

isolated tubercles scattered in the scar tissue. Two years after the discontinuation of the treatment with amphotericin B, the patient was hospitalized again because of the reappearance of the edema facialis of the eyelids. The concomitant administration of amphotericin B with hydrocortisone intravenously and also of dexamethasone intralesionally in the isolated tubercles, was also without effect, producing only the resolution of the facial edema. The cultures remained positive.

Intramuscular injections of emetine hydrochloride have been used successfully in the treatment of amebic liver abscess. Since ameba and leishmania are both protozoa, and emetine hydrochloride was found to be highly effective against ameba, it was expected that it might also be effective against the leishmania parasite. For the first time we used, successfully, intralesional injections of emetine hydrochloride in some lesions of a patient suffering from leishmaniasis cutanea diffusa which had also proved resistant to amphotericin B treatment (2). The second patient is the case presented here. Interestingly, these 2 patients belong to the two poles of the leishmaniasis spectrum.

Emetine hydrochloride is a toxic substance causing nausea, vomiting and muscular weakness. Electrocardiographic changes and hypotension have also been reported (3). All these side effects can be avoided when small amounts of emetine hydrochloride are given intralesionally. There is, however, the possibility of inducing sensitivity and an anaphylactic reaction may appear. These side effects, not observed in our two patients, can be overcome if steroids are given simultaneously—systemically or intralesionally.

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Antibiotic Sensitivity of Comedonal *Propionibacterium acnes*

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Abstract. Previous studies on antibiotic MIC levels for *P. acnes* and *P. granulosum* have shown them generally sensitive to therapeutic levels in both blood and surface lipids. The clinical response of acne vulgaris to antibiotic therapy is slower than one would anticipate from in vitro studies. It is also delayed well beyond the time tetracycline is known to appear in surface lipids. The concentration of antibiotics in comedonal material could be important, but it is not known. To determine the sensitivity level required for such an assay, MIC studies on *P. acnes* and *P. granulosum* from comedonal material were carried out from 0.05 to 25 µg/ml. The assay would need to detect 0.05 µg/ml or less in comedonal material. Interestingly, two organisms were found to be resistant to one or more antibiotics at the 25 µg/ml level and another had a MIC level of 12.5 µg/ml for tetracycline.

Key words: Acne; *P. acnes*; Ampicillin; Clindamycin; Erythromycin; Oxytetracycline; Tetracycline

Propionibacterium acnes has been associated with acne vulgaris for over 80 years. However, we still do not completely understand the part this organism plays in the pathogenesis of acne, or its response to antibiotic therapy. *P. acnes* is susceptible in vitro to a variety of antibiotics at modest concentrations (3, 7, 12). Such concentrations occur in surface lipids as well as in serum of patients on therapeutic dosage (9). However, the clinical response does not parallel in vitro sensitivity and this has not been adequately explained. It might be partly due to

Table 1. MIC levels for *P. acnes* organisms isolated from comedones of 14 untreated acne patients
Seven strains were group I and seven were group II

MIC level $\mu\text{g/ml}$	0.05 or <	0.1	0.2	0.4	0.8	1.6	3.13	6.25	12.5	25	>25
Tetracycline	4	4	1	1	1				1		2
Oxytetracycline	8	6									
Clindamycin	8	3									1
Ampicillin	4	4	1	1	1						1
Erythromycin	8	5									1

technical problems in determining the minimum inhibitory concentration (MIC) of antibiotics. Another possible explanation is the altered physiology of *P. acnes* with microenvironmental change (4). Non-antimicrobial properties of these agents could also be important.

Data presented by Resh & Stoughton (10) suggest another possibility. Over a period of several weeks, their patients on topical clindamycin had a gradual fall in *P. acnes* levels within comedones which paralleled clinical improvement in their acne. Erythromycin also improved inflammatory lesions but did not cause a corresponding drop in *P. acnes* counts. Clindamycin is lipophilic and could, perhaps, be better taken up into comedones or be more bactericidal in this milieu.

Anaerobic bacteria are reduced in number by oral tetracycline therapy (5). Yet, Smith & Waterworth (11) could not detect tetracycline in comedonal material from patients on oral therapy. Obviously, there is a need for further study of antibiotic levels within comedones. Before this can be done, it is first necessary to determine what constitutes a significant level. To try to answer this second question, we determined agar diffusion MIC levels for comedonal *P. acnes* and *P. granulosum* taken from 14 previously untreated acne patients.

MATERIAL AND METHODS

Cultures were taken from comedonal material in the following manner: The skin was wiped twice with 70% alcohol and dried. Two partially open comedones were extracted with a sterile Schamberg comedo extractor. Comedones were selected which demonstrated darkening toward the surface, but not deeper. This material was placed in a sterile 9×150 mm tissue grinder (Pyrex no. 7725) and homogenized with 5 ml of 0.05% Triton X-100 in a mixture of M/15 monobasic and M/15 dibasic sodium phosphate solution buffered to a pH of 7.9. Four tenfold serial dilutions were made up, using 0.025% Triton X-100 in the same phosphate buffer. 0.25 cc of each dilution was plated

on Stoughton's isolation medium consisting of pre-reduced Brain Heart Infusion (BHI) agar with 10% dextrose added.

To prepare the media, dextrose solutions were filtered through a 0.2 μm filter and added to the BHI agar which had been autoclaved. Cultures were incubated 5 days in an anaerobic Gas Pak jar. Single colonies grossly representing the predominant organism were selected and subcultured anaerobically in chopped meat glucose broth. Organisms were also examined by Gram stain and the API 20A series was used for identification reactions.

Antibiotics were added to BHI agar with 10% dextrose in a concentration of 25 mg/ml and serially diluted to produce concentrations of 12.5, 6.25, 3.13, 1.6, 0.8, 0.4, 0.2, 0.1 and 0.05 mg per cc.

Tetracycline, erythromycin, clindamycin and ampicillin were obtained from Scientific Products, McGaw Park, Ill. Oxytetracycline was obtained from Charles Pfizer & Company.

10^5 organisms from each isolate were inoculated on BHI agar with 10% dextrose containing serial dilutions of all five antibiotics. MIC levels were read as the lowest concentration of the antimicrobial agent that completely inhibited growth.

RESULTS

The results are outlined in Table I. With the exception of tetracycline, half or more of the organisms studied showed no growth at the 0.05 $\mu\text{g/ml}$ level. Only four organisms were inhibited by tetracycline at this level and four more were inhibited at the 0.1 $\mu\text{g/ml}$ level. One organism identified as *P. granulosum* was resistant to tetracycline, clindamycin and erythromycin at the 25 $\mu\text{g/ml}$ level. One *P. acnes* organism had a MIC of 12.5 $\mu\text{g/ml}$ with tetracycline and another was resistant at 25 $\mu\text{g/ml}$. These last two organisms had MIC's of 0.2 $\mu\text{g/ml}$ for ampicillin and 0.1 $\mu\text{g/ml}$ for the other antibiotics in the study.

DISCUSSION

Clinicians and investigators have long been puzzled by their failure to correlate in vitro antibiotic effec-

tiveness with clinical improvement in acne patients. This may partly be due to non-antimicrobial properties of antibiotics, e.g. chemotactic inhibition (2). However, much, if not most, of the chemotaxis is probably caused by materials within or derived from *P. acnes* organisms (8).

Antibiotic therapy sometimes requires many weeks to be effective in the treatment of acne, regardless of its mechanism of action. Yet, tetracycline has been reported to appear in surface lipids 4–8 days after the onset of oral therapy and to remain 3–8 days after the drug was discontinued (9). Maximum improvement from topical clindamycin also required many weeks of treatment (10). The return to usual levels of *P. acnes* following discontinuance of antibiotics is variable. Resh & Stoughton had one patient who did not grow *P. acnes* in comedones on the back for 10 weeks. In other patients, lesions on the face regrew bacteria one week later.

According to Robert T. Pfeifer, M.D., The Upjohn Co., Kalamazoo, Michigan, topical clindamycin produces comedonal levels of 0–33.8 µg/mg with a mean of 7.0 µg/mg. However, for patients on oral antibiotics, comedonal levels are not known. Smith & Waterworth (11) looked in vain for antimicrobial activity in crushed comedones from patients on oral tetracycline. It also is not certain what concentrations might be significant. The agar dilution MIC would seem a reasonable approach but obviously does not exactly reproduce what happens in vivo. Antibiotic levels within comedones might possibly be quite low and still be significant. Therefore, any assay method selected for this purpose must be sensitive at the lower MIC values for most organisms which occur within comedones. According to our data, the required sensitivity level would be 0.1 µg/ml for tetracycline and 0.05 µg/ml for erythromycin and oxytetracycline. For clindamycin, it must be even more sensitive.

An interesting finding in this study was the relative resistance of three organisms to tetracycline. One of these was also resistant to clindamycin and erythromycin. At least two other studies have reported one or more organisms resistant to erythromycin (1, 6) and Crawford et al. (1) have found that some patients develop resistant strains while on topical erythromycin and clindamycin. However, the 'wild' strain returns after topical antibiotics are replaced by oral tetracycline therapy. The MIC levels for clindamycin and erythromycin in

this study paralleled each other within one serial dilution. Ampicillin was again found to be a highly effective antibiotic in vitro. However, it reportedly does not significantly suppress *P. acnes* counts in vivo (5).

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