hydrogen bonds may well prove as important as cleavage of disulphide bonds in investigating load-rotation curves of hair fibres.

(*Received : 24th November 1958*)

REFERENCES

[1] Speakman, J. B. *Melliand Textilber.*, **33** (1952) 823.
[2] Fuhrmann, W. *Melliand Textilber.*, **33** (1952) 911.
[3] Zahn, H. *Textil-Praxis*, **9** (1954) 515.
[4] Richter, R., and Fuhrmann, W. *Arch. Dermatol u. Syphilis (D)*, **198** (1954) 274.
[5] Astbury, W. T., and Woods, H. J. *Phil. Trans. Roy. Soc. London. Ser. A.*, **232** (1933) 333.
[6] Harrison, W. *J. Textile Inst.*, **28** (1937) 110.
[7] Pauling, L., Corey, R. B., and Branson, H. R. *Proc. Nat. Acad. Sci. U.S.*, **37** (1951) 205.
[8] Hirsch, F. *Kosmetik-Parfum-Drogen Rundschau* (1955) (7/8) 81.
[9] Hirsch, F. *Das Haar des Menschen in Gesundtheit und Krankheit unter spezieller Berücksichtigung der Keratinchemie* (1956) (Haug-Verlag, Ulm).
[10] Hirsch, F. *Heilkunst*, **65** (1952) 2.
[11] Heilingötter, R. *Kosmetik-Parfum-Drogen Rundschau* (1956) (9/10) 121.
[12] Heilingötter, R. *Am. Perfumer Aromat.*, **68** (1956) (November) 17.
[13] Mercer, E. H. *Textile Research J.*, **23** (1953) 388.
[14] Hirsch, F. *Angew. Chem.*, **67** (1955) 715.
[15] Hirsch, F. *Beitrag zur Aufklärung der Keratinstruktur* (1952). Dissertation, München.
[16] Hirsch, F. *Münch. med. Wochschr.*, **42** (1951) 2105.
[17] Hirsch, F. *Münch. med. Wochschr.*, **44** (1953) 1175.
[18] Hirsch, F. *J. Medizinische Kosmetik*, (1955) 155.
[19] Crick, F. H. C. *Nature*, **170** (1952) 882.
[20] Pauling, L., and Corey, R. B. *Nature*, **171** (1953) 59.
[21] Pauling, L. *Angew. Chem.*, **67** (1955) 244.
[22] Goldworthy, J. E., and Lang, J. *J. Textile Inst., Trans.* **44** (1953) 230.
[23] Huggins, O. O. *Proc. Nat. Acad. Sci. U.S.*, **43** (1957) 204.

THE EFFECT OF DECAMETHYLENE-BIS-4-AMINOQUINALDINIUM (DEQUALINIUM) SALTS ON THE GROWTH OF *PITYROSPORUM OVALE*

P. F. D'ARCY, B.PHARM., PH.D., M.P.S.; W. A. COX, B.Sc.; M. J. HEDGE, B.PHARM., F.P.S., and G. R. WILKINSON, F.P.S.*

Dequalinium salts restrict the growth of *Pityrosporum ovale* and other micro-organisms contaminating skin and hair. Preparations of Dequalinium are highly effective in the treatment of seborrhoea and infective dandruff.

INTRODUCTION

DANDRUFF IS characterized by the presence of loosely adhering scales on the scalp; the scales are flakes of the stratum corneum or horny layer of the

* Research Division, Allen & Hanburys Ltd., Ware, Herts.

skin; frequent brushing, combing or more often scratching will remove these. The latter procedure, however, frequently damages the surface of the scalp and leads to associated skin infections. Microscopical examination of dandruff scales often reveals the presence of flask-shaped micro-organisms. These were first recognized by Malassez[1], and were incorrectly identified as bacteria; further studies have established that this organism, *Pityrosporum ovale*, is a yeast-like fungus.

There is a great deal of contentious opinion as to whether *P. ovale* is the causative organism of dandruff or whether it is merely a harmless saprophyte of human scurf. Sabouraud[2] held quite firmly that *P. ovale* was the cause of dandruff or pityriasis simplex of the scalp, whereas Darier[3] was of the opinion that the organism was in no way responsible for the disease; it merely flourished on the fertile environment of the kerose. Macleod and Dowling[4] cultured an organism which they thought to be *P. ovale*, and believed to be the cause of dandruff of the scalp and seborrhoeic eczema of the skin. However, it was later acknowledged by Dowling[5] that this organism was not *P. ovale* but *M. pinoyi*. Rocha *et al.*[6] examined 50 adult patients and were unable to show that *P. ovale* was pathogenic, and furthermore were unable to demonstrate any allergic reactions in 200 others when inoculated intracutaneously with an antigen prepared from this organism. Whitlock[7] has reviewed a great deal of the earlier work and in his own investigations found that there was no evidence that *P. ovale* was the causative organism of dandruff; he concluded that it was a harmless organism on the scalp, thriving in the medium provided by a heavy flow of sebum. On the other hand, Barber[8] concluded that *P. ovale* was the cause of simple dandruff, and Reddish[9] held that *P. ovale* fulfilled all the requirements of Koch's postulates to prove it was an infectious organism, namely

(*a*) it was present in every case of infectious dandruff,

(*b*) it had been isolated from infectious dandruff and grown in pure culture,

(*c*) when inoculated in pure culture into rabbit skin or human scalp it caused infectious dandruff, and

(*d*) it could again be recovered in pure culture from these cases of experimental infectious dandruff.

Recently, Hughes and Hamilton[10] have shown that *P. ovale* is the cause of an allergy to human scurf and that the conditions of eczema, rhinorrhoea and asthma frequently found in association with dandruff are in fact caused by the development of a skin sensitivity to the presence of this fungus. These allergic reactions can be caused not only by the fungus infected scales from the sufferer's own scalp but also by those from other contacts. These authors also drew attention to the extreme difficulty of treating *P. ovale* skin

sensitization because of the widespread contamination of clothing, bedding and upholstery with the fungus and its spores, which serve as a reservoir for re-infection of the individual or as a source of infection to others.

Whether *P. ovale* is a specific infective agent causing dandruff, or whether it be a saprophyte causing allergic skin reactions, it is very certain that its continued presence on the human scalp is far from desirable. This present work was therefore initiated to determine the effects of Dequalinium salts on the growth of this fungus. It has been established that these quaternary ammonium compounds have potent anti-microbial activity against a wide spectrum of pathogenic fungi and bacteria, and are without toxic effect when applied to the skin and eyes of experimental animals[11]. In clinical observations Coles *et al.*[12] have shown that Dequalinium is highly effective in the treatment of dermatological conditions and is not irritant or toxic to skin or mucous membranes.

Experimental Methods and Results

Pityrosporum ovale was cultured for 3 days on malt extract agar at a temperature of 37° C and thereafter maintained at room temperature. Due to the lipophilic nature of this fungus the media was enriched with 0·05 per cent sterile cream. Initial exploratory experiments were then carried out using this and other culture media to determine the optimal conditions for evaluating the fungistatic activity of Dequalinium against *P. ovale.*

In the first experiments the fungistatic activity of Dequalinium was

TABLE 1

The inhibitory activity of Dequalinium chloride against *Pityrosporum ovale* in different liquid culture media.

Culture medium	Added Fat Source	Geometric mean M I.C. in μg/ml at 7 days
Modified Curries medium	None	insufficient growth
Modified Curries medium	oleic acid 0·5 per cent	insufficient growth
Modified Curries medium	sterile cream 0·05 per cent	insufficient growth
Sabouraud's broth	None	insufficient growth
Sabouraud's broth	oleic acid 0·5 per cent	> 200
Sabouraud's broth	sterile cream 0·05 per cent	6·25
Malt extract broth	None	insufficient growth
Malt extract broth	oleic acid 0·5 per cent	> 200
Malt extract broth	sterile cream 0·05 per cent	5·1

examined by a cup plate method on malt extract agar, to which sterile cream had been added. The plates were flooded with sterile cream diluted 1 in 20 with normal saline, re-dried and then inoculated with a 3 day old culture of the fungus. After 3 and 7 days incubation at 37° C it was found that Dequalinium chloride had not inhibited the growth of the fungus at concentrations extending to 400 μg/ml. This absence of activity was thought to be due to the inactivation of Dequalinium by the agar culture media. Further experiments were performed by tube dilution methods in a modification of Curries medium[13] and also in two other broth culture media. The results of these experiments are summarized in *Table 1*, which shows the composition of each culture medium, the concentration of added lipoid and the minimum inhibitory concentration (M.I.C.) of Dequalinium chloride in μg/ml.

It is apparent from these results that the fungistatic activity of Dequalinium cannot be evaluated in a medium containing agar. From the experiments using liquid media it is clear that a lipoidal substance must be added to the culture fluid to permit the normal growth of the fungus ; the addition of 0·05 per cent sterile cream or 0·5 per cent oleic acid would appear to satisfy the nutrient requirements of the fungus ; however, oleic acid markedly inhibits the activity of Dequalinium chloride.

A malt-extract broth containing 0·05 per cent sterile cream in emulsified form was used in later experiments. This medium was selected from those liquid media found to be suitable because it consistently supported a luxurious growth of the fungus and did not noticeably reduce the fungistatic properties of Dequalinium.

Dequalinium chloride and Dequalinium stearate were then examined for their activity against *P. ovale*. These results are summarized in *Table 2*, which lists the M.I.C. value for the salts in μg/ml.

TABLE 2

The inhibitory activity of some Dequalinium salts against *Pityrosporum ovale*.

Compound	Culture Medium	Added Fat Source	Geometric mean M.I.C. in μg/ml at 7 days
Dequalinium chloride	Malt-extract broth	Sterile cream 0·05 per cent	5·1
Dequalinium stearate	Malt-extract broth	Sterile cream 0·05 per cent	8·8

Both the salts were found to show marked fungistatic activity against the organism.

Infectious dandruff of the scalp is in many cases further complicated by

the presence of other bacteria and fungi in the hair and skin. Dequalinium was therefore examined for its anti-microbial activity against some of these organisms ; the results are shown in *Table 3*, which lists the M.I.C. value for Dequalinium in μg/ml.

TABLE 3
The inhibitory activity of Dequalinium against certain micro-organisms of hair and skin.

Micro-organism	Geometric mean M.I.C. in μg/ml at :	
	5 days	14 days
Staphylococcus aureus	0·20	—
Staphylococcus saprophyticus	0·28	—
Sarcina lutea	0·17	—
Streptococcus pyogenes	1·10	—
Escherichia coli	9·50	—
Bacillus subtilis	2·50	—
Proteus vulgaris	>100	—
Candida albicans	0·63	—
Microsporum canis	—	0·60
Trichophyton mentagrophytes	—	<0·08
Trichophyton rubrum	—	0·59
Trichophyton verrucosum	—	1·67

Since Dequalinium exerts its anti-microbial action not by killing the micro-organism but by preventing its growth, it is obvious that the time of contact between drug and hair is of importance, therefore the degree of adsorption of the compound by hair was studied. Samples of human hair were allowed to soak for varying times in graded concentration of Dequalinium ; the hair samples were then washed in distilled water and allowed to dry for 30 min in warm air. The amount of the compound adsorbed on to the hair was then evaluated by determining the bacteriostatic activity of the hair sample against *S. aureus*, after 24 hours' incubation at 37° C. These results are shown in *Table 4*.

TABLE 4
The adsorption of Dequalinium by samples of human hair. Results expressed as μg. Dequalinium per mg. of hair.

Dequalinium concentration per cent	Contact time min.		
	10	30	60
4·0	2·5	2·5	2·5
2·0	1·25	1·25	1·25
1·0	0·62	0·62	1·25

Discussion

It is very evident that in these *in vitro* experiments Dequalinium salts are highly effective in restricting the growth of both *P. ovale* and the many micro-organisms associated with hair and skin. In order that Dequalinium preparations could be tested in cases of seborrhoea and infective dandruff, a study was made into the formulation of preparations which would be capable of conveying the quaternary ammonium compound in a sufficiently high concentration, and from which Dequalinium would be freely absorbed by the hair and the tissues of the scalp.

Dequalinium salts are highly reactive and therefore somewhat difficult to incorporate into anti-dandruff preparations; for example, the bacteriostatic activity of Dequalinium is reduced in the presence of some soaps and anionic surface active agents. However, this activity is retained, and may even be potentiated in the presence of certain non-ionic or cationic detergents. A hair shampoo containing Dequalinium chloride 0·25 per cent with a non-ionic detergent and a suitable hair conditioner, such as a long chain fatty acid, has been found to be acceptable from both anti-bacterial and cosmetic aspects. The shampoo has high anti-bacterial activity *in vitro* and initial studies have shown that it is highly effective when tested clinically in cases of seborrhoea and infective dandruff[14].

Even when the infection is eradicated, there is still a danger of re-infection of the scalp with *P. ovale* due to further contact with the fungus and its spores. Prophylactic treatment carried out at regular intervals is therefore the only really effective means of control. An anti-dandruff lotion or cream, which at the same time serves as a hair dressing and fixative, is a very convenient means of effecting this treatment. The formulation of two such preparations has been investigated and a hair lotion has been prepared containing 0·4 per cent Dequalinium stearate solubilized in mineral oil, and an oil in water emulsified hair cream containing Dequalinium chloride 0·4 per cent has been formulated with Collone N.I.

Conclusions

The results reported in this work show that Dequalinium salts are highly effective in restricting the growth of *P. ovale in vitro*; in addition, they exert considerable anti-microbial activity against the organisms contaminating skin and hair. Clinical and experimental observations reported elsewhere[11,12,14] have shown that repeated applications of Dequalinium to skin and mucous membranes are without toxic or irritant effect and that preparations are highly effective when tested clinically in cases of seborrhoea and infective dandruff.

Acknowledgements

We thank the Directors of Allen and Hanburys, Ltd. for permission to

publish this work, and Mr. E. L. Robins and Miss J. A. Moore for their assistance.

The organism used in the experiments was kindly supplied by Miss P. Stockdale, of the Mycological Reference Laboratory, London School of Hygiene and Tropical Medicine.

[*Received : 25th May 1959*]

REFERENCES

1 Malassez, L. *Arch. de physio. norm et path.*, **1** (1874), 203.
2 Sabouraud, R. *Maladies du Cuir Chevelu* (1902) (Paris).
3 Darier, J. Pollitzer, S. transl. *Precis de Dermatologie* (1920). (Lea and Febiger, Philadelphia.)
4 Macleod, J. M. H., and Dowling, G. B. *Brit. J. Dermatol.*, **40** (1928), 139.
5 Dowling, G. B. *Brit. J. Dermatol.*, **51** (1939), 1.
6 Rocha, G. L., Silva, C., Lima, A. O., and Goto, M. *J. Invest. Dermatol.*, **19** (1952), 289.
7 Whitlock, F. A. *Brit. Med. J. i* (1953), 484.
8 Barber, H. W., in MacKenna, R. M. B. *Modern Trends in Dermatology* (1948). (Butterworths Scientific Publications, London.)
9 Reddish, G. F. *J. Soc. Cosmetic Chemists*, **3** (1952), 90.
10 Hughes, H. W., and Hamilton, E. D. *Brit. Med. J. ii* (1958), 542.
11 Babbs, M., Collier, H. O. J., Austin, W. C., Potter, M. D., and Taylor, E. P. *J. Pharm. and Pharmacol.*, **8** (1956), 110.
12 Coles, R. B., Grubb, C., Mathuranayagam, D., and Wilinson, D. S. *Brit. Med. J. ii* (1958), 1014.
13 Benham, R. W. *Proc. Soc. Exp. Biol. Med.*, **58** (1945), 199.
14 Colin-Jones, E. Private communication (1959).

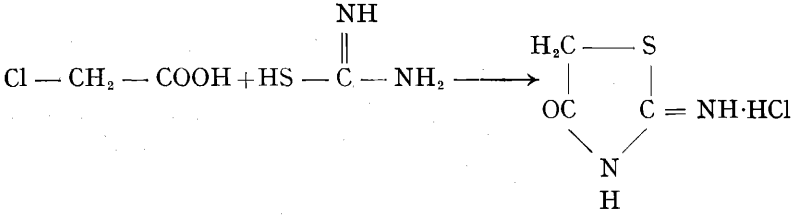
ÜBER DIE SPALTUNG DER SENFÖLESSIGSÄURE MIT AMMONIAK UND AMINEN

(The effect of ammonia and amines on 2,4-thiazoledione)

G. A. ERLEMANN, Dr. rer. nat. and H. KNETSCH, Dipl. Chem.*

According to the authors, thioglycollic acid and thioglycollic acid amide are formed when 2,4-thiazoledione is treated with ammonia.

DIE SENFÖLESSIGSÄURE wurde zuerst von Volhard[1] durch Einwirkung von Chloressigsäure auf Thioharnstoff dargestellt :



(1)

* Schwarzkopf, Hamburg-Altona, Germany.