anhydrous magnesium sulfate, filtered, and the benzene removed by distillation under reduced pressure. The oil remaining (48.3 Gm., 91.7%) was converted to the dihydrochloride which, after recrystallization from absolute ethanol–anhydrous ether, melted at 232.5–233.0°.

Anal.—Calcd. for $C_{28}H_{48}Cl_2N_4O_2$: C, 61.86; H, 8.90; Cl, 13.04; N, 10.31. Found: C, 61.60; H, 8.74; Cl, 12.79; N, 10.31.

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Antimicrobial Properties of a Propylene Glycol Based Topical Therapeutic Agent

By I. OLITZKY

Propylene glycol is shown to have antimicrobial activity when used as a dermatological vehicle for fluocinolone acetonide.

THE ANTIMICROBIAL activity of propylene glycol (PG) has been studied in great detail with primary interest in air disinfection. Ancillary test tube studies of concentrated PG solutions have revealed significant antimicrobial activity with lower concentrations having virtually no effect. In studies conducted to determine minimal killing or inhibitory concentrations, the concentration–activity relationship was characterized by rather sharp end points. (1–4).

Because of its low toxicity and apparent lack of skin sensitizing properties (5), PG can be used safely in concentrated form as a vehicle for therapeutic agents for topical application. The purpose of the present study was to investigate the *in vitro* antimicrobial activity of a topical steroid preparation in which the active therapeutic ingredient is dissolved in 100% PG.

The microorganisms used were those which might be involved in primary or secondary infections of dermatological lesions. The addition of normal human serum to the test solutions was used to determine the effect of protein material on the antimicrobial activity.

EXPERIMENTAL

The solution[1] tested had the following composition: fluocinolone acetonide, 0.01 Gm.; citric acid, 0.01 Gm.; and propylene glycol, 100% *q.s.* 100.0 ml. [referred to as S-PG (steroid–propylene glycol)].

The microorganisms employed included *Escherichia coli*, *Mycobacterium balnei* (ATTC 11564), *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* (Group A), *Staphylococcus aureus* (phage type 80/81, penicillin resistant), *S. aureus*, (phage type 53, penicillin resistant), *S. aureus*, (phage type 42E, penicillin sensitive), *Candida albicans*, *Microe sporum canis*, *M. audouini*, *Trichophyton mentagrophytes*, *T. rubrum*, and *T. tonsurans*.

Bacteriostatic and Fungistatic Tests.—The bacterial cultures were grown in trypticase soy broth. Various dilutions of S-PG made in the same broth were seeded with 0.1-ml. amounts of 18–24-hr. cultures. The tubes were incubated at 35° and observed for 7 days.

The fungi were grown on Sabourad's agar. Plugs (7-mm. diameter) were cut from the confluent growth and dropped into tubes of Sabourad's broth containing various concentrations of S-PG. Incubation at 30° was continued for 21 days.

Bactericidal and Fungicidal Tests.—Based on the data gathered in preliminary studies, two solutions were selected for further tests. Solution *A* consisted of S-PG (90%) and normal human serum, NHS (10%), v/v. Solution *B* was made up of S-PG

TABLE I.—BACTERIOSTATIC AND FUNGISTATIC ACTIVITY OF A S-PG SOLUTION

Microorganisms	Growth in (% S-PG in v/v)	But Not in Broth,
E. coli	10	20
Ps. aeruginosa	10	20
P. vulgaris	10	20
Streptococcus pyogenes, group *A*	10	15
Staphylococcus aureus, 42E	20	30
S. aureus, 80/81	20	30
S. aureus, 53	20	30
Mycobaclerium balnei	4	5
C. albicans	10	20
Microsporum audouini	5	10
Microsporum canis	5	10
T. mentagrophytes	5	10
T. rubrum	5	10
T. tonsurans	5	10

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[1] Marketed as Synalar Solution (lot 1730133C) by Syntex Laboratories, Inc.

TABLE II.—MICROBICIDAL ACTIVITY OF A S-PG SOLUTION

Culture	Time for Inactivation of Inocula by Soln.[a] A	B
E. coli	Less than 5 min.	Between 45 and 60 min.
Ps. aeruginosa	Less than 5 min.	Less than 5 min.
P. vulgaris	Less than 5 min.	Less than 5 min.
Streptococcus pyogenes, group *A*	Less than 5 min.	Between 5 and 15 min.
Staphylococcus aureus, 42E	Less than 5 min.	Less than 5 min.
S. aureus, 80/81	Less than 5 min.	Less than 5 min.
S. aureus, 53	Less than 5 min.	Between 24 and 48 hr.
Mycobacterium balnei	Less than 5 min.	Less than 5 min.
C. albicans	Less than 5 min.	Between 5 and 15 min.
Microsporum audouini	1 hr.	2 hr.
Microsporum canis	30 min.	1 hr.
T. mentagrophytes	1 hr.	4 hr.
T. rubrum	2 hr.	4 hr.
T. tonsurans	2 hr.	Greater than 24 hr.

[a] Solution *A*, S-PG, 90%, and NHS, 10% (v/v); solution *B*, S-PG, 65%, NHS, 10%; and sterile distilled water, 25%.

(65%), NHS (10%), and sterile distilled water (25%).

The cultures were grown as described previously. Test vessels containing 2 ml. of the test solutions were seeded with 0.1 ml. of 18–24-hr. broth cultures of the bacteria and *C. albicans*. At appropriate intervals, using a calibrated loop, a 0.025-ml. portion of the medication–microorganism mixture was removed and placed into a tube of broth. Based on viable counts of the inocula, it was calculated that the absence of growth in the subculture tubes indicated that, at the particular exposure period, the test solutions had killed greater than 99.999% of the inocula.

For the fungi, the test vessels containing 3 ml. of test solution were prepared in replicate, one for each time period. The inocula consisted of a plug cut as described above. At the end of each exposure period, 7 ml. of sterile water was added, the mixture centrifuged to save the free conidia, and the supernatant liquid was discarded. This wash was repeated and finally 7 ml. of Sabourad's broth added. The tubes were incubated and observed for 21 days.

RESULTS

A limited number of parallel dilutions and tests were made with propylene glycol (1,2-propanediol, Eastman). Within the limits of experimental error, no differences in antimicrobial activity were seen between solutions made of pure propylene glycol and those made with S-PG.

Results of the tests are summarized in Tables I and II. The inhibition studies (Table I) revealed that, of the microorganisms tested, *M. balnei* and the cultures representing the Microsporum and Trichophyton genera were most sensitive to S-PG. The three staphylococcus cultures were the most resistant; after a slightly lengthened lag stage, growth in the 20% S-PG broth tubes was equivalent to that in the control tubes. Continuous contact with 30% S-PG broth solutions prevented multiplication of all the cultures tested.

The rate of germicidal action is shown in Table II. Even in the presence of 10% serum, the 90% S-PG solution was rapidly germicidal to the bacterial cultures and to *C. albicans*. Complete inactivation occurred in less than 5 min. The fungal cultures were more resistant, requiring from 30 min. to 2 hr. to inactivate completely both the mycelial and conidial stages. Some germicidal efficiency is lost as the propylene glycol concentration is lowered to 65%. Also, at this concentration some surprising differences in sensitivity were noted. *E. coli* was decidedly more resistant to the action of propylene glycol than were the pseudomonas and proteus cultures. The staphylococcus culture, phage type 53, was particularly resistant to the action of S-PG, remaining viable even after 24-hr. exposure to the solution. Elements of *T. tonsurans*, although completely inactivated by the 90% S-PG solution in 2 hr., remained viable even after 24-hr. contact in the 65% S-PG solution.

DISCUSSION AND SUMMARY

The results reported here are in agreement with previous reports of the marked germicidal efficiency of concentrated propylene glycol solutions. Since its low toxicity permits its use in concentrated form, it is surprising that so little attention has been given to its potential value as a topical antimicrobial agent. The effectiveness of the 90% S-PG solution against the spectrum of microorganisms tested suggests that there might be therapeutic benefit when concentrated propylene glycol solutions are used as a vehicle for topically applied medication. The solution used in this study is a steroid preparation used topically for the therapy of acute and chronic dermatoses. Since dermatologic lesions frequently are infected initially or develop infections during treatment, it is conceivable that the use of concentrated propylene glycol as a vehicle may provide a potential for preventing or treating the initial or secondary microbial infections.

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