THE ACTIVITY OF POLYMETHYLENE-BIS-4-AMINO-QUINALDINIUM SALTS AGAINST *PITYROSPORUM OVALE* AND *CANDIDA ALBICANS*

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A homologous series of polymethylene-bis-4-aminoquinaldinium salts has been shown to possess marked fungistatic activity against the potential pathogens *Candida albicans* and *Pityrosporum ovale*. The peak of antifungal activity against *C. albicans* occurs at a chain length $(CH_2)_{12}$, whilst against *P. ovale* the most active member of the series has a chain length $(CH_2)_6$. The decamethylene member (dequalinium), shows a potent and rapid fungicidal activity against both yeasts; quantitative data, from experiments involving short periods of drug-yeast contact, indicates that this activity of dequalinium is a function of the concentration of the quaternary compound and of the time of exposure.

THE polymethylene-bis-4-aminoquinaldinium salts (B.A.Q.D.) have been reported by Babbs, Collier, Austin, Potter and Taylor (1956) to have potent antibacterial activity, of this series the decamethylene member, dequalinium, has been found to be highly effective as a topical antibacterial agent (Babbs and others, 1956; Collier, Cox, Huskinson and Robinson, 1959).

In this present work dequalinium and some other members of this series have been examined for their activity against two yeasts, *Pityrosporum ovale* and *Candida albicans*.

Pityrosporum ovale has been isolated from human scurf and although regarded by some workers to be the causative agent of dandruff (Sabouraud, 1902; Macleod and Dowling, 1928; Barber, 1948; Reddish, 1952), it is held by others to be a mere harmless saprophyte (Whitlock, 1953; Rocha, Silva, Lima and Goto, 1952). However, Hughes and Hamilton (1958) have recently shown that this fungus is the cause of an allergy to human scurf, and in fact responsible, to a considerable degree, for the conditions of eczema, rhinorrhoea and asthma frequently found in association with dandruff.

Candida albicans has long been known as one of the more common causes of superficial fungus infections, and is responsible for the varied conditions commonly classified under the general terminology of moniliasis.

EXPERIMENTAL

Materials and Methods

Fungistatic Activity

P. ovale. Serial twofold dilutions of test compounds were prepared in malt extract broth with an added fat source (0·05 per cent sterile cream). Inoculations were made with a standard suspension of the fungus grown on malt extract agar with added cream. The tube dilutions were incubated for 7 days at 37° and the minimum inhibitory concentration was determined for each compound both by visual observation and by subculture.

C. albicans. Double strength aqueous solutions of the compounds were prepared and mixed with double strength Sabouraud's broth (Glucose 4 per cent; A. & H. Eupeptone No. 2 1·0 per cent). Twofold serial dilutions were made each of a final volume of 2 ml., these were sterilised by autoclaving at 10 lb./sq. in. for 10 min. Tubes were inoculated with 0·02 ml. of an 18-hr. broth culture of *C. albicans*, grown at 37° and standardised to an arbitrary opacity (approx. 500,000 cells/ml.); the tubes were then incubated at 37° and the minimum inhibitory concentration for each compound was determined visually after 7 days.

Fungicidal Activity

P. ovale. The fungus was cultured for 3 days at 37° on slopes of tauroglycocholate agar (sodium tauroglycocholate 10 per cent; Oxoid mycological peptone 5 per cent; agar 1·5 per cent, adjusted to pH 5·0). A washed cell suspension was prepared in sterile distilled water to a

TABLE I

FUNGISTATIC ACTIVITY OF B.A.Q.D. SERIES AGAINST *P. ovale* AND *C. albicans*

Polymethylene chain length	M.I.C. μg./ml. base	
	P. ovale	*C. albicans*
B.A.Q.D. 4	4·4	>100
6	2·2	50
8	6·3	2·5
10	8·8	1·25
12	—	0·32
14	25	0·63
16	—	2·5
18	35	10
20	100	10

standard turbidity. The number of viable units/ml. of suspension was determined by plating out after suitable dilution following the technique described in detail below.

Culture suspensions (approximately 5×10^5 viable cells/ml.) were mixed with solutions of test compounds for contact times of 1, 5 or 30 min.; samples were then withdrawn and inactivated by Lubrol W (polyethylene oxide condensate), 1 ml. of the sample was diluted × 10 by a 1 per cent solution of this inactivator, and plated out on to tauroglycocholate agar plates following the technique of Miles and Misra (1938). The plates were incubated at 37° for 48–60 hr. and the colonies of fungi counted. The fungicidal activity of a specific concentration of test compound at a stated time of contact was expressed as a mean percentage kill.

C. albicans. The yeast was grown on Sabouraud's agar at 37° for 18 hr.; the growth was then suspended in water, centrifuged (R.C.F. 600 *g.*/10 min.) and resuspended in distilled water to produce a standard opacity of approximately 1×10^7 viable cells/ml. (Wellcome opacity tube 5).

An inoculum of 1·0 ml. of this washed cell culture was added to 9·0 ml. of an aqueous solution of the test compound; samples of 1·0 ml. were withdrawn after contact times of 1, 5 or 30 min. and were inactivated by pipetting into 9 ml. of a 2·0 per cent oxgall solution (Difco). The number

of surviving yeast cells were determined by a plating out technique in Sabouraud's agar; the number of resulting colonies was counted after incubation at 37° for 3 days.

Fungicidal activity was expressed by the percentage loss of viability when compared with the control count of the standardised yeast suspension.

RESULTS

The fungistatic activity of compounds B.A.Q.D. 4, 6, 8, 10, 14, 18 and 20 against *P. ovale* and compounds B.A.Q.D. 4, 6, 8, 10, 12, 14, 16, 18 and 20 against *C. albicans* is summarised in Table I. The compounds are listed according to the length of their polymethylene chain, and the minimum inhibitory concentration for each member of the series is expressed in μg./ml. calculated as the base.

TABLE II

FUNGICIDAL ACTIVITY OF B.A.Q.D. 10 AGAINST *P. ovale*

Concentration per cent	Contact min.	Mean kill per cent*
0·01	1	100
0·01	5	100
0·01	30	100
0·005	1	98·083
0·005	5	99·833
0·005	30	100
0·004	1	92·115
0·004	5	98·404
0·004	30	100
0·0025	1	87·886
0·0025	5	98·688
0·0025	30	100
0·001	1	33·579
0·001	5	61·599
0·001	30	100

* *P. ovale* suspension 5×10^5 viable cells/ml.

There is a well defined gradation of activity within the B.A.Q.D. series against both fungal species. Potent fungistatic activity against *P. ovale* is shown by compounds B.A.Q.D. 4, 6, 8 and 10 and is maximal with the hexamethylene member (B.A.Q.D. 6). Compounds B.A.Q.D. 8, 10, 12, 14 and 16 exhibit high activity against *C. albicans*, and this effect is maximal with compound B.A.Q.D. 12.

Fungicidal tests were confined to a study of the activity of the decamethylene member of the series. Dequalinium was selected from those compounds showing good fungistatic activity because of its potency against other microbial species (Babbs and others, 1956, Collier and others, 1959).

The fungicidal activity of dequalinium against *P. ovale* and against *C. albicans* is shown in Table II and in Table III respectively; fungicidal activity is expressed by the figure for the mean percentage kill. In the former tests the compound was examined at concentrations from 0·001 to 0·01 per cent and in the latter 0·001 to 0·02 per cent at drug-organism contact times of 1, 5 and 30 min.

The results of the fungicidal tests show that dequalinium is 100 per cent effective against *P. ovale* at a concentration of 0·01 per cent for a contact time of 1 min., and has the same activity against *C. albicans* at a concentration of 0·02 per cent for 1 min. This fungicidal action is also evident at lower concentrations of the compound when the contact time is

TABLE III

FUNGICIDAL ACTIVITY OF B.A.Q.D. 10 AGAINST *C. albicans*

Concentration per cent	Contact min.	Mean kill per cent*
0·02	1	100
0·02	5	100
0·02	30	100
0·01	1	99·702
0·01	5	99·999
0·01	30	100
0·005	1	99·162
0·005	5	99·947
0·005	30	99·977
0·0025	1	71·833
0·0025	5	96·660
0·0025	30	99·979
0·001	1	25·882
0·001	5	61·176
0·001	30	84·705

* *C. albicans* suspension 1 × 10^6 viable cells/ml.

increased. Although both fungal species show a very similar degree of sensitivity to dequalinium, it would appear that the compound, at low concentrations, is more effective against *P. ovale* than against *C. albicans.*

DISCUSSION

Candida species and *P. ovale* have been regarded by most taxonomists as being sufficiently closely related to be included together in the subfamily Cryptococcoideae of the yeasts, although below this level of classification the morphology and physiology of *C. albicans* and *P. ovale* are different in a number of respects; they differ, for example, in their ability to ferment sugars and to utilise ammonium salts as a source of nitrogen. Certain species of *Candida* notably *C. lipolytica* and *C. rugosa* appear to lie intermediate between *C. albicans* and *P. ovale* in that they utilise fats for growth and are unable to ferment sugars. In view of these points of dissimilarity between the two species studied it is interesting to note that the peak of fungistatic activity of the B.A.Q.D. series is n = 6 against *P. ovale* and n = 12–14 against *C. albicans.* This peak of activity against *P. ovale* is surprising in view of the previous studies on the bacteriostatic and fungistatic activity of this series which reveal a general pattern of maximal activity between the decyl and the hexadecyl members (Babbs and others, 1956; Cox and D'Arcy, 1959). It would seem likely that this difference in the most effective length of the polymethylene chain could be attributed to either an essential difference in the distribution of the receptor groups, susceptible to these quaternaries, or differences in lipid solubility between *P. ovale* and other fungi including *C. albicans.*

Dequalinium, which showed good activity against both species in the fungistatic tests, was examined for fungicidal activity because it has been shown to be effective in the treatment of local infections with *C. albicans* (Coles, Grubb, Mathuranayagam and Wilkinson, 1958; Stockdale and Banks, 1959; Roddie, 1958; Levinson, 1959), and in initial clinical investigations has proved highly effective in cases of seborrhoea and infective dandruff (Colin-Jones private communication). Some initial laboratory observations on the effects of dequalinium salts on the growth of *P. ovale* have been published elsewhere (Cox, D'Arcy, Hedge and Wilkinson, 1960).

Dequalinium has potent fungicidal activity against both species, the slightly more potent effect against *P. ovale* is in direct contrast to the fungistatic results, in which dequalinium is about eight times more active against *C. albicans* than against *P. ovale.*

This anomaly may be partly explained by the inactivation of this type of quaternary ammonium compound by the fat source in the media, which is essential for the growth of *P. ovale.* Because of this, it was in fact, necessary to perform the fungistatic tests on this series of compounds in media, in which the constituents did not neutralise antifungal activity. Media containing bile salts (Martin Scott, 1952), and fatty acids (oleic acid) were found to be unsatisfactory whilst a malt extract media with added cream was found to be the most suitable.

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