

An *in vitro* Study on the Effect of Benzoyl Peroxide 20%, Gentamicin and Phenoxyethanol on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus species*

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Received June 1, 1979

We read, with interest, the paper by W. E. Pace (1976) on the treatment of leg ulcers with benzoyl peroxide 20%. This substance probably acts as an antimicrobial, and therefore we thought it important to compare the antimicrobial action of benzoyl peroxide with Gentamicin and Phenoxyethanol, two standard preparations which are used in this part of the United Kingdom.

METHODS

The three antimicrobial preparations were 2% phenoxyethanol in 0.5% Lassar's paste, 0.3% gentamicin and benoxyl 20 (20% benzoyl peroxide); in all experiments a control of phosphate-buffered saline pH 7 was used. Bacterial strains were obtained from leg ulcers using a sterile swab. Pure cultures were identified and used in the test. The following bacteria were used: *Staphylococcus aureus*, *Proteus species*, *Pseudomonas aeruginosa*.

Each strain was grown overnight in 10 ml brain-heart infusion broth at 37°C pH 7.4. The cells were then har-

vested, washed in phosphate-buffered saline (PBS) pH 7.0 and resuspended to 5 ml volume. A 0.3 ml drop from each suspension was placed on a sterile coverslip. This procedure was repeated for the number of materials and controls. The coverslips with the bacteria were inverted onto the control/test materials which were evenly spread on a sterile slide. The slides were then incubated at 37°C for an hour in a moist chamber to negate dehydration. After incubation the coverslip and slide were individually removed to 10 ml PBS with 0.05% Triton X-100 (dispersing agent) and agitated on a Rotamix for 1 minute to disperse the bacteria. Serial 10-fold dilutions were made from each dilution onto agar plates. The fluid was evenly distributed over the surface of the agar using sterile glass spreaders. Heated blood agar was used for *S. aureus*, nutrient agar (3% agar incorporated to stop swarming) for *Proteus species*, and nutrient agar for *P. aeruginosa*. All cultures were incubated aerobically for 48 hours at 37°C and the colonies were then counted. The viable count per coverslip was calculated from dilutions which gave 30-300 colonies per plate.

RESULTS

The results are shown in Table I and this indicates the number of log. cycles decrease in the viable count.

The control data indicate that there was no significant reduction in the viability of the bacterial cells during the incubation. Benzoyl peroxide 20% has greater effect than gentamicin or phenoxyethanol, in reducing the viability of the organisms. Both gentamicin and benzoyl peroxide (20%) killed all 3 strains of *P. aeruginosa*, whereas phenoxyethanol killed only one of the 3 strains; phenoxyethanol had negligible effect on the other 2 strains of *P. aeruginosa*. Benoxyl 20 killed all 3 strains of *S.*

Table I. The effect of antimicrobials on the survival of test bacteria

Organism	Log. cycle decrease of viable bacteria			
	Control, no antimicrobial added	Benzoyl peroxide, 20%	Gentamicin	Phenoxyethanol
<i>S. Aureus</i>				
1	No reduction	Total kill	No reduction	No reduction
2	No reduction	Total kill	1.9	1.8
3	No reduction	Total kill	0.5	1.0
<i>Proteus Sp.</i>				
1	No reduction	4.9	4.2	No reduction
2	No reduction	3.0	4.0	1.2
3	No reduction	3.1	4.0	1.3
<i>P. Aeruginosa</i>				
1	No reduction	Total kill	Total kill	0.6
2	No reduction	Total kill	Total kill	1.0
3	No reduction	Total kill	Total kill	Total kill

aureus, but both gentamicin and phenoxyethanol caused only a small reduction in viability of the bacteria in 2 of the 3 strains. The viability of the *Proteus* species was decreased by all 3 antimicrobials but the effect was most marked with gentamicin and benzoyl peroxide 20%.

DISCUSSION

It should be noted that this test indicates the bactericidal effect of the materials in contact with the bacteria in a non-nutrient environment. The test system used in this work is only one of several test systems that could be utilized; for example, the minimum inhibitory concentration (bactericidal or bacteriostatic) in a nutrient environment with or without protective debris could be used. Results of such tests are merely indicators and would not necessarily predict the efficiency of the material in clinical use. Such tests do not measure but can indicate bacteriostatic effects or predict the potential of the active material when diluted by spreading on the skin. In a leg ulcer many bacteria must receive some protection by being in an environment with extracellular macromolecules and dead eukaryotic cells. Therefore, a material showing promise in this test would need to be tested in a clinical situation.

There are two published investigations on the treatment of ulcers with benzoyl peroxide. In an uncontrolled study, Pace (1976) found 20% benzoyl peroxide to be of considerable help in the management of pressure sores. However, Lookingbill et al. (1978) showed in a double-blind study that 10% benzoyl peroxide had no effect on leg ulcer flora. This is in contrast to our *in vivo* data and can be explained by the important differences between *in vivo* and *in vitro* studies, though the concentration of the benzoyl peroxide may also be important.

The mode of action of phenoxyethanol and gentamicin is antimicrobial. Benzoyl peroxide is antimicrobial but because it releases by weight 6% oxygen this could also be of help in healing of ulcers which are generally oxygen depleted.

REFERENCES

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Inhibition of Hyperhidrosis by Topical Application of a Local Anesthetic Composition

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Received June 25, 1979

Abstract. An eutectic mixture of 5% lidocaine and 5% prilocaine applied locally to the skin inhibited epinephrine-induced sweating in healthy subjects. It also inhibited palmar and axillary hyperhidrosis from 1 to 6 hours after application to 15 of 17 patients tested. A placebo lotion was without effect. When used daily for 3–4 weeks, only 25% of the patients were very pleased with the treatment. The reason for the decrease of efficacy is uncertain.

Key words: Hyperhidrosis; Inhibition of sweating; Topical anesthetic; Antiperspirant

Excessive sweating can be severe, discomforting, and interfere with many social and occupational activities. Emotional and mental factors trigger hyperhidrosis in most subjects and it is especially evident on the palms and in the axillae. Such sweating does not occur during sleep and a hereditary tendency is sometimes seen. In other patients there is an increased sensitivity of the hypothalamic heat-regulating centre resulting in hyperhidrosis, especially in warm weather. This type can be worse at night and is often generalized, but may also be most apparent for the eccrine glands in the palms and axillae. The various metallic compounds and anticholinergics used as topical antiperspirants have recently been reviewed (8). The most successful is the application of a 20% solution of aluminium chloride or zirconyl chloride in absolute alcohol under occlusive dressing when the axilla is dry (7, 8). Axillary hyperhidrosis can be treated by surgical excision of the glands (3, 9). For palmar sweating there is no surgical remedy except cervicthoracic sympathectomy (1), but this is only recommended for special cases and has not gained wide acceptance.

Injection of procaine is known to reduce the sweat response induced by acetylcholine and