
were a cinnamon-buff in colour.

Large numbers of broad, spindle-shaped macroconidia
with blunt ends which appeared echinulate and thin
walled on microscopy.  Club-shaped microconidia were
usually present.


x.  Epidermophyton floccosum:


Suede-like colonies appeared khaki-green in colour.
White tufts of growth appeared on the surface of mature
isolates.

Microscopy revealed blunt, club-shaped macroconidia
frequently occurring in groups.  No microconidia were
observed.


Special investigations


Special media were used for identification,
differentiation and confirmation of dermatophyte
strains (see page 60).


i.  T.violaceum:


The partial requirement of T.violaceum for thiamine was
investigated by culturing the organisms on diagnostic

Trichophyton agars (see Table 1). A basal medium (with no additives) was used as a control medium and growth obtained was compared to that achieved on the basal medium with thiamine as an additive. Media were prepared according to the methods described by C.N.Young (1980).

T.rubrum was used as a control organism, showing equal growth on both media (no growth requirement).

This test was performed on atypical, as well as random, typical strains of T.violaceum to confirm identification.

ii.  T.verrucosum:

Trichophyton Test Agars were similarly used for the identification of T.verrucosum. Additional tests for this organism included the routine incubation of cultures at two temperatures, 25'C and 37'C. Improved growth at 37'C was indicative of T.verrucosum, differentiating it from the morphologically similar T.schoenleinii.
Examinations for chains of chlamydospores produced at this temperature were undertaken.
A medium described by Kane et al (1978) was successfully used to rapidly confirm isolation of this

TABLE 1.

VITAMIN REQUIREMENTS OF SELECTED TRICHOPHYTON SPP.

| Species | Agar 1<br>BM | Agar 2<br>BM + Inos | Agar 3<br>BM + Th + Ino | Agar 4<br>BM + Th |
|---|---|---|---|---|
| T.violaceum | + | + | ++++ | ++++ |
| T.rubrum | ++++ | ++++ | ++++ | ++++ |
| T.verrucosum | + | +/- | ++++ | |
| T.yaoundei | ++++ | ++++ | ++++ | ++++ |
| T.schoen. | ++++ | ++++ | ++++ | ++++ |
| T.tonsurans | + | + | ++++ | ++++ |

Key : BM - Basal Medium, Inos - Inositol, Th - Thiamine

T.schoen. - Trichophyton schoenleinii.

slow growing organism.

The hydrolysis of casein by T.verrucosum is shown on
Bromo Cresol Purple Casein Yeast Extract Agar (BCPCYA)
within 2 - 3 days.  Colonies on this medium showed
restricted growth, surrounded by zones of hydrolysis
and microscopy revealed chains of flattened
chlamydospores at 37'C.  These features were not
observed with T.schoenleinii.  All cultures of
T.verrucosum were successfully tested on this medium.


iii.  T.yaoundei:


The absence of a requirement for thiamine distinguishes
T.yaoundei from T.violaceum, a morphologically similar
organism.  Trichophyton Agar No.4 was again utilized to
demonstrate this differentiating property.
A diffusing brown pigment was readily observed in
mature cultures of T.yaoundei on Sabouraud's Agar.
This feature easily differentiated this organism from
colonies of T.violaceum.


All strains of T. yaoundei tested, satisfied the above
requirements.


iv.  T.mentagrophytes:.


T.mentagrophytes was morphologically similar to

<u>T.rubrum</u>. A brown to tan pigment was produced by the vegetative mycelium of <u>T.mentagrophytes</u>. A red to maroon pigment was produced by <u>T.rubrum</u>, differentiating this organism from <u>T.mentagrophytes</u>. Pigment formation was examined by culturing suspected cultures of <u>T.mentagrophytes</u> on Potato Dextrose Agar (PDA) (see Table 2).

Urease production was examined using Urea Agar. <u>T.mentagrophytes</u> was urease positive (see Fig.6). All strains of <u>T.mentagrophytes</u> isolated in this survey produced a tan pigment and split urea.

v. <u>T.schoenleinii</u>:

Suspected isolates of <u>T.schoenleinii</u> were subjected to identification procedures as for <u>T.verrucosum</u>.

Cultures of <u>T.schoenleinii</u> did not grow well at 37'C and did not produce typical chains of chlamydospores. Growth on BCPCYA was not restricted and no zones of hydrolysis were observed.

vi. <u>T.tonsurans</u>:

<u>T.tonsurans</u> similarly has a partial requirement for thiamine (see Table 1). This property was investigated using the technique described above.

TABLE 2.

PIGMENT PRODUCTION ON POTATO-DEXTROSE AGAR.

Trichophyton mentagrophytes -    Mahogany-brown

Trichophyton rubrum          -    Wine-red


Microsporum canis            -    Canary-yellow

Microsporum audouinii        -    Salmon-pink

FIG.6

UREASE ACTIVITY OF TRICHOPHYTON MENTAGROPHYTES.

Colonies of T.tonsurans typically produce a
mahogany-red pigment on the undersurface.  This is an
important feature in the identification of this
organism and was determined by culturing the fungus on
potato-dextrose agar medium.  This medium enhances
pigment production (see Table 2).


All strains of T.tonsurans were examined using the
above mentioned media.


vii.  M.canis:.


The canary-yellow pigment produced on the undersurface
of colonies of M. canis was an important feature in the
identification of this organism (see Fig.5).  Not all
strains of this species produced this vivid colouration
and cultures needed to be differentiated from
morphologically similar dermatophytes.  All suspected
growths of M.canis were cultured on P.D.A. to enhance
natural pigment production.  A bright yellow pigment
was demonstrated in all cases.


Atypical strains morphologically resembling M.audouinii
were cultured on Rice Grain Medium.  M.canis grew well
on this medium, covering the surface with a yellow
thallus.  M.audouinii failed to germinate on this
medium.

viii.  **M.audouinii**:.

This dermatophyte typically produced a salmon-pink
pigment on the undersurface of the colony.  Cultures
could be confused with those of **M.canis** and thus these
organisms were subjected to all tests mentioned for
**M.canis** above.  Salmon-pink colouration was observed on
P.D.A. plates and no growth was noted on Rice Grain
Medium.

ix.  **M.gypseum**:

No special investigations were undertaken to identify
cultures of **M.gypseum** as morphological and
microscopical examinations adequately confirmed
isolation of this characteristic species (see Fig.7).

x.  **E.floccosum**:

Likewise no special investigations were performed on
this isolate.

FIG.7

MACROCONIDIA OF MICROSPORUM GYPSEUM.



x40 Magnification.

MEDIA

## BROMO CRESOL PURPLE CASEIN YEAST EXTRACT AGAR
### (Kane, 1978)

| | |
|---|---|
| Casein (powdered skim milk) | 40 gm |
| Yeast extract powder | 5 gm |
| Agar | 15 gm |
| Bromo Cresol Purple(1,6% in alcohol) | 1 ml |
| Distilled water | 1000 ml |
| Cycloheximide | 0,5 ug/ml |
| Chloramphenicol | 50 ug/ml |
| Gentamycin | 20 ug/ml |

Dissolve yeast extract powder in 50 ml of the distilled water and filter sterilize. Combine remaining ingredients and heat to dissolve. Adjust pH to 7,0 and autoclave medium for 10 mins at 118'C.

Add antibiotics and yeast extract to medium, dispense into McCartney bottles and slant for slopes.

MYCOSEL AGAR (BBL 11462)

| | |
|---|---|
| Phytone peptone | 10 g/L |
| Dextrose | 10 g/L |
| Agar | 15,5 g/L |
| Cycloheximide | 0,40 g/L |
| Chloramphenicol | 0,05 g/L |

Final pH ± 6.9

Suspend 36 grammes dehydrated material in 1 litre of distilled water.  Mix thoroughly, heat with frequent agitation and bring to the boil. Dispense and sterilize at 118'C / 15 minutes.

POTATO-DEXTROSE AGAR (Young, 1980)


Potatoes (washed, chopped, not peeled)   200 gm

Dextrose                                  20 gm

Agar                                      20 gm

Distilled water                         1000 ml


Boil potatoes in water for 1 hour, filter hot and make up to 1 litre.  Add dextrose and agar.

Autoclave at 121'C for 10 minutes.

RICE GRAIN MEDIUM (Young, 1980).


Long grain rice                          8 gm

Distilled water                          25 ml


Put in 25 ml Erlenmeyer flask, plug with cotton,
autoclave at 121'C for 15 minutes.  Use smaller
quantities in McCartney bottles (approximately half
full).

SABOURAUD'S DEXTROSE AGAR (Oxoid CM 41)

| | |
|---|---|
| Mycological peptone | 10 g/L |
| Dextrose | 40 g/L |
| Agar No. 1 | 15 g/L |

pH  5.6  ±  0.2

Suspend 65 grammes of dehydrated material in
1 litre of distilled water.  Boil until
completely dissolved.  Sterilize by autoclaving
at 121'C  for 15 minutes.
Dispense and solidify.

TRICHOPHYTON   AGARS
(Young,  1980)


Trichophyton Agar No. 1   (Basal Medium)


| Casamino acid (vitamin free) | 2,5gm |
| --- | --- |
| Glucose | 40,0gm |
| $MgSO_4$ | 0,1gm |
| $KH_2SO_4$ | 1,8gm |
| Agar | 20,0gm |
| Distilled water to give | 1000,0ml |

Adjust pH to 6,8


Dissolve by heating;   aliquot;   autoclave

at 121'C for 15 minutes.



Trichophyton Agar No. 2


| Inositol | 0,5ug/ml |
| --- | --- |

Melt 100ml basal medium, add 2ml inositol

solution prior to autoclaving

Dispense and solidify.

Trichophyton Agar No. 3

Inositol                          0,5 ug/ml

Thiamine                         0,2 ug/ml


Melt 100 ml basal medium ;   add 2ml each

of inositol and thiamine solutions prior to

autoclaving.

Dispense and solidify.




Trichophyton Agar No. 4


Thiamine supplement            0,2ug/ml

Melt 100 ml basal medium ;   add 2ml

thiamine supplement prior to  autoclaving.

Dispense and solidify.

UREA AGAR (Young, 1980).


A.  Christensen's urea agar base     29 gm

    Distilled water                 100 ml

Dissolve urea in water and sterilize by filtration.


B.  Agar                             15 gm

    Distilled water                 900 gm


Autoclave agar B at 121'C for 15 minutes;   cool
slightly, them mix A and B aseptically and dispense
into sterile tubes, slope.   pH 6,8.

STAINS
(Young, 1980)


### 15% KOH Solution

KOH                 15,0 gms

Glycerol            20,0 ml

Distilled water     80,0 ml

Add KOH to distilled water and mix over gentle
heat. Do not boil.  Add glycerol.

## LACTO PHENOL COTTON BLUE STAIN
### (Young, 1980)

Phenol crystals                20,0 gms

Lactic acid                    20,0 gms

Glycerol                       40,0 ml

Distilled water                20,0 ml

Dissolve ingredients by gently heating container in a 56'C waterbath.

Cotton blue crystals           0,05 gms

When lactophenol solution has dissolved, add cotton blue crystals.

3.2   The Carrier State of Scalp Ringworm.

Patient Group:

Eight institutions, all situated in the Cape
Peninsula, were included in this survey.  A total of
324 pupils were examined.

Four "Child Care" homes were visited.  Two of them
accepted only boys, one of them only girls and the
remaining institution housed both sexes.
Two institutions were convalescent homes and
similarly housed both males and females.
A reformatory for boys was also included as well as
a boarding school for deaf pupils.  The latter
school was mixed.  All children accommodated by these
institutions were permanent boarders.

School A:  St Joseph's General Convalescent Home.
           Forty-four patients in two paediatric
           wards were studied, comprising 21 males
           (47,7%) and 23 females (52,3%).

School B:  Brooklyn Chest Convalescent Hospital.
           Thirty-one patients, including 14 males
           (45,2%) and 17 females (54,8%), were

examined.  Similarly, these children
constituted two paediatric wards.


School C:  Leliebloem Child Care Centre.
           Two dormitories housing 21 children were
           sampled.  Eleven pupils (52,4%) were male
           and 10 (47,6%) were female.


School D:  St George's Child Care Home for Girls.
           All pupils in this home were examined.
           Samples from 39 children were collected
           including one from a young male
           accommodated on compassionate grounds.
              (Females 97,4%)


School E:  Bruce Duncan Child Care Home for Boys.
           All children from this home were included
           giving a total of 54 males.


School F:  St Patrick's Place of Safety for Boys.
           Thirty-two boys living at this shelter
           were studied.


School G:  Bonnytoon Reformatory for Boys.
           Fifty-seven junior inmates, all male,
           were sampled.

School H:   Wittebome School for Deaf Children.

The junior dormitory only was assessed.
A total of 46 pupils comprising 27 males
(58,7%) and 19 females (41,3%) were
examined.

All schools were initially visited to assess fungal
carriage.  Follow-up studies (see Section 3.3)
necessitated second visits to all institutions at a
later date.  Results of these collections were
correlated with above mentioned pilot study findings
enabling the possible transience or persistence of
fungal carriage to be studied.  Random visits were
undertaken thus the time elapsed between visits to each
school varied from 1,5 months to 8 months.

Time Elapsed Between First and Second Specimen
Collections:

School A:   8,0   months

    B:   1,5   months

    C:   3,0   months

    D:   6,0   months

    E:   6,0   months

    F:   6,0   months

School G:  1,5  months

  ‘H:  7,0  months


Specimen collection


Samples of skin, scales and hair stubs were collected
using the brushing technique described in Section 3.1.


Clinical Assessments


To differentiate carriers from clinical ringworm cases,
patients were classified as follows:


Ringworm Infection   –   any degree of scalp scaling
       and/or        –   a colony count of >10 on
                         culture.


      Carrier    –   no discernable scaling
        and      –   a colony count <10 on culture.


A pilot study at School A was undertaken to investigate
the clinical presentations of scalp ringworm prevalent
in local institutions.  Scalps of all children were

thoroughly examined and the degree of scaliness recorded.

Degrees of scaling were defined as follows:

    0 Scaling      - no discernable scaling

    + Scaling      - mild, diffuse or patchy scaling

    +++ Scaling    - profuse, diffuse scaling.

No inflammatory reactions were encountered in any of the schools investigated.

Degree of scaling was not recorded in schools B to H. For purposes of differentiating carriers from clinical cases it was decided to merely separate "scalers" (including all degrees of scaling) from "non-scalers".

Laboratory Methods

All laboratory procedures were performed as for Section 3.1.

3.3 Antifungal Shampoo Studies.

Group I - Selenium-sulphide vs Control Shampoo

Patient Sample

A total of 90 children attending the St. Josephs
Convalescent Home (School A)and the Wittebome School
for the Deaf (School H) were included in this group.
All were pre-pubertal pupils and the estimated mean age
was 6 years. Forty-eight (53,3%) were male.

Shampoos

Selenium-sulphide, a preparation with known antifungal
properties, was selected for evaluation by this group.
Colgate shampoo, a bland preparation, was used as a
control. Unlabelled containers were distributed to the
supervisors to avoid possible bias.

Selenium-sulphide shampoo was allocated to 47 pupils
and the control shampoo to 43. Non-statistical random
allocations of shampoos to approximately equal numbers
of "carriers", "infected" and "negative" pupils, as
well as all new pupils, were made. (Allocations were

based on earlier pilot study results.)


## Shampoo regimens


Sufficient supply of both selenium-sulphide and the
control shampoo was provided for a twice weekly shampoo
for four weeks. Hostel staff were instructed to
supervise all applications ensuring that a single wash
was immediately followed by thorough rinsing.


## Clinical evaluation


Scalps of all children in each group were clinically
assessed immediately prior to commencement of the
shampooing regimen. As defined in Section 3.2,
children were recorded as being "scalers" or
"non-scalers" and similarly "carriers", "infected" and
"negative" patients were defined by the criteria
described earlier.


## Specimen Collection


All specimens from each group were collected using the
brushing technique. Samples were taken immediately

prior to the commencement of the regimens and again 3 to 4 days following the final application of shampoo.


Laboratory Investigations


After sampling, the plastic brushes were inoculated onto Mycosel agar (see Section 3.1). All specimens, brushes and plates were randomly numbered for the laboratory evaluation to ensure that the identity of the patients and shampoos remained unknown to the laboratory investigator.

Isolates were identified on the basis of cultural characteristics and microscopical appearance (see Section 3.1).


Group II - Selenium-Sulphide Shampoo: Timed Exposures


Patient sample


Twenty children occupying two dormitories at the Leliebloem Child Care Home (School C) were investigated. The mean age was 12,8 years with a range of 6 - 17 years. Half the pupils were male.

Shampoos

As with Group I, selenium-sulphide shampoo was tested
in this trial. Comparisons of the effects of
selenium-sulphide when left on the scalp for differing
periods of time were made in this group. No control
shampoo was used. The shampoo was distributed to 20
pupils but no attempts were made to disguise the
shampoo in this trial.

Shampoo regimens

Pupils participating in this study were subjected to a
twice weekly shampoo for four weeks. Supervisors,
however, were instructed to ensure that the selected
shampoo remained on the scalp for a predetermined
period of time before rinsing.

Seven boys in dormitory A allowed the shampoo to remain
on the scalp for 5 minutes prior to rinsing.

Three males and ten females in dormitory B were
instructed to allow a 30 minute waiting period before
rinsing.

The exposure periods tested in these trials were

originally designed to include a 15 minute exposure
time. It proved difficult, however, to find an
institution with suitable numbers of pupils and
dormitories to participate. Consequently, the 15
minute group was excluded as the purpose of the trial
was to find a regimen that was simple to implement and
hence encourage good compliance (thus the 5 minute
group) but also to ensure maximum effect and benefit of
the antifungal product, hence the 30 minute groups. In
addition, earlier in-vitro investigations were
undertaken prior to the formulation of the survey
protocols (see Section 3.4). These pilot studies
revealed that fungal growth was only partially
inhibited following both 15 and 30 minute exposure
periods, suggesting that similar results would be
achieved with both time periods but that greater
deposition on the scalp surface might be achieved
following a longer application time. It was not
considered practical, however, to increase exposure
periods to longer than half an hour.

Group III- 3 Antifungal Shampoos versus Control Shampoo
(Econazole-nitrate, Selenium-sulphide, Povidone-iodine)

Patient sample

Dormitories and wards of five institutions were
visited.

School B:-  Thirteen males (56,5%) and ten females
            (43,5%) at the Brooklyn Chest Hospital
            were included in this group
            (total 23).  The range of ages was
            1 - 12 years with a mean of 4,8 years.

School D:-  All pupils in the St George's Home
            were sampled.  Thirty-eight were
            female (97,4%) and the remaining pupil
            was male (total 39).  The range of
            ages was 4 - 17 years with a mean of
            11,7 years.

School E:-  Thirty-nine pupils of the Bruce
            Duncan Home, all males, were
            examined.  Their mean age was 11,3yrs
            with a range of 6 - 16 years.

School G:-  Specimens were collected from 40

males at the Bonnytoon Reformatory.
The mean age was 13,3 years with a
range of 7 - 16 years.


School F:- All children frequenting the St.
Patrick's Shelter were investigated.
Twenty-one males with a mean age of
13,2 years (range 5 - 17 years) were
included.


Shampoos


Three shampoos with antifungal components were selected
for comparison in this group. 1% Econazole-nitrate
(EN)*, 2,5% selenium-sulphide (SS)* and 4%
povidone-iodine (PI)*. A preparation with no known
antifungal properties was used as a control, Baby
Johnson's Shampoo (CS)*.


---------------------------------------------------------------


*  EN - Pevaryl Foaming Solution, Fisons Laboratories.
   SS - Selsun Shampoo, Abbott Laboratories.
   PI - Betadine Shampoo, Adcock-Ingram Laboratories.
   CS - Baby Johnson's Shampoo, Johnson & Johnson.

Shampoo regimens


Each of the five homes was randomly allocated a
different shampoo.


Econazole-nitrate shampoo   -   School E   -   39 pupils


Selenium-sulphide shampoo   -   School D   -   39 pupils


Povidone-iodine shampoo     -   School G   -   40 pupils


Control shampoo             -   School B   -   23 pupils
                     and    -   School F   -   21 pupils


As with the previously mentioned regimens, all five
participating schools were allocated sufficient supply
of the shampoos for a month of twice weekly shampoos.
Supervisors in this group were instructed to ensure
that shampoos remained on the scalp for at least 15
minutes prior to rinsing.

## 3.4 Mycological and Ultrastructural Investigations

### Specimens

Infected hair stubs were collected from children with clinical evidence of "black dot" ringworm attending as outpatients at the Dermatology Clinic at the Red Cross War Memorial Children's Hospital. Hairs were examined microscopically for evidence of endothrix spores and were cultured to confirm infection with T.violaceum. Only samples that were microscopically and culturally positive were included in the study. Twelve specimens were tested.

### Shampoos

The four shampoos selected for evaluation by Group III (see Section 3.3) were studied in this project. Each shampoo was tested on samples from three patients.

### Cultural methods

Specimens were processed using a modified method described by Gip and co-workers (1966) to determine the

in-vitro activity of topical antimycotics.

Sampling Procedure

Specimens were collected from patients using sampling methods described in Section 3.1.  Scrapings were similarly collected into sterile petri dishes.

Processing of Samples

1 ml of shampoo was placed onto a sterile glass slide. Using slightly moistened needle tips, infected hairs were selected and immersed in the shampoo droplet. Specimens remained submerged in the allocated shampoos for predetermined periods.

Each specimen was treated as follows:-

    Culture A : hairs not exposed to any shampoo

                    (specimen control)

    Culture B : hairs exposed to shampoo

                    for 15 minutes.

Culture C : hairs exposed to shampoo

for 30 minutes.

Following this, all hair stubs were well rinsed by passing through a series of petri dishes containing sterile distilled water.
The specimen control was only subjected to the rinsing procedure.

Culture of Specimens

Rinsed hair stubs were pressed onto a small block (10mmx10mmx4mm) of Sabouraud's Dextrose Agar (Oxoid, CM.41) positioned centrally on a glass slide. A strip of sellotape was placed (sticky side down) over the inoculated medium in order to secure the block to the slide, to prevent excessive dehydration and to facilitate the microscopical examination of the culture. All slides were incubated in moist chambers at 25'C in darkness.

Microscopical examination

Cultures were examined microscopically on a daily basis. Following adequate growth of the control

specimen, the degree of germination in the test samples was graded. (see Table 3). This occurred between Day 3 and Day 5.

The grading of growth was as follows:-

Normal growth    -    germination equal to control culture slide.

Suppressed growth -    short, fat, deformed and/or curved hyphae.

No growth        -    no germination of spores.

Specimens were then examined by transmission electron microscopy.

Electron Microscopy

Hair cultures were trimmed out of SDA leaving a thin layer of media around the periphery of the hair and spores. The samples were fixed in 4% collidine-buffered gluteraldehyde (pH 7,4) and then washed in 0,1M collidine buffer. The specimens were post-fixed

TABLE 3.

FUNGAL GROWTH FOLLOWING EXPOSURE TO SHAMPOOS.

| Test shampoo | Specimen control | 15 min | 30 min |
|---|---|---|---|
| Control shampoo | +++ | +++ | + |
| Econazole nitrate | +++ | + | - |
| Selenium-sulphide | +++ | + | + |
| Povidone-iodine | +++ | - | - |

Grading of germination :   normal growth       +++

suppressed growth   +

no growth           -

in 1% collidine-buffered osmium tetroxide (pH 7,4),
dehydrated in graded alcohols, processed and embedded
in Spurr's resin.  Semi-thin sections (0,5u) were
stained with toluidine blue and suitable areas
containing keratin with adjacent spores were selected
for ultramicrotomy.  Ultrathin sections were double
stained with uranyl acetate and lead citrate and
examined with a Phillips 201 transmission electron
microscope.  The ultrastructural examination was
undertaken without the identity of the shampoos being
known to the laboratory investigator.

Mycological evaluation and ultrastructural results were
correlated only at the completion of all studies.

The criteria selected for ultrastructural examination
were general spore morphology, cell wall structure and
thickness, cytoplasmic membrane integrity and the
presence and condition of cytoplasmic organelles.

Spore degeneration was graded as follows:-


Normal      -

Mild        +

Moderate    ++

Severe      +++.


(Refer Table 4).

TABLE 4.

ULTRASTRUCTURAL COMPARISONS

|  | Cell wall degen. | Cytoplasmic degen. | Morphology |
|---|---|---|---|
| **Control shampoo:** | | | |
| Specimen control | – | – | – |
| 15 Minutes | + | + | + |
| 30 Minutes | ++ | + | + |
| **Econazole nitrate:** | | | |
| Specimen control | – | – | – |
| 15 Minute | + | ++ | + |
| 30 Minute | ++ | ++ | ++ |
| **Selenium-sulphide:** | | | |
| Specimen control | – | – | – |
| 15 Minutes | – | – | – |
| 30 Minutes | + | + | + |
| **Povidone-iodine:** | | | |
| Specimen control | – | – | – |
| 15 Minutes | + | + | + |
| 30 Minutes | +++ | +++ | +++ |

----------------------------------------------------------------

Grading of spore degeneration :  normal      –

mild        +

moderate    ++

severe      +++

CHAPTER 4.

4.    RESULTS

4.1    Epidemiology and Aetiology of Scalp Ringworm in the

        Southern and Western Cape.


Total Patient Sample


A total of 1440 children were included in this trial,

1332 (92,5%) were coloured, 103 (7,2%) were black, while

whites numbered only 5 (0,3%).   Females constituted just

over half (54,7%) of the patient sample.   The majority

of children were from low socio-economic backgrounds.


Direct microscopy was performed on 1142 (79,3%) samples

and 558 (48,9%) of these showed fungal elements on

examination.   Of the remainder that were negative, over

half (51,1%) yielded dermatophytes on culture.


A total of 867 (60,2%) specimens yielded positive

cultures and covered a range of nine dermatophytes (see

Table 5).   All dermatophyte species were confirmed by

macroscopic and microscopic methods and by the specific

identification tests previously described.   The

predominant organism was T.violaceum constituting 91,5%

of isolates.   The proportion of this organism in

positive samples from the Cape Peninsula and the West

TABLE 5.

RANGE OF DERMATOPHYTES ISOLATED

|  | Group A<br>No.(%) | Group B<br>No.(%) | Group C<br>No.(%) | Total<br>No.(%) |
|---|---|---|---|---|
| T. violaceum | 603(90,9) | 40(93) | 163(92,6) | 806(91,5) |
| M. canis | 25(3,8) | 0 | 0 | 25(2,8) |
| M. audouinii | 15(2,3) | 0 | 0 | 15(1,7) |
| T. verrucosum | 5(0,8) | 1(2,3) | 3(1,7) | 9(1,0) |
| T. yaoundei | 0 | 2(4,7) | 7(4,0) | 9(1,0) |
| T. mentagrophytes | 4(0,6) | 0 | 2(1,1) | 6(0,7) |
| T. schoenleinii | 5(0,8) | 0 | 0 | 5(0,6) |
| T.tonsurans | 2(0,3) | 0 | 1(0,6) | 3(0,3) |
| M. gypseum | 2(0,3) | 0 | 0 | 2(0,2) |
| E. floccosum | 1(0,1) | 0 | 0 | 1(0,1) |
| Microsporum spp | 1(0,1) | 0 | 0 | 1(0,1) |
| Total | 663(100) | 43(100) | 176(100) | 882(100) |

Cape Coast was not significantly different (p>0,1).

T.yaoundei (1,0%) was isolated only from West Coast patients while T.schoenleinii (0,6%), M.canis (2,8%), M.audouinii (1,7%), M.gypseum (0.2%) and E.floccosum (0,1%) were cultured only from patients attending the Dermatology Clinic. Two patients who yielded T.schoenleinii, however, originated from Bitterfontein on the West Coast.

Group A

Direct microscopy was performed on 883 (82,3%) of 1073 specimens. Insufficient material was collected in 5,1% of samples and brushings, precluding direct examination, were collected from the remaining 12,6% of children. Of the 883 specimens tested, half were positive on direct microscopical examination. Almost 35% of samples which were negative on direct examination yielded positive cultures.

All specimens in this group were cultured. Positive cultures were obtained from 657 (61,2%) children. Six cultures yielded two dermatophytes each thus the total number of isolates was 663.

A range of eight dermatophyte species was cultured, with

T.violaceum constituting the overwhelming majority of isolates (90,9%), (see Fig.8). One Microsporum species (identified on cultural microscopy) failed to germinate on subculture, thus further identification of species could not be undertaken.

Patients in this group presented with varying clinical signs of tinea capitis. The vast majority presented with mild diffuse scalp scaling (see Fig.9). Classical ring-shaped lesions were rarely observed. All patients infected with T.schoenleinii presented with crusty lesions (scutula) and patchy alopecia (see Fig.10). Infections with T.verrucosum were characterised by severe inflammatory reactions and evidence of permanent scarring was noted in most cases.

Group B

Fungal elements were demonstrated in 35 (72,9%) specimens while positive cultures were obtained from 43 (89,6%).

Three dermatophyte species were isolated from this group, including T.yaoundei(4,7%), and T.verrucosum (2,3%) with T.violaceum again being the predominant organism (93%) (see Fig.11). All specimens yielded single dermatophytes on culture.

FIG.8

RANGE OF DERMATOPHYTES — GROUP A.
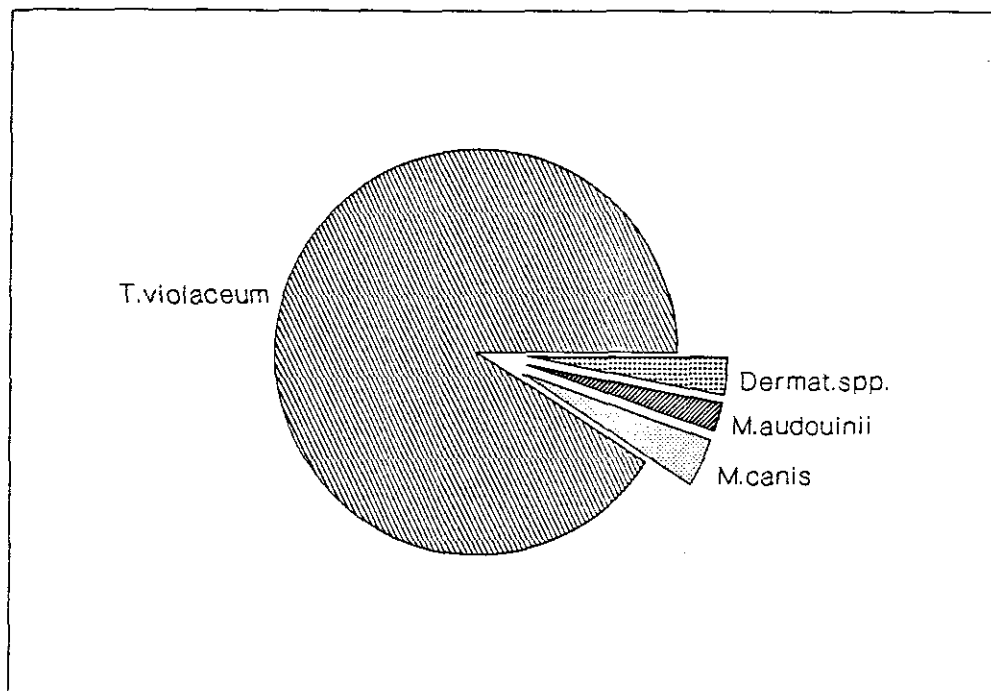
FIG.9

CLINICAL PRESENTATION OF TINEA CAPITIS BY
TRICHOPHYTON VIOLACEUM SHOWING MILD, DIFFUSE
SCALP SCALING.

FIG.10

CLINICAL PRESENTATION OF FAVUS DEMONSTRATING

NUMEROUS SCUTULA.
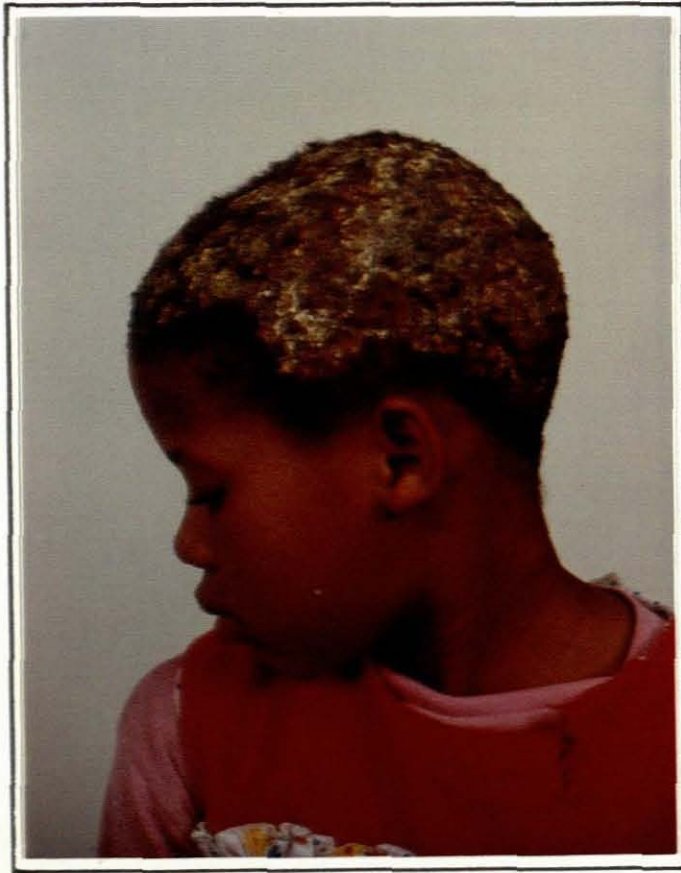
FIG.11

RANGE OF DERMATOPHYTES - GROUP B.
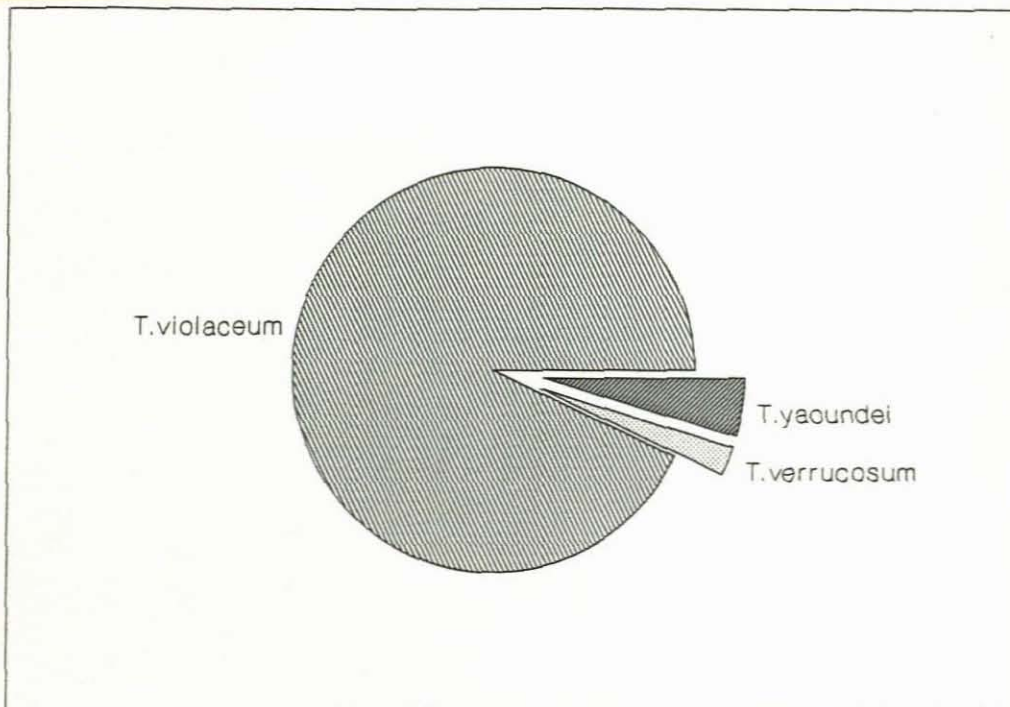
As in group A, all children presented with obvious
lesions suggestive of scalp ringworm.  Profuse, diffuse
scaling was the most common manifestation of infection.
A single inflammatory reaction in response to
T.verrucosum infection was observed in this group.


Group C


Thirty-four percent of specimens submitted proved
insufficient for direct microscopy.  Positive microscopy
results were obtained for 83 (26,0%), the remainder were
negative.


No clinical information was submitted for these
samples.  Fifty-two children, however, were recorded as
being asymptomatic suggesting that the remainder
presented with discernable signs of ringworm infection.


All specimens were cultured.  One hundred and
sixty-eight (52,7%) yielded dermatophytes.  Eight
samples grew two dermatophytes each.


The spectrum of organisms isolated included five
dermatophytes including T.violaceum, T.verrucosum,
T.yaoundei, T.mentagrophytes and T.tonsurans (see Fig.

12). <u>T.violaceum</u> constituted the majority of isolates
(92,6%).

Samples from 52 asymptomatic children were included in
the above-mentioned group. Notwithstanding the poor
quality of these samples (due to the lack of scaling or
visible signs) dermatophytes were isolated from 8
(15,4%) of these children.

* As the patient groups in 4.1 were not identical only
limited statistical analysis could be undertaken.

4.2 The Carrier State of Scalp Ringworm.

Total Group - Primary Assessment

In keeping with classifications defined in this section,
95 children (29,3%) presented with overt tinea capitis
infections (see Fig.13).
A further 79 (24,4%) were identified as ringworm
carriers. Just under half (46,3%) of all children
examined yielded negative results both clinically and on
culture.

School A had the highest infection rate of 52,3% (see

FIG.12

RANGE OF DERMATOPHYTES – GROUP C.
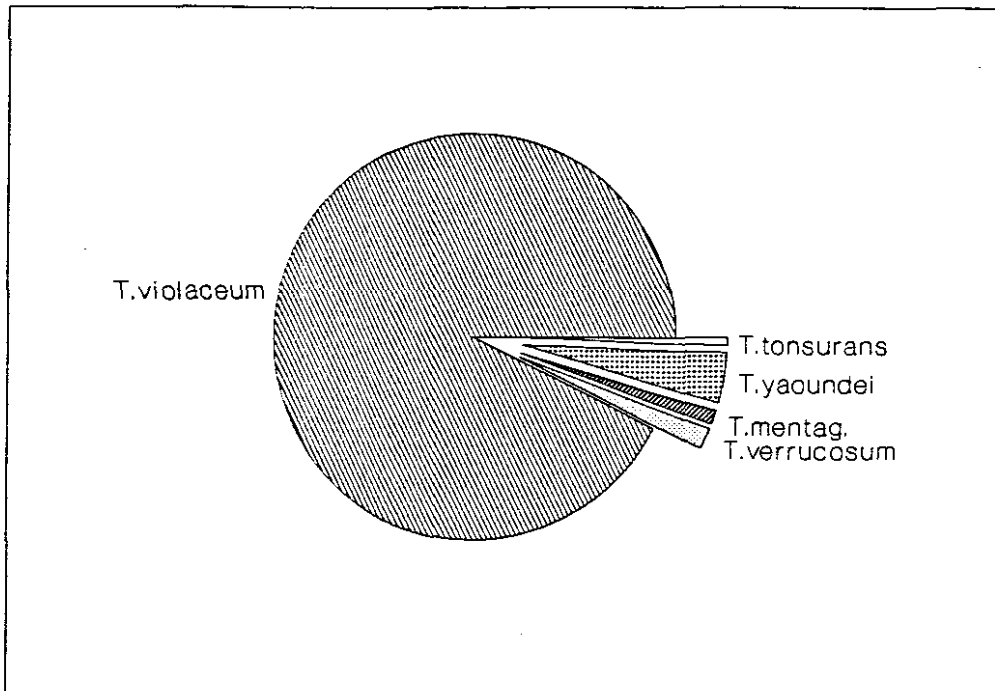


T.mentag. • T.mentagrophytes

FIG.13

CLASSIFICATION OF CHILDREN AT 1ST AND 2ND ASSESSMENTS.
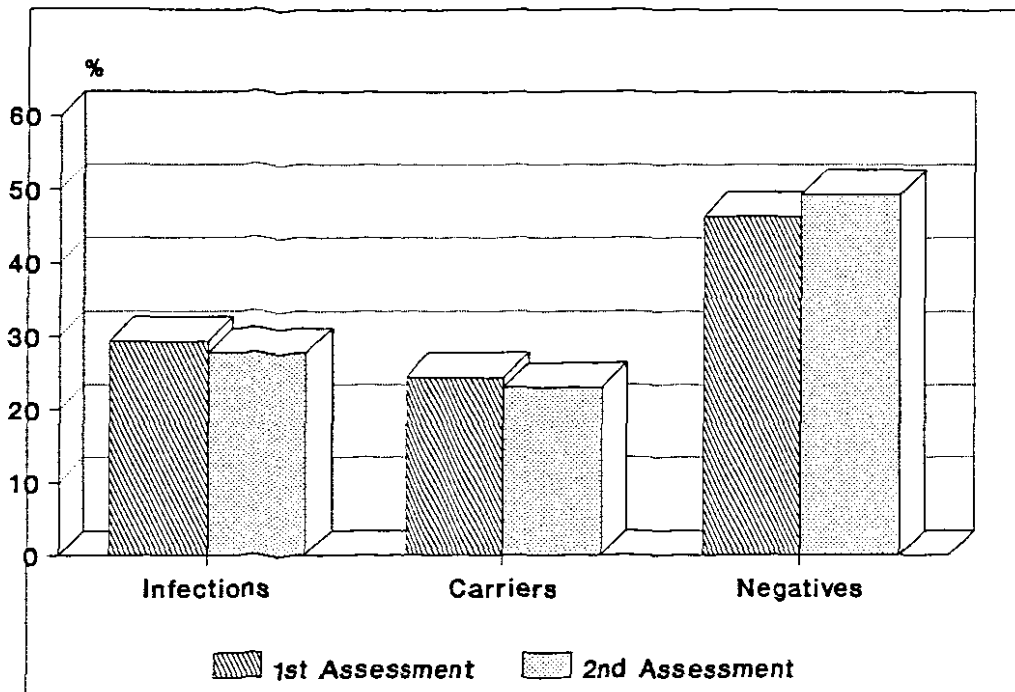
Fig.14). The carriage rate at this institution was lower (20,5%) than the estimated average (24,4%) for all schools.

School F yielded the highest percentage of carriers at 37,5% and the corresponding infection rate for this home was 15,6%.

With the exception of School B, all schools were infected exclusively with Trichophyton violaceum.

Four dermatophyte species were isolated from School B.

These included:

|  | | | |
|---|---|---|---|
| i) | T.violaceum | - | 19 (73,1%) |
| ii) | M.canis | - | 4 (15,4%) |
| iii) | M.audouinii | - | 2 (7,7%) |
| iv) | T.yaoundei | - | 1 (3,8%) |

Twenty-four patients in group B yielded positive cultures. Two children yielded two dermatophytes each, one including T.violaceum and M.canis and the other M.audouinii and M.canis. The total number of isolates was 26.

T.violaceum was isolated from both carriers and infected children.

FIG.14

COMPARISON OF CLINICAL STATUS OF CHILDREN

IN PARTICIPATING INSTITUTIONS.
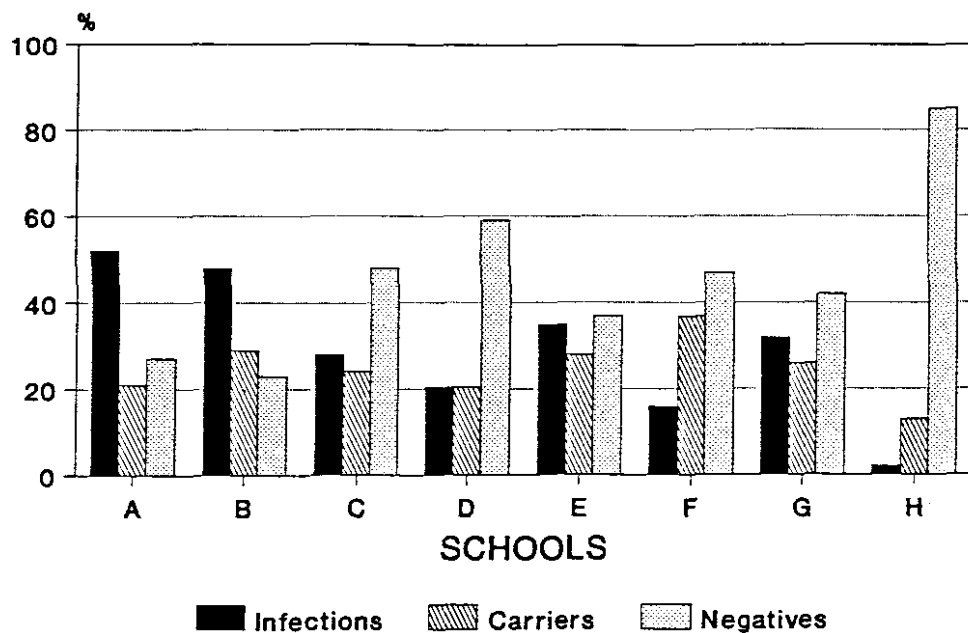
M.canis was isolated from: 1 overt infection; 1 carrier; and in small numbers from 1 dual infection (mixed with a predominant growth of T.violaceum) and 1 dual carrier (mixed with a scanty growth of M.audouinii).

M.audouinii was isolated from: 1 overt infection, and in small numbers from 1 dual carrier (mixed with a scanty growth of M.canis) as mentioned above.

T.yaoundei was isolated from one heavily infected case. There appeared to be no obvious difference in the range of dermatophytes colonising carriers and those causing clinical ringworm.

No discernable trends appeared evident when comparing the number of carriers to infections in each home (see Fig.14).

Total Group - Second Assessment

A total of 217 children were examined on each of two random visits.  (Due to the constant migration of pupils, not all children included in the primary study were available for reassessment.)  Results of the second visitation to schools A to H revealed that of

the remaining patients previously examined, 50 (23,0%) were carriers, a similar finding to the pilot study (24,4%), (see Fig.13). Of these children, 19 (38,0%) had remained persistent carriers over varied time periods.

The second sampling also showed that 30 children (13,8%) had become "new" carriers. Fourteen pupils whom had previously been negative became carriers and, similarly, 16 children who had been "infected" reverted to carrier status. Only one child in this group had been administered griseofulvin prior to the second sampling.

Twenty-three children (10,6%), classified as carriers in the pilot study, were negative on reassessment.

4.3  Antifungal Shampoo Studies

Group I : Selenium-sulphide vs Control Shampoo

Clinical evaluation

On clinical examination immediately prior to implementation of the shampooing regimen eleven children (12,2%) were found to have discernable scalp scaling.  No pupils presented with inflammatory reactions.

Pre-shampoo assessment

Total Group :
Twenty-five children (27,8%) had clinical scalp ringworm as shown by laboratory culture.
A further fifteen (16,7%) proved to be asymptomatic carriers and the remainder (55,6%) were both clinically and culturally negative (see Table 6).

SS Group :
Of 47 children selected to use Selsun shampoo, 17 were classified as infected, 8 were carriers and 22 were negative.

CS Group :

Of forty-three pupils allocated to this group, eight were recorded as infected, 7 as carriers and the remaining 28 as negative.

Post shampoo assessment

Total Group :

Laboratory investigations following the completion of the regimen revealed that an increased total of 28 children were infected, 25 were carriers and 37 were negative.

SS Group :

Thirteen pupils remained infected following the shampoo protocol suggesting an 8,5% improvement. The number of carriers doubled, however, increasing the numbers from 8 (17%) to 16 (34%), (see Table 6).

CS Group :

Similarly, the number of infected pupils increased by 7 to 34,9%. Carriers likewise increased from 7 (16,3%) to 9 (20,9%) reducing the number of negatives from 28 (65,1%) to 19 (44,2%), (see Table 6).

TABLE 6

Pre- and Post-Shampoo Assessments

|          |            | Pre-<br>No. | Post-<br>No. |
|----------|------------|------|-------|
| SS Group | Infections | 17   | 13    |
|          | Carriers   | 8    | 16    |
|          | Negatives  | 22   | 18    |
| CS Group | Infections | 8    | 15    |
|          | Carriers   | 7    | 9     |
|          | Negatives  | 28   | 19    |
| Total    | Infections | 25   | 28    |
|          | Carriers   | 15   | 25    |
|          | Negatives  | 50   | 37    |

Analysis of the overall results revealed that a total of 6 (6,7%) carriers became negative after treatment.

In the Selsun Group two infected pupils became clinically and culturally negative and 4 infected children showed reduction in fungal load i.e. were

classified as carriers (see Table 7).

In the Colgate Group one infected pupil became negative and no infected children were converted to carrier status. Two carriers became negative (see Table 7).

TABLE 7

Analysis of SS Trial Results

|  |  | Post-shampoo | | |
|  |  | Infections | Carriers | Negatives |
|  |  | No. | No. | No. |
|  | SS Group |  |  |  |
| Pre- | Infections | 11 | 4 | 2 |
| shampoo | Carriers | 1 | 3 | 4 |
|  | Negatives | 1 | 9 | 12 |
|  | CS Group |  |  |  |
| Pre- | Infections | 7 | 0 | 1 |
| shampoo | Carriers | 2 | 3 | 2 |
|  | Negatives | 6 | 6 | 16 |

Cultural results

Trichophyton violaceum was the only dermatophyte

isolated from all children in Group I.

Group II - Selenium-sulphide : Timed Exposures.

Clinical evaluation

Pre-shampooing evaluation showed that only one of the

twenty pupils had scalp scaling. This single case of

scaling persisted even following the shampoo

applications. The remaining children were all

asymptomatic.

Pre- and Post-shampoo Assessments

Total Group

Pre- examination : Twenty-five percent of the pupils in

this group were classified as infected and a further

quarter were asymptomatic carriers. Ten children (50%)

were clinically and culturally negative.

Post- examination : Laboratory investigations following
the shampooing regimen revealed that the number of
infections had been reduced from 5 to 3.  The figure
for carriers was also minimally reduced from 5 to 4 and
thus the number of negative patients was increased to
13 (65%).

5 Minute Exposure Group

Pre- examination : This sample consisted of 7 of the
above-mentioned children.  Two were infected, three
were carriers and a further two were negative prior to
treatment (see Table 8).

Post- examination : Following this brief exposure
period 2 children remained infected, carriers were
reduced in number from three to one and the number of
negatives increased to 4 (see Table 8).

TABLE 8


Results of 5-Minute Shampoo Exposures


|  | Pre-shampoo No.(%) | Post-shampoo No.(%) |
|---|---|---|
| Infections | 2 (28,6) | 2 (28,6) |
| Carriers | 3 (42,8) | 1 (14,3) |
| Negatives | 2 (28,6%) | 4 (57,1) |


30 Minute Exposure Group


Pre- examination : A total of thirteen children
constituted this group.  Pre-shampoo assessment
revealed that three were infected, two were classified
as carriers and eight were negative (see Table 9).


Post- examination : This longer exposure period
resulted in the number of infections being reduced from
three to one.  The number of carriers, however,
increased from two to three and similarly the number of
negatives were increased from 8 to 9 (see Table 9).

TABLE 9

Results of 30-Minute Shampoo Exposures

|  | Pre-Shampoo No.(%) | Post-Shampoo No.(%) |
|---|---|---|
| Infections | 3 (23,1) | 1 (7,7) |
| Carriers | 2 (15,4) | 3 (23,1) |
| Negatives | 8 (61,5) | 9 (69,2) |

Analysis:

Total Group : General analysis of the results revealed that a total of 3 carriers became negative following treatment and two infected pupils were converted to carrier status.

5 Minute Exposure Group : The status of all pupils remained the same with the exception of 2 carriers who became negative (see Table 10).

TABLE 10

Analysis of 5-Minute Exposure Results

|  |  | Post-Shampoo | | |
|---|---|---|---|---|
|  |  | Infections | Carriers | Negatives |
|  |  | No. | No. | No. |
| Pre- | Infections | 2 | 0 | 0 |
| shampoo | Carriers | 0 | 1 | 2 |
|  | Negatives | 0 | 0 | 2 |

30 Minute Exposure Group : One carrier became negative and 2 infected children became carriers. No other changes were noted in this group (see Table 11).

TABLE 11

Analysis of 30-Minute Exposure Results

|  |  | Post-Shampoo | | |
|  |  | Infections | Carriers | Negatives |
|  |  | No. | No. | No. |
| Pre- | Infections | 1 | 2 | 0 |
| shampoo | Carriers | 0 | 1 | 1 |
|  | Negatives | 0 | 0 | 8 |

Insufficient samples in this group precluded meaningful statistical analysis. The results obtained, however, suggest that no overwhelming difference in the effects of selenium-sulphide shampoo occurred following the differing exposure periods used in this trial. This outcome suggested that shorter exposure periods to this product could result in a similar or equal response.

Cultural results

As in Group I, all positive samples yielded cultures of T.violaceum.

Group III : Three Antifungal Shampoos vs Control

Clinical evaluation:

Econazole nitrate Group - Seven children (17,9%)
presented with discernable scalp scaling.  Thirty-two
were asymptomatic and no inflammatory reactions were
noted.
Four children (10,3%) were recorded as "scalers"
following completion of the trial.

Selenium-sulphide Group - Scalp scaling was noted in
four pupils (10,3%) prior to shampooing.  Thirty-five
were asymptomatic.
Following treatment, four children remained "scalers".

Povidone-iodine Group  -  Pre-shampoo assessment
revealed four pupils (10,0%) with evidence of scalp
scaling.  Thirty-six were asymptomatic.
No discernable scaling in any of the pupils was noted
following the regimen.

Control shampoo Group  -  Seven children (15,9%) were
noted as "scalers" and thirty-seven were asymptomatic
prior to shampooing.
Following treatment six pupils (13,6%) presented with

scaling.

Pre- and post-shampoo assessments

Econazole nitrate Group

Pre- examination : Investigations showed that 16 pupils (41,0%) were infected, eight (20,5%) were carriers and the remaining 15 (38,5%) were clinically and culturally negative (see Table 12).

Post- examination : Following treatment, the number of infections diminished to 12 (30,8%) while the number of carriers remained constant at 20,5%. The number of negatives increased to 19 (48%), (see Table 12).

TABLE 12

Pre- and Post-Shampoo Results

|  | Pre-shampoo No.(%) | Post-shampoo No.(%) |
|---|---|---|
| Infections | 16 (41,0) | 12 (30,8) |
| Carriers | 8 (20,5) | 8 (20,5) |
| Negatives | 15 (38,5) | 19 (48,7) |

TABLE 13

Analysis of EN Shampoo Results

|  |  | Post shampoo | | |
|---|---|---|---|---|
|  |  | Infections | Carriers | Negatives |
|  |  | No. | No. | No. |
| Pre- | Infections | 9 | 3 | 4 |
| shampoo | Carriers | 2 | 1 | 5 |
|  | Negatives | 1 | 4 | 10 |

* Statistical evaluation : Using the McNemar test of symmetry, no statistically significant improvement was noted following shampoo applications (ie. p>0.05).

Post-shampoo results were also compared statistically to those obtained following use of the control shampoo using the Yates-corrected Chi squared test. No significant difference or improvement was shown (p>0.05).

* All statistical analyses were undertaken by the Institute for Bioststistics, Medical Research Council, Johannesburg, using the BMDP Statistical Package.

Selenium-sulphide Group.

Pre- examination : Eight children were documented as infected, a further eight (20,5%) as carriers and 23 (59%) as negative (see Table 14).

Post- examination : The number of infected patients as well as the number of carriers was reduced by one i.e. to 7 pupils (18%). A slight increase in the number of negatives was noted i.e. 25 children (64%) (see Table 14).

TABLE 14

Pre- and Post-Shampoo Results

|  | Pre-shampoo No.(%) | Post-shampoo No.(%) |
|---|---|---|
| Infections | 8 (20,5) | 7 (17,9) |
| Carriers | 8 (20,5) | 7 (17,9 |
| Negatives | 23 (59,0) | 25 (64,1) |

TABLE 15

Analysis of SS Shampoo Results

|  |  | Post Shampoo | | |
|---|---|---|---|---|
|  |  | Infections | Carriers | Negatives |
|  |  | No. | No. | No. |
| Pre- | Infections | 6 | 1 | 1 |
| shampoo | Carriers | 0 | 3 | 5 |
|  | Negatives | 1 | 3 | 19 |

* Statistical Evaluation :  Pre- and post-shampoo results were again analysed using the McNemar test of symmetry.  No statistical improvement was noted following shampoo applications (p>0,05).

Post-active shampoo results were also statistically compared to those obtained following application of the control shampoo using the Yates-corrected Chi squared test. No significant difference or improvement was noted (p>0,05).

Povidone-iodine Group

Pre- examination : Twenty percent of this group were infected (8 pupils), thirty percent were assessed as carriers (12 pupils) and the remaining half were negative (see Table 16).

Post- examination : No infections were noted following completion of the regimen i.e. a reduction of 20%. The number of carriers was also significantly lower (p<0.05) at 7,5%. The number of negatives increased from 50% to 92,5% (see Table 16).

TABLE 16

Pre- and Post-Shampoo Results

|  | Pre-Shampoo No.(%) | Post-Shampoo No.(%) |
|---|---|---|
| Infections | 8 (20) | 0 |
| Carriers | 12 (30) | 3 (7,5) |
| Negatives | 20 (50) | 37 (92,5) |

TABLE 17

Analysis of PI Shampoo Results

|  |  | Post-Shampoo | | |
|  |  | Infections | Carriers | Negatives |
|---|---|---|---|---|
| Pre- | Infections | 0 | 3 | 5 |
| shampoo | Carriers | 0 | 0 | 12 |
|  | Negatives | 0 | 0 | 20 |

* Statistical Evaluation :  Pre- and post-shampoo results were analysed but zero values precluded effective use of the standard McNemar test of symmetry.  A modified McNemar test, using a two-by-two table rather than a three-by-three table, was performed and yielded highly significant results ($p \ll 0,001$). Post-active shampoo results were similarly compared to those obtained following use of the control shampoo. Due to numerical values less than 5, the Fisher's Exact test was utilised and indicated that significantly more children improved on Betadine shampoo than on Johnson's shampoo ($p < 0,05$).

Control Shampoo Group

Pre- examination : Infections were noted in eleven pupils (11,0%), 10 children (22,7%) were classified as carriers and a further 23 (59,0%) were negative (see Table 18).

Post- examination : Infections were noted in 7 children following treatment i.e. a reduction of 2,5%.
Increases occurred in the number of carriers and in the number of negatives to 22,7% and 59,1% respectively (see Table 18).

TABLE 18

Pre- and Post-Shampoo Results

|  | Pre-Shampoo No.(%) | Post-Shampoo No.(%) |
|---|---|---|
| Infections | 11 (25) | 7 (15,9) |
| Carriers | 10 (22,7) | 11 (22) |
| Negatives | 23 (52,3) | 26 (59,1) |

TABLE 19


Analysis of CS Shampoo Results


|  |  | Post-Shampoo | | |
|  |  | Infections | Carriers | Negatives |
|---|---|---|---|---|
| Pre-shampoo | Infections | 6 | 5 | 0 |
|  | Carriers | 1 | 3 | 6 |
|  | Negatives | 3 | 0 | 20 |


* Statistical evaluation :  The McNemars test of symmetry was likewise used to compare pre- and post-results.  No statistical significance was proven following use of this shampoo (p>0,05).


Cultural results


All positive cultures from the econazole nitrate, the selenium-sulphide and the povidone-iodine groups yielded T.violaceum exclusively.


School B, however, included in the control shampoo group, yielded cultures of T.violaceum and M.audouinii.

Three children (one infected child and two carriers) yielded pure growths of M.audouinii. All cultures became negative following shampooing although scalp scaling remained discernable in the infected pupil.

One previously negative child became a carrier of M.audouinii following treatment.

## 4.4 Mycological and Ultrastructural Investigations.

### Cultural Results

### Controls

The specimen control displayed prolific growth for each sample tested (see Table 3). No distortion or degeneration was noted. Growth appeared consistent with normal germination noted in cultures of T.violaceum.

15-Minute exposure group

Samples subjected to the control shampoo all displayed
normal growth with no discernable degeneration.
Econazole nitrate and selenium-sulphide shampoos both
produced suppressed germination in the specimens
tested.  Povidone-iodine shampoo completely inhibited
all growth in all the samples examined following this
15 minute period.

30-Minute exposure group

Poor growth and degeneration of fungal hyphae and
spores was noted with all shampoos.  Some degree of
germination was noted in the samples processed wih the
control shampoo and the selenium-sulphide product.
Hyphal elements, however, appeared gnarled and
distorted.  A total absence of growth was noted in
specimens subjected to treatment with econazole nitrate
and povidone-iodine shampoos.

Ultrastructural Results

Control specimens

By ultrastructural examination control spores appeared rounded to slightly oval in shape with no pleomorphism. The cell walls had a predominantly light, homogenous coat of uniform thickness, which only occasionally revealed multi-layering. The cytoplasmic membrane was smoothly contoured and integral with the inner surface of the spore walls. Cell contents consisted of nuclei, mitochondria, endoplasmic reticulum, vacuoles and occasional lipid droplets (see Fig.15). Some spores contained a cytoplasmic matrix almost too dense for detailed observation. These spores were nevertheless considered viable, as the cell wall, cytoplasmic membrane and visible organelles appeared normal. Their dense appearance could be as a result of culture malnourishment or age. Degenerate spores were occasionally found but numbered less than three per low power field.

FIG.15

CONTROL SPORES OF TRICHOPHYTON VIOLACEUM.
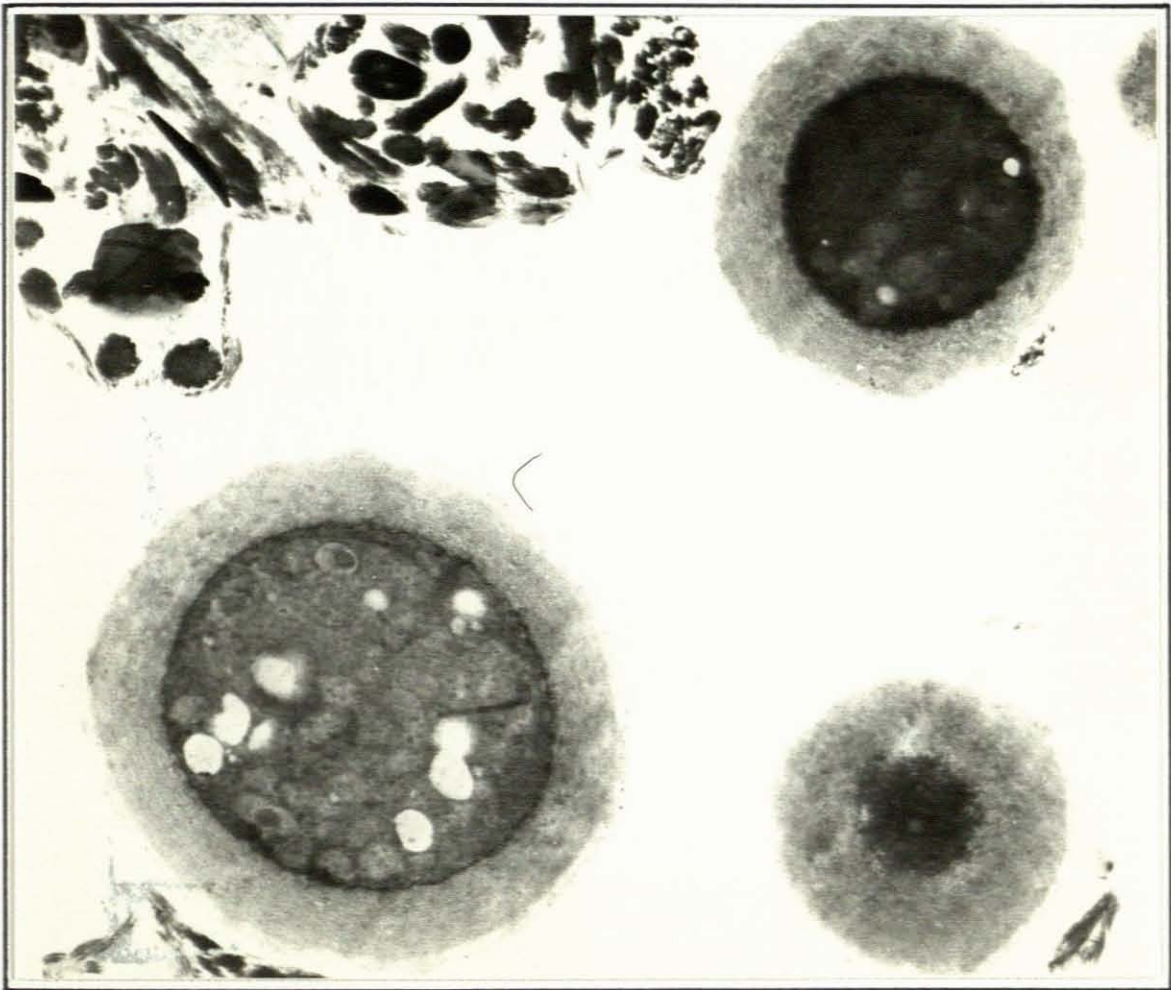


(Original magnification x 2000).

Treated specimens (Econazole nitrate, selenium-sulphide and control shampoos)

T.violaceum spores treated with the above mentioned shampoos all showed comparable degenerative and morphological effects on the cell wall, plasma membrane and cytoplasmic organelles in both exposure periods (see Table 4). The cell wall appeared thin, moth-eaten and irregular. The plasma membrane showed a tendency to separate from contact with the spore wall and appeared ragged and had lysed in some areas. Lysis of the cytoplasmic contents was present (see Fig.16). Selenium-sulphide showed the smallest degree of degeneration with normal cells being noted in the 15-minute group and only mild changes in the 30-minute group. All changes noted with these preparations were mild to moderate with no severe degeneration noted.

Povidone-iodine shampoo

Spores that were treated with povidone-iodine were more severely affected, exhibiting greater damage with severe lysis of the cell wall. These walls were extremely thin, moth-eaten, irregular and appeared permeable. Dissolution of the cytoplasmic content appeared complete with only little membranous and

FIG.16

SPORES OF TRICHOPHYTON VIOLACEUM FOLLOWING
30-MINUTE EXPOSURE TO ECONAZOLE NITRATE.
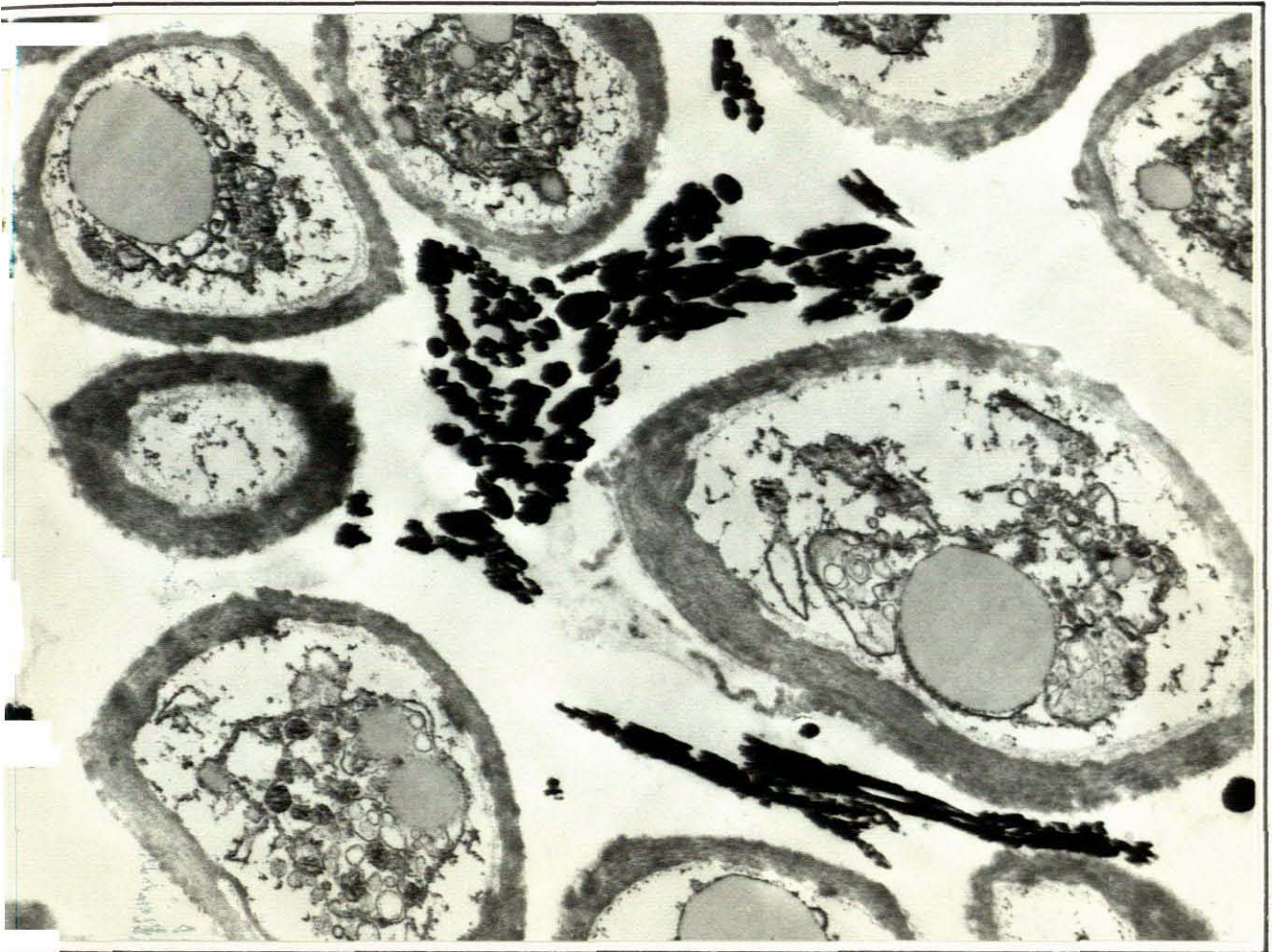


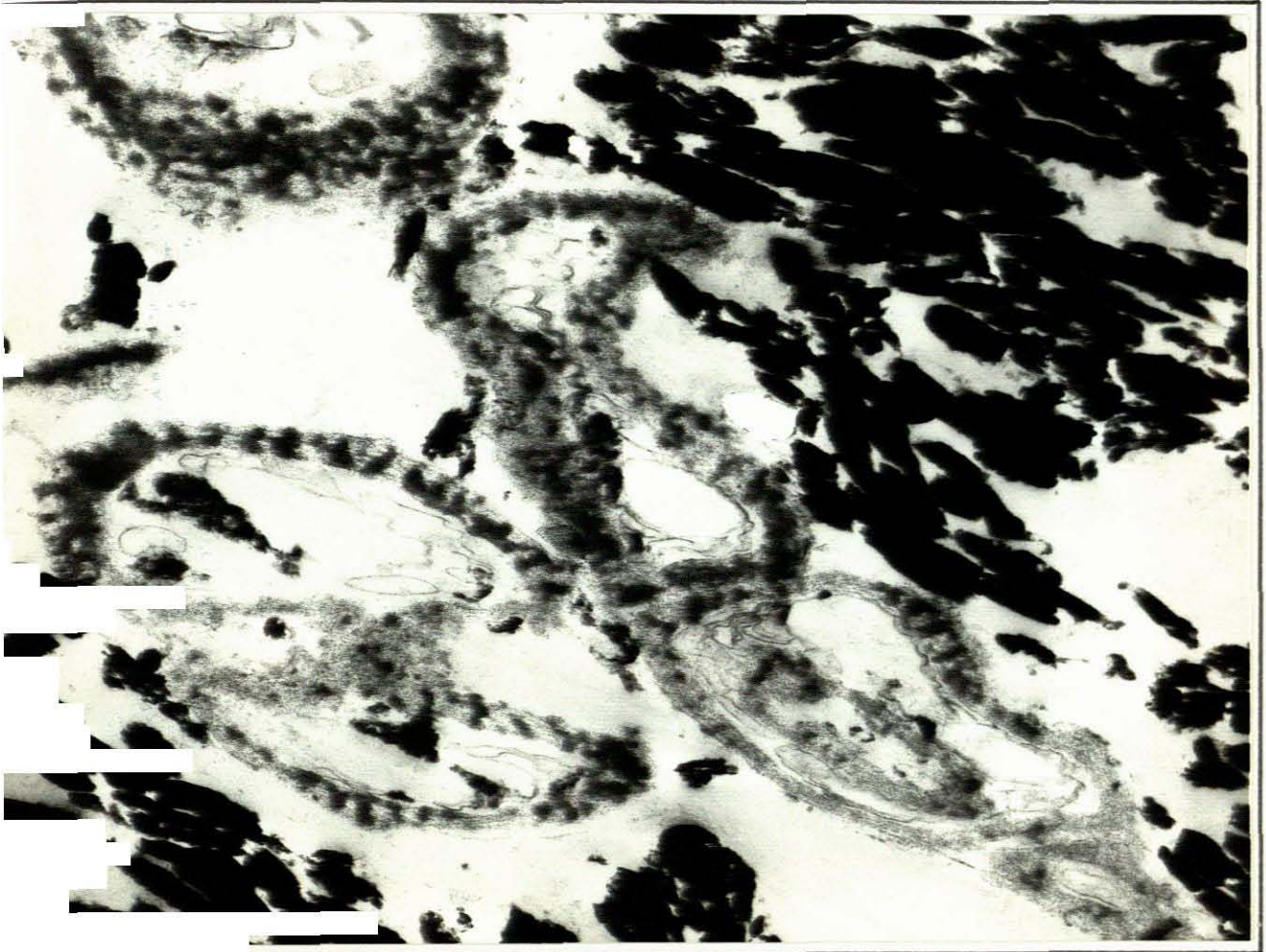(Original magnification x 7000).

non-specific, osmophilic material remaining (see

Fig.17).

FIGURE 17

SPORES OF TRICHOPHYTON VIOLACEUM FOLLOWING

30-MINUTE EXPOSURE TO POVIDONE-IODINE.



Original magnification x 7000).

CHAPTER 5.

5.      CONCLUSIONS


5.1   The Epidemiology and Aetiology of Scalp Ringworm in the
      Southern and Western Cape.



      The total patient sample consisted almost exclusively

      of coloured children.  This result was expected as the

      population ratios in the Cape Peninsula show this race

      as the predominant group in the region (Readers' Digest

      Atlas, 1987).  Coloureds and asians together constituted

      approximately 55% of the local indigenous population,

      whites 33% and blacks 12%.  Notwithstanding their

      predominance in numbers, the infection rate among the

      coloured peoples appeared to be discernably elevated

      (92,5%) when compared to that of the white group.


      In general, this group, constituting the major labour

      source in the area, consisted largely of families of

      low socio-economic background.  Inadequate housing

      facilities, overcrowding and poor personal hygiene were

      common features of this group.  As the majority of

      children were shown to be infected with T.violaceum

      (91,5%), a strict anthropophile, these conditions were

      considered highly contributory to the spread and

      persistence of scalp ringworm in this community.

Similar findings for the black population i.e.
increased incidence of disease when compared to the
white group were noted. As the economic status of this
group was considered to be even lower than that of the
coloured group, similar factors were assumed
responsible for the propagation of this infection.

The relatively high standard of living enjoyed by the
white population groups and their correspondingly low
infection rate further enhanced the theory that
ringworm of the scalp was primarily a disease of the
economically poorer groups.

The mean age for each group under examination was
similar, ranging from 6,4 years to 7,5 years. It is
widely accepted that tinea capitis is a paediatric
disease  infecting mainly pre-pubertal children
(Emmons, 1977; Rippon, 1988), similarly, this local
survey has shown that the disease infected most
children between the ages of 6 and 8 years. As this is
approximately the officially accepted age for school
attendance, and thus increased opportunity for social
interaction, it appeared that this event increased the
likelihood of spread occurring. The greater physical
contact observed amongst young scholars was almost
certainly responsible for the higher prevalence of
disease amongst this age group.

A small group of patients between the ages of 13 and 16 years were included in the study. Although these children could be considered adolescent and post pubertal, it was noted that in general, many appeared both mentally and physically immature for their age, a possible predisposing factor. T.violaceum has, however, been noted to cause chronic infection and even to persist into adulthood (Barlow, 1988).

Approximately 3% of patients seen at the Dermatology Outpatient's Department at the Red Cross Children's Hospital attend specifically for tinea capitis infection. These figures alone would suggest a low prevalence of scalp ringworm in the South Western Cape. Later studies, however, revealed that the vast majority of tinea capitis victims remain undetected and untreated (Neil, 1987). Random visits to schools along the West Coast, with samples collected from all pupils in each class, revealed an infection rate as high as 52,7%. The community health worker responsible for the collection of the above mentioned samples recorded only 52 of the 319 school pupils under examination as asymptomatic. This would suggest that the remainder had scalp scaling or other lesions indicative of possible tinea capitis, all of which had remained undiagnosed, untreated and overlooked by families and staff.

Similar findings were noted during local studies in the
Cape Peninsula (Neil, 1990). The average incidence of
tinea capitis in 8 local children's homes was 29,3% and
similarly, most cases appeared to have been overlooked
by hostel supervisors. These findings showed that
scalp ringworm was far more widespread than official
hospital and clinic records indicated. In urban
environments it seemed that the incidence was slightly
lower (29,3%) than that occurring along the
predominantly rural West Coast (52,7%). Improved
living conditions and sanitation, a higher standard of
education and improved and accessable medical
facilities in the urbanised areas were considered
contributary to the lower incidence in the S.W. Cape.
Conversely, extremely poor housing, lack of sanitation,
minimal education and the spartan medical services
encountered in the rural villages along the West Coast
seemed to promote the spread and persistence of tinea
capitis.

The infection rate of female children was slightly
higher than that noted in males in these regions. On
close scrutiny, it appeared that the intricate braiding
frequently noted in girls' hair exposed extensive areas
of scalp along the hair partings. Furthermore, hair
was generally tautly pulled often resulting in minor
trauma, folliculitis and traction alopecia. It has

been widely accepted that trauma followed by adequate inoculum of fungal spores frequently resulted in clinical infection, this phenomenon was a probable explanation of the slightly elevated rate of infection in females. In addition, this ornate braiding of the hair often lead to less diligent hair care practices, particularly less frequent hair shampooing. This, too, could have been a contributary factor.

In keeping with earlier reports, T.violaceum was found to be the predominant causative organism (91,5%) of scalp ringworm amongst coloured children in both regions under study. M.canis was the second most common cause (2,8%), and M.audouinii third (1,7%). A range of dermatophytes including T.verrucosum (1,0%), T.yaoundei (1,0%), T.mentagrophytes (0,7%), T.schoenleinii (0,6%), T.tonsurans (0,3%), M.gypseum (0,2%) and E.floccosum (0,1%) was also recorded (refer pg 93). The isolation of this organism from a scalp infection was an unusual finding. E.floccosum is frequently isolated from tinea cruris and tinea corporis infections and involvement of the hair and scalp has not been documented. Rippon (1988) reports occasional involvement of the face and forehead and as the exact site of the lesion was not recorded, the finding could suggest that the infection was restricted to the hairline. No hair invasion on microscopical

examination was discerned. Inadequate records preclude any meaningful evaluation of the case. One case from the <u>Microsporum</u> group (identified on cultural microscopy) failed to germinate on subculture and could not be identified specifically.

In spite of the diverse geographic and climatic influences experienced by the S.W. Cape and the W. Cape Coast, <u>T.violaceum</u> was shown to be the primary cause of tinea capitis in both regions. Contrary to the varied theories on the influence of these factors, no significant difference in the prevalence of this dermatophyte in either the southern or western region was noted (90,9% and 92,6% respectively).

It thus appeared that neither geographical, with particular reference to altitude, nor climatic conditions exerted any discernable effect on the prevailing dermatophyte species in any particular region.

The numbers obtained for the remaining dermatophyte species were regretably too small to allow any significant evaluation of their distribution in the areas under study.

M.canis was isolated exclusively from children in the S.W. Cape. Although this organism has been extensively reported amongst the white population groups, analysis of the racial distribution revealed that 76% of isolates were obtained from coloured children. A further 12% occurred in blacks leaving a lower prevalence rate of M.canis infection among whites (12%) than initially anticipated. The zoophilic nature of M.canis indicated that most infections originated from domestic pets, particularly cats and dogs. The incidence of pet owners was generally considered higher within urbanised communities thus providing a possible explanation of the exclusive isolation of this organism in the S.W. Cape. The improving standard of living and the relatively successful urbanisation of the coloured peoples in this area suggested that pet ownership had also increased amongst this group thus leading to an increase in M.canis-related tinea capitis.

In contrast, however, T.mentagrophytes var granulare, similarly a zoophile, occurred more frequently along the W. Coast than in the S.W. Cape. Although similarly reported to occur amongst domestic animals, this organism has also been isolated from farm animals, in rural areas. No conclusions could be drawn, however, as the number of isolates was too small for meaningful statistical evaluation (only 4 cases in the S.W. Cape

and 6 in the W.Cape).

Analysis of the prevalence and occurrence of the
remaining dermatophytes was similarly restricted to
numerical trends as figures were too small for
statistical evaluation. M.audouinii (15 cases),
M.gypseum (2 cases), E.floccosum (1 case) and
T.schoenleinii (5 cases) were all isolated exclusively
in the S.W. Cape during this survey. Previous
investigators have, however, reported the occurrence of
these organisms in widespread areas of the country,
suggesting that more extensive investigations would
need to be undertaken to draw any meaningful
conclusions on the significance of the distribution of
these dermatophytes.

5.2   The Carrier State of Scalp Ringworm.


Sampling of entire dormitories or wards in 8 different
institutions in the Cape Peninsula showed that
approximately one quarter of all children were asymp-
tomatic carriers of scalp dermatophytes (24,4%) (refer
pg 103).  A further 29,3% were found to be infected,
predominantly with T.violaceum, (clinically evident and
culturally proven) while less than half (46,3%) were
neither infected nor colonised.  The actual prevalence
of carriers in each home ranged from 13% in School H
(Deaf School) to 30% in School F (Place of Safety).

Contrary to expectation, no correlation was found in
any residential school between the percentage of
carriers and the percentage of infected cases.

It has been suggested that carriage of scalp ringworm
fungi is merely a transient event and thus not a major
contributory factor to the spread of tinea capitis.
Interpretation of our trial results, however, showed
that as many as 38% of carriers remained persistent
vehicles of fungal carriage over prolonged periods.
These findings suggest that fungal carriage is not an
occasional event but a condition that may persist for
lengthy periods of time, constituting a constant

nidus of infection.

Brushes, towels, clothing and bedding, acting as
possible transmission fomites, were not investigated as
previous studies have suggested that these items are of
minor significance in the spread of this disease.  It
was also felt that in-house practices relating to
cleaning and sterilisation of the above mentioned
articles were unlikely to be changed and that the
communal use of these items was unlikely to be stopped
amongst children of this age group.

It has also been proposed that the carrier state of
scalp ringworm is a pre-infection state and that clin-
ical tinea capitis usually develops in time (Polonelli,
1982).  The above results, however, suggest that fungal
carriage is not a form of subclinical infection but
rather a benign state of colonisation.  Twenty-three of
the asymptomatic carriers reverted to negative status
after various time periods.  It was noted that seven
children (14%) classified as carriers had developed
infection on reassessment but many more of the carriers
(47%) had become negative on reassessment.  It appeared
that certain children were more predisposed to becoming
heavily colonised and only approximately 14% of
carriers are likely to develop clinical infection.  The
chance of remaining a carrier (39%) or reverting to

146. 

negative status (47%) was similar in the remaining
patient sample.


Examination of the varied clinical presentations showed
that infection due to T.violaceum manifested with
minimal to severe scalp scaling. Approximately 40% of
infected children presented with minimal scaling and
only 2,1% showed severe scaling. Furthermore, fungal
cultures revealed that 55 clinically asymptomatic
children were classified as infected only following
laboratory investigations (i.e. produced colony counts
>10 per plate). These findings illustrate the often
subtle and easily overlooked clinical manifestations
encountered in T.violaceum infection. It has been
stated that the milder the inflammatory response to
infection with dermatophytes, the greater the tendency
towards chronic and continuous infection. Rippon
(1988) noted that certain dermatophytes, including
T.violaceum, appeared to reach an equilibrium with
regards to growth and lack of irritation within various
hosts. He stated that these fungi were usually
physiologically less active, especially in the
production of proteolytic enzymes, and also produced
fewer arthroconidia when growing on their preferred
hosts. It was also suggested that some inhibitor in
the keratin of the preferred hosts could "switch-off"
the production of inflammatory agents by the infecting

fungus. Although no enzymatic studies were undertaken, the findings of this survey suggest possible support for Rippon's findings.

Although the dermatophytes M.audouinii and M.canis were isolated from pupils in one of the institutions, the clinical presentation was similar to that of T.violaceum. No inflammatory responses were noted in any of the schoolchildren. Three of four pupils in school B were carriers of M.canis i.e. grew less than 10 colonies on culture. The fourth child was heavily infected (>40 colonies) and presented with scalp scaling only although M.canis is generally reported to invoke inflammatory disease. As only a single case was observed no conclusions could be drawn from this information.

The acute discomfort and high visibility of an inflammatory response to scalp ringworm would generally assure prompt medical attention. No such cases were noted during visits to the various institutions and this could have explained the absence of any hypersensitivity reactions during this trial and the development of a chronic infective cycle which passed unnoticed by the school supervisors.

5.3  Antifungal Shampoo Studies.


Group I : Selenium-sulphide vs Control Shampoo


Examination of the effects of selenium-sulphide on the
carrier state revealed that no real improvement had
occurred with the number of carriers in this group
doubling (refer pg 111).  As the study was designed to
investigate the effects of various agents on the
carrier state in particular, these results showed that
no beneficial response was obtained.  However, overall
analysis of these results showed that the number of
infected cases had been reduced resulting in this
increased number of carriers.  Similar results were
noted following the use of the control shampoo.  The
overall result, however, showed this shampoo to be even
less effective in reducing fungal load.


The poor effect of the sporicidal shampoo, Selsun,
could have been attributed to poor compliance or too
short an exposure period prior to rinsing.


These results suggested that only minimal benefit could
be achieved by regular use of these shampoos.

Group II : Selenium-sulphide : Timed Exposures

A numerical improvement was noted in the 5-minute group following the use of selenium-sulphide shampoo (refer pg 114). A reduction in the number of carriers with a resultant increase in the number of negatives was noted, suggesting some degree of beneficial response. Due to the small number of pupils tested, however, no meaningful statistical analysis could be performed.

Interpretation of the results following 30 minute exposure periods proved difficult (refer pg 115). The number of infections was reduced by two-thirds resulting in a slight increase in the number of carriers and in the number of negative pupils. The overall result of this trial thus suggested an improvement but as this study was aimed at reducing the carrier state in institutions, it can be concluded that minimal inhibitory response was achieved in this group by selenium- sulphide shampoo following the 30-minute exposure period.

No obvious explanation for these conflicting results was apparent when comparing the influence of exposure periods. Following in-vitro investigation, Allen et al (1982) reported that selenium-sulphide was sporicidal and that it also had the property of deposition on the

scalp surface. Rinsing reportedly did not affect this property. Consequently it was anticipated that significantly better results would be achieved following the longer contact times. The results obtained, however, indicated findings to the contrary. As with the 5-minute group, the number of patients tested was small and thus meaningful interpretation questionable.

Following completion of the regimens, supervisors reported fairly good compliance but noted that a few children in the 30-minute group complained of pruritis or burning during the exposure period.

Group III : Three Antifungal Shampoos vs Control

A schematic representation of the overall results obtained (Table 18) suggests that both selenium-sulphide and povidone-iodine shampoos produced results superior to those achieved with the econazole preparation. Furthermore, it would appear that the poorest effect was produced with the control shampoo, this deduction in keeping with expected findings.

TABLE 18

Schematic Representation of Results

| Econazole nitrate | | Selenium-sulphide | |
|---|---|---|---|
| Infections | - | Infections | - |
| Carriers | NC | Carriers | - |
| Negatives | + | Negatives | + |

| Povidone-iodine | | Control Shampoo | |
|---|---|---|---|
| Infections | - | Infections | - |
| Carriers | - | Carriers | + |
| Negatives | + | Negatives | + |

Key : +   Increase

\-   Decrease

NC  No Change

The trends suggested by numerical evaluation, however, indicated that econazole nitrate, selenium-sulphide as well as the control shampoo all produced similar beneficial results i.e. there was a discernable reduction in the number of infected children or carriers (or both) following use of all these products

although statistical analysis showed no real improvement (refer pg 120-26). It would seem likely that the slight reduction in fungal load was due mainly to the physical removal of fungal debris from the scalp surface.

When considering the effects of the shampoos on carriers alone, it became evident that similar results were achieved by the use of econazole nitrate, selenium-sulphide and the control shampoo. The results obtained following the use of povidone-iodine shampoo, however, showed a marked decrease in number in both of the culture positive groups. The findings for the latter were proven to be highly significant, confirming earlier deductions (refer pg 124). (Using a modified McNemar test - $p \ll 0,001$). Following the use of Betadine shampoo fungal carriage was erradicated in 100% of colonised children. It could thus be concluded that the povidone-iodine shampoo was the product most effectively contributing to the elimination or reduction in the fungal load of colonised children.

5.4   Mycological and Ultrastructural Investigations.

Evaluation of fungal germination following both time
exposures to the shampoos showed similar results
following the use of econazole nitrate, selenium-
sulphide and the control shampoo (refer pg 87).  These
results appeared more sensitive but correlated
reasonably well with the corresponding preceding
in-vivo trial findings (see Section 4.3).

Spores appeared to have been least affected by the
control shampoo which was the only preparation showing
normal growth following the 15-minute exposure.  At 30-
minutes, however, suppressed growth was noted.  Results
for econazole nitrate suggest that this preparation
might have been slightly more effective than
selenium-sulphide or the control shampoo.  No
germination was detected in the 30-minute group, while
suppressed growth was noted following the use of the
remaining two shampoos.  Numbers of samples tested,
however, were too small for meaningful statistical
evaluation.

Interpretation of these results and comparison with the
in-vivo tests in Section 4.3 indicate that the mild
beneficial effects observed earlier were not due only
to improved hair hygiene and the physical removal of

fungal debris but due in part to the effects of these preparations on the fungi. This was further emphasised by the poorer results obtained with the _in-vivo_ testing of the control shampoo. Following both time exposures, all specimens tested against Betadine shampoo failed to germinate. These results suggested that the povidone-iodine preparation successfully inhibited fungal germination following even 15-minutes of immersion. These results correlate well with the _in-vivo_ studies undertaken earlier. A significant number of infected children showed a decrease in fungal load while carriage of _T.violaceum_ was completely eradicated. These findings suggested that Betadine shampoo was a highly effective antifungal shampoo and that even shorter periods of exposure were likely to result in beneficial results. More studies were clearly indicated to confirm these conclusions.

Transmission electron microscopy was subsequently undertaken to investigate the ultrastructural effects of these products on spores of _T.violaceum_.

Control spores appeared normal in all categories examined.
Cells exposed to the control shampoo showed mild degeneration of the cell wall, cytoplasmic contents and morphology following both the 15- and 30-minute

exposures (refer pg    ).  Although strict comparison
could not be made between the 30-minute exposure cells
and in-vivo tests, the 15-minute results correlated
well.  The fungal load was reduced in only 9,1% of
infected children with increases noted amongst the
carriers.  The in-vivo tests showed only mild degrees
of fungal degeneration.

The results of the econazole nitrate treated cells, in
the comparable 15-minute in-vivo groups, showed mild to
moderate degeneration.  They also showed acceptable
correlation.  In-vivo trials showed a drop in the
number of infected pupils as well as in the original
number of carriers while the cells in the in-vitro
tests showed mild changes to morphology and cell wall
and moderate cytoplasmic degeneration.

Selenium-sulphide showed poor E.M. results with normal
cells noted following the 15-minute exposure period.
(Mild changes were noted following 30-minutes).
In-vivo findings for this shampoo showed that both the
number of infected pupils and carriers were reduced by
only one.  Although cultural results showed slightly
suppressed growth suggesting some degree of mild effect
(as yet undetected by E.M.) the overall effect of this
preparation on spores of T.violaceum was regarded as
insignificant with the main source of benefit being the

removal of fungal debris.

The 15-minute results for the povidone-iodine shampoo showed that in spite of only mild degenerative changes to the morphology, cell wall and cytoplasmic organelles demonstrated by E.M., total suppression of germination was noted in culture and fungal carriage was totally eradicated in the in-vivo tests. (Infections also significantly reduced $p \ll 0,001$). Following the 30-minute period, however, the severest degree of degeneration was noted with this product. these findings suggested that T.E.M. was not a sensitive means of predicting cellular response to the antifungal properties and that the cultural techniques more accurately paralled the in-vivo situation.

In summary it appeared that the results of these technical investigations supported the findings in Section 4.3 which could be considered acceptable in spite of the lack of cross-over trials and the variables of poor compliance etc. in the different institutions. These in-vivo trials have thus emphasised the validity of earlier results and similarly suggested that Betadine shampoo has a role to play in the control of fungal carriage on the scalp.

Shortcomings of the Study.

As most sections of this study were undertaken out of
interest and a desire to solve immediate problems, and
were not initiated with a view to including in a
thesis, many shortcomings were clearly evident.

In Section I, "Epidemiology and Aetiology of Scalp
Ringworm in the Southern and Western Cape", a more
satisfactory and conclusive result could have been
attained had similar groups of children been included
from both the Cape Peninsula and the West Coast.
Retrospectively, a more general sample of children in
their natural environments would have given a clearer
picture of the prevalence of the entire range of tinea
capitis-causing dermatophytes with particular reference
to species other than T.violaceum.  It would also have
allowed for statistical analysis which, due to the
diversity of the groups, was not attainable in this
section of study.

The study on transience and persistence of fungal
carriage was similarly undertaken retrospectively and
thus included varying time periods between repeat
samplings.  Ideally, standardised intervals between
specimen collections covering a wider range, e.g. 3-

months, 6-months, 12-months and 2-years, would have
produced more conclusive results. Further studies into
this important aspect of tinea capitis would be highly
beneficial.

A major shortfall in the shampoo trials undertaken by
various institutions was the general reluctance of the
staff to participate in further studies. This aspect
of the investigation precluded any "cross-over" trials
which would have provided a successful gauge of the
standard of compliance of all included schools.
Although all participants reported satisfactory
implementation of the regimens, it was implied that
most found it tedious to perform. These implications
questioned both the actual degree of compliance and the
suitability and acceptability of the tested regimens
and suggested that simpler regimens with longer periods
between washes and shorter exposure periods would be
necessary to ensure regular usage. This would appear
possible to achieve with the povidone-iodine shampoo
but further in-vivo and in-vitro trials are necessary.

CHAPTER 6.

6.    DISCUSSION.                    Pages 160 - 201.

6.  DISCUSSION

Tinea capitis has a high prevalence in both the South Western Cape and along the Western Cape Coast.

Studies of scalp ringworm began as early as 1955 (Lurie, 1955) suggesting that an increasing number of infections in South Africa had warranted these investigations.  Throughout the ensuing decades research in the form of geographical surveys continued, inferring the persistence of cutaneous mycoses in this country (Findlay, 1959; Clarke, 1959; Brede, 1962; Marshall, 1963; Ross, 1966; Dogliotti, 1970; Bentley-Phillips, 1972; Brede, 1972; Findlay, 1974; Scott, 1973; Daynes, 1974; Young, 1976).

A study, restricted to the southern reaches of the country, was among the most recent of current investigations to be undertaken in South Africa (Neil, 1987).  In some local communities studied (Neil, 1990), including only children with clinical lesions suggestive of tinea capitis (a high index of suspicion was used incorporating even the mildest suggestion of scaling), the isolation rate of scalp dermatophytes was 60%, while studies undertaken along the West Coast, including a cross section of children (even those with

no discernable signs of infection), showed an almost

equally high prevailing rate of approximately 53%. The

ensuing surveys undertaken at child care institutions,

including all children in specific dormitories, showed

a similar prevalence of 53,7%. These high figures

served to highlight the importance of further studies

into all aspects of this disease.

Similar studies to those undertaken locally along the

West Coast and at local child care institutions were

performed by Ive in Nigeria (1966) and Wright et al in

Zimbabwe (1986). Examination of all pupils in a rural

Nigerian school by Ive showed that scalp dermatophytes

were isolated from approximately 30%. Wright et al in

Zimbabwe examined the prevalence of scalp ringworm in a

home for the physically handicapped and demonstrated an

isolation rate of 39% (Wright, 1986). Comparison of

results shows a significantly higher prevalence in the

southern regions of the Republic. Further comparisons

were not possible as few studies of this nature

encompassing a cross-section of the public

(incorporating diseased and apparently healthy

individuals) appear to have been undertaken elsewhere.

Apart from establishing the unaceptably high prevalence

rate of tinea capitis in local areas, these studies

confirmed the major role of Trichophyton violaceum as a

causative agent of this disease (92%). Our findings
verified earlier studies which showed this organism to
be of a ubiquitous nature among the black and coloured
populations of the Cape (Marshall, 1964; Brede, 1972).
Social conditions have been deemed responsible for the
rampant spread of this anthropophilic dermatophyte
amongst these groups with overcrowding, poor hygiene
practices and poverty considered contributory to the
persistence of this disease in these communities.

Microsporum canis, the second most common organism
isolated, accounted for only 2,9% of dermatophytes.
Its zoophilic nature and its exclusive isolation from
children in the South Western Cape suggested a higher
incidence of domestic pets in this region.
Approximately three quarters of the children infected
with this organism (76%) were coloured and indicated
that successful urbanisation and an improved standard
of living had resulted in pet ownership in this group.
Only 12% of M.canis infection occurred amongst blacks
with a similar, unexpectedly low percentage occurring
amongst whites.

The remaining dermatophytes isolated included
M.audouinii, T.verrucosum, T.schoenleinii,
T.mentagrophytes, T.tonsurans, M.gypseum, and
E.floccosum. The small numbers isolated, however,

precluded further investigation into the significance of these organisms.

Documentation of these locally prevalent dermatophytes was undertaken to enlighten and assisst all associated medical and health workers with regard to the possible source of infection and the likely mode of spread.

For centuries mycologists have been endeavouring to prove conclusively that certain factors are responsible for the occurrence of various dermatophyte species in particular regions. Most studies have been focused on theories of geographical, climatic and racial influences. It would seem that little progress has been achieved in this field. Correlation of results from numerous studies on the effects of geographical influences on fungal spectra would seem to suggest a minor role of questionable significance. Physical position, altitude and climate of regions have long been quoted as the dominating factors determining dermatophyte flora in particular regions.

Within South Africa, Brede in 1962 confidently explained the differing spectra of ringworm fungi present in the Cape and those reported by Lurie (1955) on the Witwatersrand comparing these results to those

found in European areas of similar climate. Brede made
particular reference to comparable statistical material
published by Neves (1960) on the occurrence of
T.violaceum in the Mediterranean regions of Portugal.
References by Neves to work by Levy-Lebhar and Herman
in Morocco, who reported an 80 persent incidence of
tinea capitis due to T.violaceum in this Mediterranean
area, were also made.

Neves (1966) continued epidemiological investigations
into dermatophyte infections in Portugal and remained
confident that each geographical area had its own
characteristic dermatophyte flora. Brede, too,
continued his research into the geographical
distribution in Southern Africa and an overview of
ringworm fungi in this region, published in 1972,
broadly divided the areas under study into climatic
zones i.e. Cape Coastal Belt, Cape Highlands and
Transvaal highlands. It appeared that the available
statistics suggested a definite influence by climate as
well as physical position on the prevailing
dermatophyte spectra.

In a major review of dermatophytes occurring on the
African continent, however, Verhagen (1974) concluded
that climate played no role in the prevalence of
dermatophytes. He demonstrated that anthropophilic

fungi predominated in all areas, but that no clear difference existed in the specific fungi isolated in different regions. The emergence of anthropophilic fungi, especially Trichophyton species, as causes of tinea capitis has also been seen in the U.S.A. (Laude, 1982; Ravits, 1983; Schokman, 1983) and in the U.K. (Payne, 1984).

Nsanzumuhire et al (1974), working in Tanzania, similarly stressed the geographical distribution in relation to the epidemiology of human dermatophytes. He believed that the survival and pathogenicity of dermatophytes was enhanced by humidity and heat and thus explained the high incidence of ringworm infections in the tropical areas. A report by Schockman et al (1983) on tinea capitis in Philadelphia supported these views stating that the likelihood of dermatophytic infection was increased, among other factors, by a tropical climate.

Subsequent studies during the ensuing years further investigated the effects of climate on ringworm fungi and seasonal comparisons were made.

A comprehensive report by Philpot (1973) on the epidemiology of tinea, referred to the findings of Sanderson and Sloper (1953) who implicated sustained

high temperature and humidity as the most important
supplementary factors influencing the incidence of
ringworm fungi. They suggested, however, that this was
an indirect factor as the resultant effects of warm and
moist skin became vital predisposing factors ensuring
the continuance of endemic species. All forms of
clinical ringworm were studied.

Ali-Shtayeh et al (1986) of Jordan reported the number
of tinea capitis infections in the West Bank varied
with season, the highest numbers occurring in winter
and spring and the lowest numbers occurring in the
summer months. The winter and spring periods were
associated with low temperature and high humidity while
the summer season was associated with high
temperature. A similar study, spanning 12 years was
undertaken by Sekhon et al (1986) in Canada and
conversely the incidence of dermatophyte infections was
highest from August to October, the summer season, and
lowest during the winter period. These results,
however, included all the dermatophytoses and were not
peculiar to tinea capitis infections. Caprilli et al
(1987) reported similar findings in Rome. His survey,
over a period of five years, showed a definite increase
in the incidence of ringworm disease during spring and
summer with a marked decrease during the winter
months. This study, too, included all forms of

dermatophyte infection.

The conclusions drawn from these varied seasonal findings suggested that humidity was the overiding climatic feature influencing prevalence of ringworm fungi. It appeared to play a greater role than the effect of elevated or decreased tmperatures as shown by increases in the number of infections in both winter and summer in different countries. Elevated humidity often leads to increased maceration of tissue, especially in the body flexures, with a resultant increase in infection. The effect on the scalp, however, is undetermined. As the range of ringworm infections was not consistent throughout each study no conclusive deductions could be made.

A recent report by Attapattu (1989) on the occurrence of tinea capitis in Sri Lanka suggested that climatic conditions, together with customs and genetic factors of population groups were largely instrumental in the incidence of dermatophytosis within a community. Her study spanned 10 years and included 462 patients from Columbo and environs.

Literature searches have also revealed that many earlier reports considered altitude the most important

factor amongst the geographical influences. In Brede's report (1972) he divided the regions of South Africa into different climatic zones which co-incided with the areas of differing altitude i.e. Cape coastal belt, Cape highveld and Transvaal highlands. With reference to surveys carried out by various researchers within these areas, he showed that the spectra of dermatophytes differed between the "high, dry" areas and the lowlands of the country. He made particular reference to T.violaceum which Marshall (1964) had reported in the Cape coastal areas and its virtual disappearance on the central plateau. He also noted its reappearance in the lowveld of the Transvaal, in the Zimbabwean midlands, Botswana and Mocambique. Brede, however, based his studies largely on statistics received by his mycology unit in Stellenbosch in the Cape, and thus little information pertaining to sex, race or social status of patients was available.

During this period research on the African continent by Vanbreuseghem (1962) and Dockx (1969) suggested that in the fertile highlands of East Africa, the frequency and prevalence of T.violaceum increased with altitude. Referring to the opposite findings by Brede, Verhagen (1974) offered anthropogeography as a plausable explanation. He ventured that the distribution of T.violaceum in these East African regions coincided

with those areas which were historically influenced and linked to the Middle East by trade, indicating that it was imported by visiting Arabs.

Scott et al (1973) investigated dermatophytoses amongst all patients attending Dermatology clinics in Bloemfontein, a town situated at high altitude on the central plateau of South Africa. He was able to demonstrate, in contrast to information supplied by Lurie (1955) and Brede (1972), that T.violaceum did indeed prevail in regions of high altitude. The results of his survey showed an incidence of 63,5% of tinea capitis infections caused by T.violaceum. He stressed, however, that this organism was restricted to the "Bantu" population group and was rarely encountered amongst the whites.

A similar situation was recorded by Daynes (1974) following work in the mountainous Tsolo district of the Transkei. Contrary to suggestions by Marshall (1964) that Microsporum infections were restricted to low-lying areas, he found M.audouinii in 75% of tinea capitis cases in local black children.

Although the patient samples in the above mentioned studies did not lend themselves to direct comparison, the general trend of the findings suggested that

altitude was of little significance in the prevalence
of dermatophyte species.

With regard to the geographical distribution of fungi,
including influence of both climate and altitude, our
findings for T.violaceum re-iterated the minor role, if
any, played by these factors. This organism was
isolated with similar frequency from two regions with
vastly different climate types (West Coast - desert;
South Western Cape - Mediterranean) and altitudes (West
Coast - 126-1322m; South Western Cape - sea-level).
The average relative humidity of the W.Coast and the
S.W.Cape was 52% and 73% respectively. In agreement
with Verhagen it would appear that neither climate nor
altitude significantly influenced the prevalence of
T.violaceum in our study. While differences for the
remaining causative organisms were found when comparing
isolates from the two regions, the total number of
isolates was too small to draw any convincing
conclusions. In summary, it seemed that most
dermatophytes had to be regarded as cosmopolitan and
that the geographical classification of these organisms
was of limited value and could not be regarded as
constant.

Differences in susceptability to fungal infections

between races has long been a cause for speculation.
In 1973, both Blank et al (1974) and Scott et al found
apparent racial predilection for blacks by M.audouinii
and T.violaceum. Each was unable to satisfactorily
explain this phenomenon and conceded that possible
reasons were poor hygiene and diet or a high exposure
rate amongst this group. Studies in the 1980's by
Lubwana (1986) and Mclean et al (1987), in East Africa
and New York respectively, showed that the distribution
of the prevailing dermatophytes in each area
(T.violaceum in East Africa and T.tonsurans in the
U.S.A.) could not be attributed to race in view of the
racial heterogenicity of the areas.

Comparison of racial predisposition was not possible
within our study as the overwhelming majority of
children examined were coloured and due to political
segregation policies all schools visited were also
exclusively coloured. A small number of blacks, whites
and Indians attending the Dermatology clinics were
examined but numbers were low. No concerted effort was
made to include all population groups thus the possible
effect of racial predisposition remains unclear. The
numerous cases of chronic, non-inflammatory disease
which appeared common to the coloured, black and indian
groups, however, suggested poor immunological response
towards the endemic dermatophytes.

The conclusively proven factors influencing prevalence and incidence of dermatophyte infections were standard of hygiene and level of nutrition. (Malnutrition is known to depress cell-mediated immunity.) In a study of tinea capitis in Philadelphia, Reid et al (1968) used case and control groups to explore the effects of economic status and hair care practices. She was able to demonstrate that ringworm of the scalp most commonly afflicted poor, overcrowded populations and that annual income per se was not associated with tinea capitis. She commented that a greater number of children in families tended to accentuate overcrowding and poor hygiene conditions, promoting spread of anthropophilic species. Similar studies worldwide strongly support these findings and most concurred that the populations at highest risk for this type of infection come from the lower socio-economic scale (Jacyk, 1982; Bugingo, 1983; Herbert,1985; Ali-Shtayeh, 1986).

Two studies of interest, however, referred to uncommonly low scalp infection rates amongst the lower income groups in the respective areas studied. Kamalam et al (1973) found scalp ringworm less common in India than in other third world countries and suggested that the regular use of vegetable oils for hair dressing was a preventative factor. Similarly, a study in Sri Lanka (Attapattu, 1989) postulated that the local custom of

frequent head baths with soap and water, especially among children, may have accounted for the low prevalence of tinea capitis in this community. In as early as 1966, following extensive work in Portugal, Neves stated that improvement of hygiene was indeed a fundamental prophylactic measure in the prevention and control of ringworm infection.

Vanbreuseghem (1957) has suggested diet as a factor influencing the spread of ringworm. He recorded the highest incidence of tinea capitis in Zaire in areas where protein deficiency was greatest. Neves (1966), however, found normal serum levels in infected children in Lisbon and felt that hypoproteinemia in many populations depended on deficient nutritional and hygiene standards of underdeveloped areas. More recent field studies of the dermatophytoses in Yugoslavia also showed no support for poor nutrition as a single predisposing factor to ringworm infections (Ozegovic, 1985).

It appeared that our findings were in keeping with worldwide opinions. Most children included in this study were from low-income, poor socio-economic groups. This was particularly true of all Western Cape Coast children who originated almost exclusively from families of farm labourers or miners. Patients

attending the Dermatology Outpatients Department in Cape Town were predominantly urban and although slightly more affluent in terms of income, were nevertheless considered of poor economic standing. The children's homes and institutions visited were of various standards relying heavily on government and charitable support as a means of subsistence. Although no formal, comparative study with more affluent patients was undertaken, the enthusiastic encouragement of concerned local health authorities in the regions studied, to investigate the prevalence of tinea capitis amongst these population groups, seemed to suggest an unacceptably high infection rate.

No studies on hypoproteinemia were undertaken but comparison of field study results from the West Coast (including children from poorly educated, low income families with a high probability of malnutrition) and results from boarding schools and homes (including reasonably well nourished children) showed little difference in incidence of tinea capitis and spectrum of causative organisms.

In general it appeared that the single most influential factor in the spread and persistence of scalp ringworm in the regions under study was poor hygiene practices. Children from the West Coast were largely from rural

settlements with no running water, electricity or
modern sanitation systems within the dwellings.
Expectedly, personal hygiene was generally of a poor
standard and hair washing was seldom practised
regularly. Overcrowding and sharing of clothing and
bedding etc. was common and effectively promoted the
spread of scalp ringworm, particularly by the
anthropophilic species. Little contact with domestic
pets was noted in this group although contact with cows
and chickens present within the settlement areas was
recorded.

Although the urban group of patients from the hospital
environs in the S.W.Cape were generally from adequately
serviced homes, many remained poorly informed on
acceptable practices of personal hygiene.
Overcrowding, too, remained a constant factor with
often several families occupying a single dwelling.
Contact with domestic pets was prevalent amongst these
children, perhaps explaining the exclusive occurrence
of M.canis in this group.

Within the schools and institutions of the Cape Town
areas, personal hygeine of children was more strictly
attended to. However, severe staff shortages in all
schools under study resulted in difficulty with close
supervision and frequently older pupils were left to

supervise the routine bathing and washing procedures.
Hair washing practises varied considerably from school
to school but the average frequency of shampooing was
one hair wash per week. A daily hair washing routine
using toilet soap was recorded in one all male
institution (school G) while an all girls home (school
D) reported a maximum of one wash per week suggesting
possibly even longer periods between washes. The
reason given for the longer than average periods
between hair shampoos was the difficulty experienced
both with the braiding and unbraiding of the girls
intricate, traditional hairstyles. This factor would
appear to support the premise that poor hygiene
practices predispose certain groups to ringworm
infection as the number of girls infected consistently
remained slightly higher than the infection rate for
boys. The intricate design of the braiding, however,
exposed large areas of the scalp along the numerous
hair partings and possibly also contributed to the ease
of contracting this infection. Bhakhtaviziam (1984)
found boys more commonly infected in Libya but noted
that in females, fungal involvement of the median
parting hairline was particularly common. Traction
alopecia and folliculitis often occurred amongst these
females and resulted in further predisposing factors to
tinea capitis. Our finding of a slightly higher
infection rate among females was in agreement with

reports by Ali-Shtayeh (1986) of Jordan and McLean (1987) of New York. Many earlier publications reported a predominant incidence of tinea capitis amongst boys, explaining the phenomenon to be a result of the very short hairstyles (then worn by males) exposing more scalp and hair follicles (Reid, 1968; Kamalam, 1973; Jacyk, 1982; Schockman, 1983). In spite of our differing findings for the sexes, the local hair fashions, braided, plaited hair for girls and longer "Afro" styles for boys, still support Schockman's theories.

Comparison of the infection rates between schools and their respective hair washing routines, however, showed no obvious trends. A paediatric convalescent home (school A) had the highest infection rate, yet was supervised by trained nursing personnel and practised an acceptable routine hair washing regimen. The all girls school (school D) with the least regular hair washing routines had the second lowest infection rate of only 5%. It would thus seem that even personal hygiene with specific regard for hair care played a lesser role than initially suspected. (No school routinely used any form of antiseptic or antifungal shampoo but various "ordinary" shampoos, frequently donated, or plain toilet soap.)

In 1966, Ive undertook a study to determine the
prevalence of fungal carriage on the scalp in a rural
Nigerian school. After elimination of diseased
children from the trial he showed that 24,5% of
asymptomatic children were carriers. Further studies
were also undertaken throughout the world in urban
centres. Clayton in 1968 reported on findings
in five, day schools in London. She reported carriage
rates of between 12% and 30% in classes where cases of
scalp ringworm were present and lower carriage rates of
between 1% and 5% in other classes in the same
schools. The importance of fungal carriers was also
noted by Polonelli et al in Rome in 1982. They
suggested that one of the greatest difficulties in the
prevention and eradication of tinea capitis was the
presence of healthy fungal carriers. They felt that
carriers represented a little known, epidemiologically
significant, source of infection. Their study included
754 children in the public and private schools of Rome
and showed that only two asymptomatic pupils were
carriers (0,27%). In 1988, Sharma et al randomly
cultured scalp scrapings of 200 healthy children in a
large Kansas metropolitan hospital. The overall
incidence of fungal carriage in this group was 4%.

Our local studies have shown an average incidence of
24,4% of carriers in 8 schools investigated (range

37,5% - 13,0%). All schools were boarding schools with all pupils permanently resident on the premises. Five day schools along the West Coast were also visited yielding a carrier prevalence rate of 15,4%. In comparison with the above-mentioned studies these local carrier rates were unexpectedly high.

One of the earliest reports on fungal carriage was made by Raubitschek in 1959. His study in Jerusalem included 150 new immigrant families housed predominantly in overcrowded huts in immigration camps. Although no figures for fungal carriers were given, his findings showed a 20% higher incidence of clinical tinea capitis in families harbouring carriers and he thus deduced that the home might be even more important as a source of infection than the schools.

Our findings locally were in complete agreement with Raubitschek's conclusions. Sporadic cases from day schools were regularly seen at the weekly Dermatology Outpatients Clinic at the Red Cross Children's Hospital while large groups of infected pupils from child care institutions, substituting the family environment, were constantly treated for primary or recurrent infection. The closed communities with larger numbers of carriers appeared to experience persistent problems with the control and eradication of tinea capitis.

Although carriers appeared to be increased in number in these homes, contrary to Clayton's findings in the London schools, the higher number of carriers did not necessarily correspond to the school or class with the highest number of infections. Conversely too, the school with the lowest number of carriers did not correspond with the lowest number of infected cases as might have been expected. This suggested that the existence of carriers was not dependent on, nor determined by, the presence or number of infections in their immediate environment.

Local authorities have expressed the opinion that the carriage of scalp fungi may be a transient condition and thus of questionable significance as a major source of infection. Although this study was not initially designed to investigate this aspect of carriage, examination of the available results showed that an average of 36% of carriers in eight institutions remained persistent carriers over periods varying from 6 weeks to 8 months. There was no correlation between the school with the highest number of persistent carriers and the shortest time period between its assessments and *vice* *versa*. These results seemed to successfully refute the theory of carriage being merely a transient condition and served to emphasise the importance of the role of the carrier in the community.

Few comparable studies were encountered that similarly examined the transience or persistence of the carrier state, a factor which suggests that the carrier has long been overlooked as an important source of infection to the predisposed child. A single survey by Ive (1966), undertaken in a rural Nigerian school, monitored 19 untreated carrier children over a four-month period. The pupils were reassessed following this interval and results showed that 4 (21%) had developed overt ringworm and 8 (42%) had remained carriers.

Polonelli et al (1982) suggested that the continued survival of anthropophilic dermatophyte species was ensured by the existence of low-grade, virtually inapparent infections. He referred to studies of Mariat et al (1976) in Alsace which revealed that the carriers of zoophilic dermatophytes showed actual infection when examined histologically and suggested that epidemiologically the anthropophilic fungi could similarly be maintained by the existence of sub-clinical infection.

Although no histological examinations of skin biopsies were undertaken on the pupils in this study, the number of carriers which reverted to negative status following various intervals (47%) would seem to suggest that most

carriers were not victims of low-grade, chronic infection but rather had experienced a benign state of colonisation. A similar response has been documented with dermatophytes causing athlete's foot (Rippon, 1988). These fungi were frequently isolated from apparently healthy feet and resulted in a condition termed "occult tinea pedis". These dermatophytes were regarded as part of the normal microflora.

Examination of our results showed that only seven carriers (14%) had developed clinical ringworm on reassessment. It was thus conceded that a small percentage of "carriers" were in fact in the sub-clinical stages of early infection but that the vast number of these patients had remained persistent carriers (39%) or became negative (47%) without ever having shown any signs of developing clinical disease.

It has long been documented that fomites, too, are possible vehicles of carriage for ringworm infections (Baer, 1966). Rippon noted that certain hair-invading species of dermatophytes have evolved a parasitic form of arthroconidia, produced only during active infection, but which would remain viable on fomites for several years. Gip reviewed a vast number of reports on the recovery of dermatophytes from floors, clothing, dust, air and soil in 1966. He surmised that

dermatophytes (including geophiles) were present in
indoor environments but questioned their role as
exogenous agents of infection and suggested further
studies. In 1972, Midgley and Clayton investigated the
occurrence of dermatophytes in the air and on healthy
sites of the body and confirmed earlier reports that
these fungi are rarely present in the microflora of the
air or skin of healthy individuals.

In spite of the strong possibility of fungal elements
occurring within the environments of the children's
centres, no concerted effort was made to examine this
aspect as a source of colonisation or infection by
scalp dermatophytes. As some form of basic health and
hygiene practice was routinely undertaken in all homes,
it was doubtful whether any further suggestions
altering existing protocols would have met with lasting
or successful implementation. Furthermore, due to the
youthfulness of the children, it seemed futile to
examine clothing, brushes or bedding for evidence of
dermatophytes as poor habits involving communal use of
these items were unlikely to be broken. The greater
amount of physical contact observed amongst this age
group, particularly with regard to the sharing of beds
and clothing, was a normal phenomenon which we felt
should not be actively discouraged. These factors,
together with the above-mentioned reports suggesting

that these vectors of spread play a relatively minor
role in the spread of this disease, discouraged further
investigation into this aspect of the ecology of
infection.  A more realistic approach was considered to
be the elimination and possible prevention of the
clinical infections as well as the unexpectedly high
number of scalp carriers.  It was hoped that this
approach would also serve to lessen the fungal load in
the environment.

All cases of T.violaceum-tinea capitis encountered in
the homes presented with some degree of scalp scaling.
The degree of scaling varied from mild to severe with
the vast majority of cases exhibiting moderate scaling
(76%) and 19% only minimal scaling.  As few as 5%
presented with easily discernable, severe scaling.
These subtle properties of T.violaceum infection were
frequently overlooked by poorly informed medical
practitioners as well as the institution supervisors
and contributed to the persistently high incidence of
infections in the schools under examination.
Inflammatory response to this organism was not noted in
any child studied in this survey.

In agreement with Rippon's observations, it seemed
likely that T.violaceum had reached an equilibrium with
regards to lack of irritation and growth in local

children. He suggested that the less inflammatory responses resulted in a greater tendency towards chronic and continuous infection and was usually as a result of the physiologically less active nature of the anthropophilic fungi. A further possible factor in the persistently subtle presentation of this particular infection was his suggestion of an inhibitor in the host which interfered with the production of inflammatory agents by T.violaceum.

Similar findings to those noted locally were made by Kamalam et al in India (1979). After the examination of two communities he concluded that the non-inflammatory, seborrhoeic form of T.violaceum infection usually occurred in groups that lived in close contact while the opposite occurred with inflammatory responses. Due to the clinically distinct types of presentation encountered in his trial, he hypothesised that epidemicity or endemicity depended mainly on the infective strain of the fungus rather than the hosts. He summarised, however, that the probable genetic predisposition to tinea capitis in the communities could also have influenced the results.

A study on differing clinical presentations as a result of T.tonsurans infection was also undertaken by Rasmussen et al in New York (1978). He tested 52

patients with tinea capitis including 16 children
showing inflammatory response and 36 with minimal
reaction. Each patient was tested with trichophytin
antigen to determine whether cell-mediated immunity was
responsible for the different clinical presentations.
He was thus able to show that mild, chronic ringworm
infection with no inflammatory response occurs in those
with no cell-mediated response to trichophytin.

No tests for cell-mediated or humoral response were
performed on local children so immunological reactions
to local species of dermatophytes were not studied.
Neither was work on possible differences between the
strains of infective organisms undertaken thus reasons
for the high local prevalence of non-inflammatory,
chronic infection remains unclear. With reference to
these earlier findings it seemed likely that many local
children may well have been genetically predisposed to
infection by T.violaceum but adaptation and evolution
of this organism had resulted in an equilibrium within
the infected host being reached. As a result, it is
possible no cell-mediated response was elicited and the
subsequent lack of irritation gave rise to the more
subtle, chronic and continuous form of infection. Only
a single child was infected by another dermatophyte,
M.canis. He, too, presented with scaling only but due
to this singular occurrence no conclusions could be

drawn.

Following the work of Gentles in 1958, which showed the antimycotic agent, griseofulvin to be useful in the treatment of animal ringworm, this drug has become the standard treatment for most forms of human and animal dermatophytoses. Since the advent of the imidazoles, numerous comparative studies have been undertaken to assess their role in the current management regimens. Ketoconazole has most frequently been evaluated, and although random conflicting reports have arisen, it has been widely shown that due to hepatotoxic reactions, long-term cure failures and the interference of rifampicin and isoniazid with this drug, griseofulvin should remain the drug of choice (Gan, 1987; Martinez-Roig,1988; Rippon, 1988; Tanz, 1988). This is of particular importance in the South Western Cape where the incidence of tuberculosis is extremely high. Itraconazole has similarly emerged as a possible replacement therapy for griseofulvin, but, as yet, little information on the long-term effects of this drug on dermatophytoses is available (Hay, 1983).

Topical therapy of tinea capitis is widely accepted as having little benefit in the treatment of this disease. However, the addition of a topical preparation to a more effective treatment regimen e.g. systemic

griseofulvin, has been shown to be beneficial and it has been suggested that some degree of response was indeed achieved by these agents (Allen, 1982; Krowchuk, 1983; Rippon, 1988).

Effective treatment of asymptomatic fungal carriers has long posed a problem to clinicians. Ethical considerations effectively prohibit the use of a registered antifungal drug, albeit with minimal side-effects, for use on asymptomatic, healthy children. Although administration of griseofulvin would appear to be the only certain means of eradication of fungal elements from these children, other factors, including expense and lengthy treatment periods with probable resultant poor compliance, were also prohibitive. As a result of the accelerated treatment periods obtained following the combined selenium-sulphide and griseofulvin regimen used by Allen et al (1982), Selsun shampoo was readily selected as a trial preparation for use on carriers. Allen showed that selenium-sulphide had the property of deposition on the scalp surface, was sporicidal to T.tonsurans and that clinical tinea capitis was eradicated in as little as two weeks following its use in combination. Our study, however, revealed little beneficial effect by Selsun shampoo with the number of carriers in the test group doubling.

Examination of the survey revealed that many factors could possibly have exercised some degree of influence over these disappointing study results. Although all institution supervisors were adult staff members resident in the hostels and appeared to fully comprehend the selenium-sulphide shampooing regimen, adequate supervision must clearly have been difficult. The large numbers of children under the surveillance of a single adult could have resulted in inadequate shampooing technique and even in the overlooking of shampoo applications amongst certain pupils. It was not possible to provide external supervision or assistance for each application. The attitude and willingness of staff was another influencing factor. Staff members reported the standard hair washing procedure to include only a single weekly shampoo. The trial protocol was reported as tedious and time-consuming and this aspect could have affected the enthusiasm and diligence of the supervisors. Supervisors also complained that the more frequent braiding and unbraiding of the girls' hair, necessitated by the additional hair washing, was also time-consuming and not all pupils were readily co-operative. Although all supervisors reported successful administration of the regimen, these factors could have contributed to the poor outcome of this study.

Manufacturers' instructions for the use of the selenium-sulphide preparation recommended allowing the shampoo to remain on the scalp for 2-3 minutes prior to rinsing. In order to standardise and simplify the shampooing regimens, the supplied directions were not adhered to and hair was rinsed immediately following each shampoo application. It was thus suspected that the exposure periods could significantly influence the investigation and a follow-up study was planned.

Due to the extra work incurred by the administration of these investigations, the institutions included in the above-mentioned trial were regretably unwilling to participate in the follow-up study.

As the initial shampooing protocol included no absorption period for selenium-sulphide on the scalp surface, the subsequent studies were designed to allow longer contact periods thus enhancing the adherence potential as described by Allen et al. Although numbers of participating pupils in this study were small (thus precluding statistical analysis) a numerical improvement was noted with a reduced number of carriers following the 5-minute exposure periods to Selsun shampoo. Interpretation of results in the 30-minute exposure group proved difficult. The number of clinical infections was reduced, resulting in an

overall increase in carriers.  Only a single carrier, however, became negative but meaningful interpretation of this result could not be undertaken due to the small numbers examined.  On conclusion of this study, results obtained showed that little advantage was gained by the increased exposure periods.  Further studies would be necessary, however, to substantiate these deductions.

As in earlier investigations, various human factors were also likely to have influenced the outcome of the studies.  Each dormitory was supervised by its own staff member and thus no consistency in the method of administration of the regimens could be obtained.  Supervisors were instructed to implement these procedures and concern was expressed as to the co-operation of the pupils with regard to the lengthy exposure periods, particularly in the 30-minute group.  Similar problems to previous groups with reference to the increased work load due to twice weekly hair washing and the more frequent need for braiding and unbraiding of hair were encountered.  Further prohibitive factors affecting compliance were sporadic side-effects noted by supervisors.  These included burning and pruritis.  These features are documented side-effects of selenium-sulphide shampoos and were noted in the 30-minute exposure group only.

On conclusion of this study, results obtained showed that minimal benefit could be achieved by using selenium-sulphide shampoo and allowing increased exposure periods before rinsing. No further conclusions were drawn due to the numerous variable factors (and the small numbers) influencing the outcome of this investigation. As a result of this and the unsatisfactory side-effects of this product, it was decided to examine the effectivenesss of other topical antifungal preparations.

The principal requirements deemed necessary for further suitable preparations to eradicate fungal carriage in local institutions were ease of application, minimal side-effects, and cost-effectiveness. It was thus decided to concentrate on shampoos for further studies as these were generally cheaper, application was non-invasive and their usage could be assimilated readily into existing hair-care routines.

It is, however, widely accepted that topical treatment of tinea capitis is without benefit and the infection often appears to be unaffected by local application of antifungal preparations. Rippon conceded, however, that the addition of these agents to systemic regimens was an important adjunct to therapy as by this means arthroconidia were killed and infected debris removed,

thereby preventing spread of infections. This
hypothesis suggested that topical agents may well have
an important role to play in the eradication and
control of non-infected, fungal carriers.

Selection of possible agents proved difficult for many
varying reasons. Literature searches revealed few
reports on the effect or efficacy of shampoos on tinea
capitis but showed that fairly extensive studies had
been undertaken on the effects of certain topical
preparations on the fungal infection, pityriasis
versicolor. Although this disease was not strictly
comparable to the dermatophytoses, these reports were
numerous and frequently alluded to the general effects
of various topical preparations on fungal elements.

Selenium-sulphide containing preparations have long
been known to be fungicidal in the treatment of this
superficial yeast infection. In addition, Allen et al
(1982) reported its beneficial effect when used in
conjunction with griseofulvin in the treatment of tinea
capitis. Results of our previous trial, too, suggested
that some degree of benefit could be achieved following
the use of this shampoo by fungal carriers.

Manna et al (1984) showed that povidone-iodine paint
(Betadine) also had a beneficial effect when used to

treat pityriasis versicolor but they also observed some curative effect in two cases of T.rubrum infection. Similarly, various topical imidazole preparations (e.g. Miconazole and Bifonazole) were reported to be of some benefit in the treatment of pityriasis versicolor and various dermatophytoses (Wright, 1986; Soyinka, 1987).

In spite of the poor correlation of these earlier studies with our proposed study to be undertaken on tinea capitis carriers, these reports, claiming some degree of positive response to the above-mentioned preparations, gave some encouragement. Thus the agents finally selected for investigation included a povidone-iodine containing shampoo, Betadine; an imidazole (econazole nitrate) containing body shampoo, Pevaryl; and a shampoo containing selenium-sulphide, namely Selsun. Johnson's Baby shampoo was selected for use as the control as it was not known to contain any antifungal properties.

Following random allocation to all schools, and a standardised regimen allowing shampoos to remain in contact with the scalp for 15 minutes, the overall comparative effect of the shampoos showed that some degree of benefit i.e. a reduction in the number of culture-positive children, was obtained by the use of each shampoo. Numerical trends showed that similar

results were achieved following shampooing with the econazole nitrate, selenium-sulphide and the control preparations. Statistical analysis of these results, however, showed no significant improvement had occurred. An average of 14% of patients using these three shampoos showed a decrease in fungal load, infected children becoming either carriers or negative and those categorised as carriers becoming negative.

In comparison, the overall effect achieved following the use of the povidone-iodine was an 85% reduction in culture positive children. Statistical analysis confirmed the significance of these results (p<0,001).

The conclusions drawn from these results suggested that the less effective reduction in fungal load achieved by the former preparations was largely due to to the improved personal hygiene and the physical removal of fungal debris from the scalp. The significantly greater reduction following the use of povidone-iodine suggested that, in addition to the removal of spores, possible fungistatic or fungicidal properties were active. These results strongly supported opinion that this preparation would be a suitable product to include in a standard regimen used routinely in institutions as a prophylactic measure to reduce the number of carriers, and thus ultimately the number of scalp

ringworm infections.

When considering the effects of the preparations on carriers alone, it was shown that an average of 61,7% of the colonised patients became negative following treatment with econazole nitrate, selenium-sulphide and the control shampoos. In contrast, fungal carriage was erradicated in 100% of colonised children in the povidone-iodine group, further emphasising the increased effectiveness of this preparation over the remaining three shampoos.

As in previous trials, the human factor was likely to have had some degree of influence on the final outcome of this survey. Both the degree and standard of compliance were areas over which little control could be exercised. Although all project supervisors reported problem-free implementation of the regimen, all schools appeared to have slightly differing standards of personal hygiene with greater general supervision and involvement of staff members noted in some dormitories. To confirm reported findings and to establish the effect of differing degrees of compliance by the various schools, "cross-over" trials were planned. The participating schools, however, were unwilling to repeat any shampooing regimen due to the heavy demands of its implementation on staff members.

This unwilling attitude further questioned the reportedly successful administration of the regimen and suggested that the twice-weekly shampooing of hair, together with a 15-minute waiting period, would not be acceptable as a standard prophylactic measure to reduce fungal carriage. The total success of the implemented povidone-iodine regimen, however, suggested that further investigation, utilising shorter exposure periods or less frequent hair washes (or both), could also result in a similarly beneficial outcome.

To verify the in vivo trial findings and in the absence of the proposed "cross-over" investigation results, the cultural and ultrastructural examination of spores of T.violaceum following comparable exposure periods to the shampoos was undertaken. A modified version of the rapid and simple screening test for determining the effects of antimycotic agents as described by Gip et al (1984) was used to assess the viability of spores and their response to the shampoos. Daily examination by light microscopy showed restricted germination induced by the econazole-nitrate and selenium-sulphide preparations and completely suppressed growth by the povidone-iodine shampoo following 15-minute exposures. Normal germination was noted following treatment with the control shampoo. Comparison of these results with the in vivo findings showed a similar outcome with the

notable exception of the control shampoo. The _in vivo_ study showed similar results following the use of the econazole nitrate, selenium-sulphide and control shampoo. This finding suggested that the reduction in fungal load obtained by the use of these three preparations was indeed largely due to the physical removal of fungal debris in response to improved hair hygiene. It appeared that the effect of the antifungal properties on the spores of _T.violaceum_ was minimal, if any. The complete lack of growth following exposure to povidone-iodine, however, was in keeping with the earlier study results which showed virtual eradication of fungi from children in this group.

Although results following the longer 30-minute exposures were not directly comparable to the _in-vivo_ findings, this period was included to investigate the possibility of improved results following extended exposure periods. Suppressed growth, observed by the microscopic appearance of favic forms, was noted with selenium-sulphide and the control shampoo. Total suppression of growth was demonstrated following use of econazole nitrate and povidone-iodine. All cultures showed some degree of inhibitory influence and it appeared that increased exposure periods would enhance the effects of all preparations, improving the end result.

To further examine the ultrastructural effects of the shampoos on the spores of _T.violaceum_ all samples examined above were submitted for transmission electron microscopy. Untreated spores of _T.violaceum_ were used as controls and structures and organelles as documented by previous investigators were identified (Yuko Ito, 1967). On ultrastructural examination of fungal cultures following shampoo exposures, it became apparent that all shampoos effected some degree of alteration to spores of _T.violaceum_. These results were more pronounced following the 30-minute treatments but effects were noted even following the 15-minute group.

Examination of micrographs revealed three predominant areas of ultrastructural alteration to spores. These included degeneration of the cell wall, cytoplasmic contents and general morphology. These findings were in keeping with similar reports on the effects of imidazoles on dermatophytes as well as _Candida albicans_ (Iwata, 1973; Borgers, 1987). Ultrastructural examination in this study revealed that 15-minute exposure periods were inadequate for optimal effect of all shampoos. All preparations, including the control, showed similar mild, degenerative changes affecting all the above-mentioned categories. Correlation of these results with _in-vivo_ trial results show similar

comparable effects for econazole nitrate, selenium-sulphide and the control shampoo but the findings for povidone-iodine contrasted sharply. Highly significant reductions in fungal load were noted in in-vivo surveys using this preparation but only mild changes, similar to those found with the remaining shampoos, were noted ultrastructurally. In spite of this finding, however, earlier cultural investigations of these spores revealed them to be non-viable, while those treated with the remaining three shampoos remained viable. Following the 30-minute exposure periods, similar mild to moderate degenerative changes were noted with econazole nitrate, selenium-sulphide and the control shampoo while gross degeneration was noted following treatment with povidone-iodine. These findings correlate well with the earlier in-vivo studies and cultural examinations.

Numbers of specimens examined in this cultural and ultrastructural investigation were small and statistical analysis was thus precluded. Various factors which may have influenced the final results were also noted. Control spores of T.violaceum were not immersed in a liquid medium for the trial time periods i.e. 15- and 30-minutes. The possible effects of this procedure on the cell wall and its permeability, apart from the influence of the

antifungal properties, was thus not taken into account.
Similarly the shampoos were tested in undiluted form
thus not accurately reproducing the in-vivo study
conditions. The dilution factor of the preparations
following application to wet hair was not reproduced in
the in-vitro investigations. This could possibly have
influenced these in-vitro results by slightly
exaggerating the effects of the products.

After conclusion of these shampooing surveys it
appeared that all studies had produced similar
end-results. All early findings had been verified by
the follow-up investigations and in summary it appeared
that, although the improvement of personal hygiene in
the form of regular hair washing would be of some
benefit in reducing the number of fungal carriers in
the community, the regular use of one of the tested
shampoos would further enhance these effects. To
achieve optimum results, however, repeated use of the
povidone-iodine containing shampoo appeared to
significantly reduce, if not eradicate, the fungal load
of ringworm carriers. Thus the ultimate goal of this
project appears to have been realised with the
identification of an easy to use, topical preparation
which could readily be assimilated into existing hair
care regimens of institutions and schools experiencing
an "endemic" tinea capitis problem.

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