In Vitro Study of Antibacterial Action of Various Chemicals on *Corynebacterium acnes*

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Six strains of *Corynebacterium acnes*, isolated directly from acne pustules, were tested for their susceptibility to various levels of hexachlorophene, bithionol, cetyl pyridinium chloride, propylparaben, *p*-chloro-*m*-xylenol, salicylic acid, and resorcinol. The zones of inhibition produced in agar cups by these agents in a seeded anaerobic medium with and without 0.25 per cent synthetic sebum were measured. In the absence of sebum, hexachlorophene and bithionol produced the largest zones over a wide range of concentrations. Although the zones produced by all the compounds were decreased significantly in the presence of the synthetic sebum, *p*-chloro-*m*-xylenol was least affected.

UNNA DESCRIBED (1) in 1896 an organism found in histological sections of acne comedones which subsequently became known as the acne bacillus. Little is known about the bacteriology and etiological role of this organism. However, during the past decade the role of bacteria in acne vulgaris has received increasing attention. For many years, there has been a considerable amount of confusion and conflicting reports concerning the types of organisms present in acne lesions and their pathological significance. The organisms most frequently implicated include different types of staphylococci, streptococci, enteric species, and diphtheroids. Much of the confusion about the bacteriology of acne vulgaris may be attributed to inadequate techniques in the isolation, culture, and identification of the type of lesion from which the organisms were isolated. The difficulty in determining whether the isolated organisms were derived from acne lesions or were simply surface contaminants has contributed to the confusion. It is now recognized that only unopened lesions are appropriate for primary isolation, and these must be sampled with a fine probe worked into the depth of the lesion. Using this technique, Pochi and Strauss (2) found *Corynebacterium acnes* to be the predominant isolate from acne lesions. They rarely recovered staphylococci. Smith and Waterworth (3) and Shehadeh and Kligman (4) studied many acne lesions and found that in addition to the acne bacillus, *Staphylococcus albus* was almost always a concomitant resident.

The role of *C. acnes* in the etiology of acne vulgaris is now receiving increasing attention by many dermatologists, but relatively little is known about its susceptibility to antibacterial agents. Previous *in vitro* studies were limited to a determination of its sensitivity to antibiotics and sulfonamides. Meyer (5), using a disk plate method, found the acne bacillus sensitive to penicillin, chlortetracycline, chloramphenicol, oxytetracycline, picromycin, and streptomycin. Pochi and Strauss (2), using a tube dilution technique, reported that *C. acnes* was sensitive to penicillin, erythromycin, demethylchlortetracycline, tetracycline, chlortetracycline, chloramphenicol, novobiocin, and oxytetracycline. The ineffective agents included streptomycin, neomycin, sulfisoxazole, sulfamethoxypyridazine, and sulfadimethoxine. Smith and Waterworth (3), using an agar cup diffusion method, tested the sensitivity of 24 strains of acne bacilli to different antibiotics and found that these strains were largely sensitive to penicillin, tetracycline, erythromycin, chloramphenicol, and sulfafurazole. Streptomycin was considerably less effective.

No attempt was made in any of these *in vitro* studies to simulate natural conditions in which agents to be applied topically were admixed with sebum. It has long been a practice when testing the antibacterial activity of compounds of therapeutic interest by *in vitro* test to add serum to the medium to detect inactivation by proteins which inevitably occurs to a greater or lesser degree. Therefore, it would seem equally logical to incorporate sebum into the medium when testing the effects of topically applied antibacterial agents against skin bacteria.

EXPERIMENTAL

The antibacterial action of seven compounds was tested against six strains of *C. acnes* in the presence and absence of a synthetic sebum using an agar cup plate diffusion method. The compounds which were solubilized in a vehicle of 10% acetone, 40% alcohol, and 50% water consisted of hexachlorophene, bithionol, cetylpyridinium chloride, propylparaben, *p*-chloro-*m*-xylenol, salicylic acid, and resorcinol. Because of the difficulty of collecting a sufficient amount of natural sebum, a synthetic sebum was substituted, based on the quantitative composition of human sebum and hair fat reported by Weitkamp *et al.* (6), MacKenna *et al.* (7), Nicolaides and Foster (8), and Wheatley (9). It consisted principally of a mixture of saturated and unsaturated fatty acids, esters, squalene, cholesterol, paraffin, higher alcohols, and several glycerides. No attempt was made to simulate the amount of sebum found on the skin. A 0.25% concentration of synthetic sebum was selected because turbidity produced by greater amounts masked the inhibition zones produced by the test compounds.

Five strains of *C. acnes* used in this study were isolated from acne pustules.[1] The sixth strain was the American Type Culture strain No. 11828. All the strains were catalase positive and were agglutinated by *C. acnes* antiserum.[2] The organisms were essentially Gram-positive and were decolorized easily, as described by previous workers. Microscopically, pleomorphic club-shaped rods, which

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[2] The antiserum was obtained through the courtesy of Dr. Albert M. Kligman, University of Pennsylvania School of Medicine.

tended to occur in pairs with the cells joined at a slight angle, were observed. These six strains had a preference for anaerobic conditions. On anaerobic solid media, surface colonies were smooth, round, and cream-colored which became distinctly pink after 2 weeks. Broth cultures developed a dense growth with a heavy sediment and creamy curd after 48 hr. at 37°.

The test employed in this study consisted of the following method. The anaerobic medium described by Evans (10) supported excellent growth of *C. acnes* on agar and in broth. A relatively large inoculum was required to produce a sharp zone of inhibition, therefore, 10 ml. of a 48-hr. broth culture was placed in 350 ml. of the liquified agar medium from which 30 ml. was placed in each Petri dish. Sebum plates were prepared by adding it to the molten agar medium maintained at 60°. This mixture was stirred magnetically for 2 min. and, while hot, passed rapidly through a sterile hand homogenizer, cooled to 44°, inoculated with the test organism and dispensed in 30-ml. quantities in Petri dishes. Three 10-mm. diameter cups were made in plates, except where the zone size was so large that only two cups could be used. The bottom of the cup was sealed with melted agar and 0.2 ml. of the test solution pipeted into each cup. The plates were incubated in MacIntosh-Fildes anaerobic jars under an atmosphere of 95% nitrogen and 5% CO_2 for 48 hr. at 37° before the zones of inhibition were measured.

RESULTS

Table I shows the mean diameter of the zones of inhibition produced by the various chemical compounds at concentrations from 2 to 0.0001% (w/v) against the six *C. acnes* strains. In the absence of sebum, hexachlorophene and bithionol produced the largest zones over a wide concentration range. At the levels of 0.25 and 0.13%, bithionol produced statistically significant larger zones than hexachlorophene, but the differences in zone size below 0.13% were not significant.

The range of concentrations for testing was selected to plot a dose–response curve, which in most cases was linear over the entire range, confirming the findings of Plein and Plein (11). Five of the compounds could not be tested above 1% concentration because of insolubility.

Although *p*-chloro-*m*-xylenol was less active than hexachlorophene and bithionol, it showed large zones at levels ranging from 1 to 0.25%. Below these levels, the size of the zone diminished rapidly. Propylparaben produced an appreciable zone at the 1% level, and cetylpyridinium chloride produced comparatively small zones, although activity was measurable for the last concentration tested of 0.0001%. Concentrations of 1 and 2% resorcinol and salicylic acid produced relatively small zones of inhibition.

In the presence of 0.25% synthetic sebum in the medium, the size of the zones produced by all the compounds was decreased significantly. It is interesting that, while *p*-chloro-*m*-xylenol was considerably less effective than hexachlorophene and bithionol in the absence of sebum, it was significantly more active than these substances in the presence of sebum at the 1 and 0.5% levels. At the 0.5% level, *p*-chloro-*m*-xylenol was comparable to 0.25% bithionol in the presence of sebum but markedly less effective than bithionol in the absence of sebum. Propylparaben produced similar zones with and without sebum. Cetylpyridinium chloride did not produce any zones of inhibition, even in the highest concentration tested. This is to be expected since fatty acids present in sebum are known to inactivate quaternary antibacterial agents.

Figure 1 provides a graphic comparison of the mean zone sizes in the absence of sebum. The slightly larger zones produced by bithionol at the higher concentrations are statistically significant. The apparently greater activity of hexachlorophene at the lower concentrations is not statistically significant, as previously noted.

In Fig. 2, the bar graphs show the comparative zones of inhibition produced by the various compounds in the presence of sebum. It will be noted that there is a marked reduction in the size of the zone for all compounds; but in general the relative activity of the various compounds remains essentially the same. Bithionol appears to give somewhat larger zones than hexachlorophene in most of the dilutions tested. Also, at the higher concentrations *p*-chloro-*m*-xylenol produced the largest zones.

DISCUSSION

While it is tempting to seek theoretical explanations for the differences in the zone size produced by

TABLE I.—COMPARATIVE BACTERIOSTATIC ACTION OF COMPOUNDS ON SIX STRAINS OF *C. acnes*[a]

Compd.	2.0	1.0	0.5	0.25	0.13	0.06	0.03	0.02	0.008	0.004	0.002	0.001	0.0005	0.0001
% Concn. (w/v) without Sebum														
Hexachlorophene	...[b]	43.7	41.2	39.6	37.1	...	33.5	...	28.2	...	23.6	...	16.7	13.4
Bithionol	...	...	...	44.8	43.0	40.0	37.3	...	32.7	...	20.7	...	13.6	8.5
p-Chloro-*m*-xylenol	...	35.7	31.7	19.7	12.5	8.3	5.3	...	...	...	...	...	...	...
Propylparaben	...	16.7	13.3	8.8	4.9	3.1	1.7	...	...	...	...	...	...	...
Cetylpyridinium chloride	...	12.0	9.7	8.7	...	7.0	...	6.4	...	5.0	...	4.5	...	...
Resorcinol	2.2	1.3	0.3	...	...	...	...	...	...	...	...	...	...	...
Salicylic acid	5.8	2.8	1.3	...	...	...	...	...	...	...	...	...	...	...
% Concn. (w/v) with Sebum														
Hexachlorophene	...	14.9	13.8	12.9	11.6	...	10.2	...	8.6	...	6.8	...	4.7	2.5
Bithionol	...	...	...	19.2	18.1	17.1	15.1	...	13.0	...	8.9	...	3.8	0.2
p-Chloro-*m*-xylenol	...	26.3	22.6	15.0	7.6	4.4	0.9	...	...	...	...	...	...	...
Propylparaben	...	12.4	9.6	5.5	2.0	0	0	...	...	...	...	...	...	...
Cetylpyridinium chloride	...	0	0	0	...	0	...	0	...	0	...	0	...	...
Resorcinol	1.9	0.8	0	...	...	...	...	...	...	...	...	...	...	...
Salicylic acid	4.5	2.5	0.8	...	...	...	...	...	...	...	...	...	...	...

[a] Mean diameter (millimeters) zone of inhibition. [b] ..., not tested.

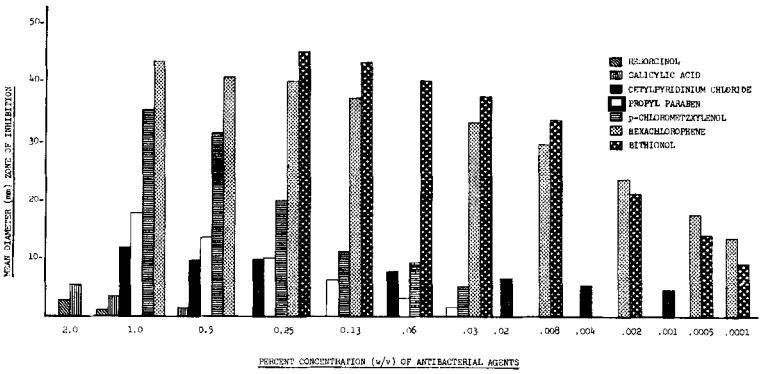

Fig. 1.—Comparative bacteriostatic action of various compounds on *C. acnes* in absence of artificial sebum.

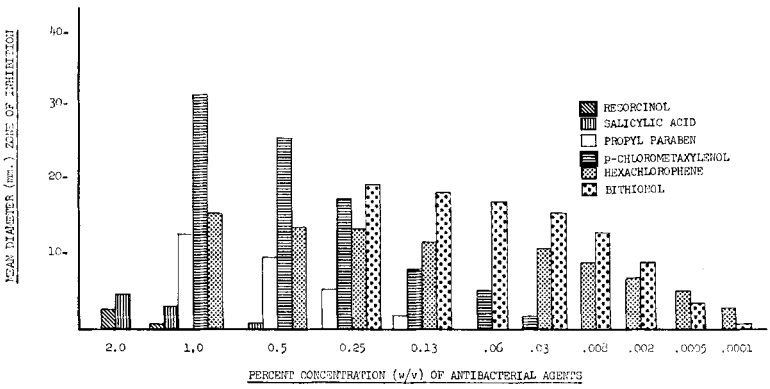

Fig. 2.—Comparative bacteriostatic action of various compounds on *C. acnes* in presence of artificial sebum.

the various compounds with and without sebum, it would be premature to attempt such an explanation without further study of the numerous factors involved. For example, the zone sizes produced by antibacterial compounds are a measure of the diffusibility of the compounds. In this study their diffusion was no doubt influenced by the nature of the solvent system used, the solubility of the compound-solvent system in the sebum and sebum-free agar, the oil-water partition coefficients in the medium containing the synthetic sebum, and by the differences in molecular weights of the compounds tested. Furthermore, the composition of the synthetic sebum may produce an effect different from that produced by natural sebum, especially since the exact composition of human sebum is unknown at the present time. It is obviously impossible to determine how closely it has been simulated in the mixture employed in this study. However, it is recognized generally that human sebum consists of approximately one-third unsaturated fatty acids, one-third saturated fatty acids, and one-third nonsaponifiable material; the sebum used in this study fulfills this requirement. It is recognized that some of the compounds (resorcinol and salicylic acid) tested for their antibacterial action may have other even more significant but unknown therapeutic action in the topical treatment of acne. Furthermore, while the technique employed here points the way to a more realistic evaluation of topical antibacterial agents because the organisms are tested in the presence of sebum, in the human host other factors, such as a penetration of material, may be important. It is hoped that subsequent studies will elucidate the importance of these other factors.

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