

Antifungal Properties of the Polymyxins. (19300)

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Previous investigations with the various polymyxins have established the marked effectiveness of these antibiotics against Gram-negative bacteria(1-3). In view of the antifungal properties possessed by certain aliphatic fatty acids, the isolation of a fatty acid component from the polymyxins(4) suggested a study of these antibiotics for similar properties. Identification of the acid as an isomer (6-methyl-octan-1-oic acid) of pelargonic acid (5) added further interest in light of the work reported by Rothman and co-workers(6,7), who found pelargonic acid to be quite prominent in a group of highly active normal aliphatic monobasic acids isolated from hair fat of adults.

In the present study, the relative antifungal activities have been determined for polymyxins A, B, D and E. Several known antifungal agents were included for purpose of comparison. While the investigation was in progress, Serri(8) reported on the activity of areosporin (polymyxin B) against a number of dermatomycetes. The antibiotic showed greatest activity against *Achorion schoenleini*, was less active but equally so against *Trichophyton gypseum asteroides*, *Microsporium audouini*, *Microsporium felineum* and *Sporotrichum gougeroti*, and least active against *Trichophyton rubrum*, *Trichophyton crateriforme* and *Achorion quinqueanum*.

Experimental. Preliminary determinations of the antifungal properties of the polymyxins* were carried out by means of the agar cup-plate method of Burlingame and Reddish (9). A serial dilution technic was used to ascertain fungicidal activity. Known antifungal agents included in the study were: benzoic acid, salicylic acid, undecylenic acid,

G-4† and Asterol‡ *Fungistatic procedure.* Sabouraud's maltose agar was used in the agar cup-plate method, each agent being tested in plain medium and in the medium containing 10% normal horse serum. The diameter of the cup was 1.5 cm. Inocula consisted of 5-day-old cultures of the respective test organisms, *i.e.*, *Candida albicans*, *Trichophyton mentagrophytes* and *Debaryomyces neoformans*. Solutions of each agent were made up at a concentration of 0.1% in 95% ethyl alcohol, from which 0.1 cc was pipetted into the cup. Control tests on the alcohol solvent were included in each run. Plates were covered with unglazed clay tops and incubated for 5 days at 28°C. The extent of growth inhibition was measured in millimeters from the edge of the cup to the periphery of the cleared zone. *Fungicidal procedure.* The medium consisted of 1% neopeptone and 4% maltose, adjusted to pH 5.6. As in the fungistatic procedure, determinations were made in both plain medium and in broth containing 10% normal horse serum. Stock solutions of the respective test materials were prepared in 95% ethyl alcohol at a concentration of 0.8%. Subsequent dilutions were made aseptically in tubes of broth so that each drug was assayed at a maximum of 25 mg %, with lower concentrations decreasing by halves. Each tube contained a final volume of 10 cc of medium plus drug at the various dilutions. Although controls on the alcohol solvent were included in each series of tests, it was found in repeated determinations that the concentration of ethyl alcohol in broth necessary to inhibit growth of the various test organisms safely exceeded that present in the initial tube of the dilution series.

The following organisms were used in the fungicidal tests: *Candida albicans*, *Trichophy-*

* Polymyxin B as the sulfate was kindly supplied by Chas. Pfizer and Co. and assayed at 7625 U/mg. The remaining polymyxins were prepared in our laboratories as the hydrochlorides, by Dr. H. A. Nash with the following potencies: polymyxin A, 9425 U/mg; polymyxin D, 1526 U/mg; polymyxin E, 9500 U/mg.

† G-4, trademark of Sindar Corp. for bis (2-hydroxy-5-chlorophenyl) methane.

‡ Asterol, trademark of Hoffmann-LaRoche, Inc. for 2-dimethylamino-6-(β -diethylaminoethoxy)-benzothiazole dihydrochloride.

TABLE I. Fungistatic Activity of Polymyxins and Various Known Agents.

Agent	— Zone of inhibition in mm —					
	<i>C. albicans</i>		<i>T. mentagrophytes</i>		<i>D. neoformans</i>	
	S*	NS†	S	NS	S	NS
Polymyxin A	2	2.5	.5	1	.5	2
B	3	3	2.5	3	4.5	4
D	2	2.5	.5	1.5	1.5	1
E	2.5	2.5	2.5	3	4.5	4
Benzoic acid	2	1.5	1	2	.5	2
Salicylic acid	1.5	2	0	1.5	2	2
Undecylenic acid	2.5	3.5	4	10	2	4.5
G-4	4	7	4.5	15	3	10.5

* Medium plus serum.

† Plain medium.

ton mentagrophytes, *Trichophyton purpureum*, *Microsporium audouini* and *Microsporium lanosum*. Cultures grown on Sabouraud's maltose agar at 28°C for 14 days served for the inocula. These were prepared by transferring the mats from the agar surface into flasks containing glass beads and neopeptone-maltose broth, shaking vigorously for 10 minutes, and then straining through several layers of cheese-cloth. The spores were counted on a hemocytometer and the suspensions diluted with broth to contain approximately 50000000 spores per cc. Each serial dilution-tube received 0.1 cc of the adjusted spore suspension. Incubation was carried out at 28°C for 14 days. Dilutions showing complete inhibition of growth were tested for fungicidal action by subculturing into 10 cc of the test broth plus serum and incubating at 28°C for at least 14 days.

Results. Antifungal response to each of the agents tested by the agar cup-plate method is shown in Table I. It is recognized that this method does not always give a true expression of antifungal activity, since inhibition will depend largely upon penetration of the test substance through the medium. The data are presented, however, to indicate the close agreement in the order of activity obtained with this procedure and the serial dilution technic (Table II). As shown by both methods, polymyxins B and E were the most active members of the antibiotic group, appearing equally effective against the respective fungi tested.

In the presence of serum, polymyxins B and

E proved equal to G-4 in fungicidal activity against *Candida albicans*, while benzoic acid, salicylic acid, undecylenic acid and Asterol were ineffective at the maximum concentration employed (Table II). G-4 was the most active agent against *Trichophyton mentagrophytes*; undecylenic acid and Asterol were approximately twice as effective as polymyxins B and E, while benzoic acid and salicylic acid failed to inhibit this organism at 25 mg %. Against *Trichophyton purpureum*, polymyxin E was again more active than the organic acids in the presence of serum. The fungicidal activity of polymyxin E was also greater than that of benzoic acid and salicylic acid against *Microsporium audouini*, equal to that of undecylenic acid, but less than that of Asterol and G-4.

Antifungal activity of the polymyxins did not appear to be significantly antagonized by serum. Benzoic acid and salicylic acid were only slightly affected, while activity of undecylenic acid and G-4 was markedly decreased. The presence of protein seemed to increase Asterol activity. Of interest in this regard was a similar observation(10) of an enhancing effect of agar upon this compound.

Discussion. Polymyxins B and E appear to possess potentialities as antifungal agents. Based on *in vitro* tests, these antibiotics might possibly be more effective than some antifungal drugs in current use. The absence of sensitizing properties of polymyxin B(11), for example, would make it ideally suited for topical application. Polymyxins B and E were the most active of the substances tested against the systemic fungus *Debaryomyces neoformans* in the presence of serum (Table I). Recent reports(12-14) favoring the parenteral use of polymyxin B for certain bacterial infections would suggest trial of this antibiotic for therapy of various systemic mycoses.

It has been the experience of clinicians that the use of antibiotics may result in overgrowth of organisms not susceptible to their action, *e.g.*, *Monilia* and certain species of Gram-negative bacteria. In view of the susceptibility of *Candida albicans* to polymyxins B and E and the marked antibacterial activity they possess against Gram-negative organisms,

TABLE II. Fungicidal Activity of Polymyxins and Various Known Agents.

Agent	Minimum fungicidal concentration in mg %									
	<i>C. albicans</i>		<i>T. mentagrophytes</i>		<i>T. purpureum</i>		<i>M. audouini</i>		<i>M. lanosum</i>	
	S*	NS†	S	NS	S	NS	S	NS	NS	
Polymyxin A	>25	>25	>25	25						
B	25	25	25	25						25
D	>25	>25	>25	>25						
E	25	25	25	25	25	25	12.5	25		
Benzoic acid	>25	>25	>25	>25	>25	25	25	12.5		25
Salicylic acid	>25	>25	>25	>25	>25	25	>25	25		25
Undecylenic acid	>25	25	12.5	6.25	>25	6.25	12.5	3.12		3.12
Asterol	>25	>25	12.5	>25	3.12	>25	6.25	25		>25
G-4	25	3.12	6.25	.78	6.25	.78	6.25	.39		.78

* Medium plus serum.

† Plain medium.

it would appear unlikely that overgrowth of such types would be encountered during systemic use of these antibiotics. However, there would be the possibility that an increase in Gram-positive flora might result.

Summary. (1) Polymyxins A, B, D and E were tested for antifungal activity against 5 species of dermatophytes and 1 systemic fungus. Varieties B and E were the most active and of equal effectiveness. (2) Activity of the polymyxins was not significantly inhibited by serum. (3) Clinical trial of the topical and systemic efficacy of polymyxins B or E in mycotic infections is suggested.

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Terramycin Hydrochloride Alone and with Mapharsen and Bismuth in Treatment of Experimental Syphilis of Rabbits. (19301)

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As shown by Hobby and her associates(1), terramycin hydrochloride is absorbed readily from the gastrointestinal tract of rabbits with high concentrations in the blood within an hour following the oral administration of the

compound. Under the circumstances we have thought it advisable to determine the therapeutic activity of terramycin hydrochloride alone and in combination with Mapharsen and bismuth salicylate in the treatment of acute