Rapid g.l.c. method for the separation of picogram quantities of morphine and codeine

M. J. KOGAN, M. A. CHEDEKEL, *The State of New York, Office of Drug Abuse Services, Testing and Research Laboratory, Brooklyn, New York, 11217, U.S.A.*

The procedure which follows is the modification of a method described by Dahlstrom & Paalzow (1975) for derivatization of morphine and codeine after extraction from biological materials.

A solution containing a small quantity of free base, 1·0 ng to 1·0 μg, of either morphine or codeine was evaporated to dryness in 15 ml conical test tube which had been treated previously with a 5% (v/v) solution of Dri-Film SC-87 in toluene (Pierce Chemical Co., Rockford, Ill.). To the tube 100 μl of glass distilled benzene (Burdick and Jackson Chemical Co., Muskegon Mich.) and 100 μl of pentafluoropropionic anhydride (Pierce Chemical Co., Rockford, Ill.) was added. The tube was capped with a size '00' thimble type stopper (A. H. Thomas Co., Phila., Pa.) then allowed to react for 25 min at 70°.

After reaction, the solvent with the reagent was evaporated to dryness at room temperature under a stream of dry nitrogen. The sample was taken up in ethyl acetate and 1 μl, containing 5 to 1000 pg μl^{-1}, was injected into a gas chromatograph (Hewlett Packard Model 5830A) equipped with a ^{63}Ni electron capture detector. The stationary liquid phase was 3% OV-22 on 80/100 Supelcoport (Supelco, Inc., Bellefonte, Pa.) in a 6 ft × 2 mm (i.d.) glass column. The carrier gas was a 90:10 mixture of argon–methane and the flow rate was set at 40 ml min^{-1}. Analysis was carried out isothermally at an oven temperature of 215°. Under these conditions the retention times for the morphine and codeine pentafluoro derivatives were 5·33 and 9·10 min, respectively.

The derivatization was carried out with several solvents: ethyl acetate, cyclohexane, toluene, and acetonitrile. The results indicated that unlike the reaction of morphine with trifluoroacetic anhydride (Wallace, Hamilton, & others, 1974) there is no critical ratio of anhydride to solvent. In fact for neat reactions there is no apparent loss in sensitivity although a higher temperature of 110° is required. For absolute standards the lower limit of sensitivity for morphine and codeine were 2 and 20 pg, respectively. This difference in sensitivity is apparently due to the fact that morphine forms a diester (Dahlstrom & Paalzow, 1975) whereas codeine most probably forms a monoester with the derivatizing agent. In addition, codeine and nalorphine are completely resolved when chromatographed under the conditions described. The retention time for nalorphine is 7·40 min. Dahlstrom & Paalzow (1975) noted that under their conditions nalorphine and codeine were not completely resolved. These derivatives are detectable on FID, however, the sensitivity is diminished.

August 21, 1975

REFERENCES

DAHLSTROM, B. & PAALZOW, L. (1975). *J. Pharm. Pharmac.*, **27**, 172–176.
WALLACE, J. E., HAMILTON, H. E., BLUM, K. & PETTY, C. (1974). *Analyt. Chem.*, 2107–2111.

Antimicrobial effects of some bis-biguanides on certain bacteria which occur in connection with acne vulgaris

IDA K. HEGNA, *Department of Microbiology, Institute of Pharmacy, University of Oslo, Blindern, Oslo 3, Norway*

It has been reported that washes and lotions which contain hexachlorophene display an *in vitro* reducing effect on *Corynebacterium acnes* and *Staphylococcus albus* (*S. epidermidis*) (Jungermann & Taber 1971, Montes & Pittillo, 1972; Cunliffe, 1973), the microorganisms most frequently isolated from acne lesions (Shehadeh & Kligman, 1963; Kirschbaum & Kligman, 1963; Hall-Smith & Marks, 1973; Cunliffe, 1973). Although chlorhexidine is commonly used as a skin disinfectant (Senior, 1973), no reports on treating acne vulgaris or the acne bacteria with chlorhexidine have been recorded.

R-NH-C(NH)NH-C(NH)-NH-$[CH_2]_6$-NH-C(NH)-NH-C(NH)-NH-R

I

Ia R = cyclohexylmethyl
Ib R = 2-norbornyl
Ic R = 1,5-dimethylhexyl
Id R = 1,3-dimethylpentyl
Ie R = 2-ethylhexyl
If R = 4-chlorophenyl

Some bis-biguanides I have been evaluated, with chlorhexidine (If) as reference compound, for their *in*

vitro effects on the following acne bacteria: *C. acnes* NCTC 737, *C. acnes* ATCC 6921, two *C. acnes* strains isolated from skin, and two *S. epidermidis* strains (also from skin). 2·2mM stock solutions of the bis-biguanides were made in sterile distilled water, and a tube dilution test method was used. Serial dilutions were made in 0·07 M phosphate buffer with pH 7·0; the *S. epidermidis* inoculum had a cell density of 10×10^6 cells ml^{-1}, and that of *C. acnes* a cell density of $40–70 \times 10^6$ cells ml^{-1}. Exposure time was 5 min at 22°. HS-T broth (Clausen, Aasgaard & Solberg, 1973; Clausen, 1973) was used as a recovery medium. The influence of pH on the effects of the various bis-biguanides on *C. acnes* NCTC 737 was recorded using the same dilution method (inoculum 40×10^6 cells ml^{-1}, exposure time 5 min at 22°) and the following pH values: 5·6 (0·05 M acetate buffer), 6·2 (0·07 M phosphate buffer), 7·0 (0·07 M phosphate buffer), 8·0 (0·07 M phosphate buffer), 9·0 (0·05 M tris-HCl buffer). The effects of 10, 20 and 50% normal horse serum on the bactericidal action on *C. acnes* NCTC 737 were recorded. The serum dilutions were made in 0·07M phosphate buffer (pH 7·0) and the inoculum (a: 13×10^7, b: 7×10^7 cells ml^{-1}) was added to the serum just before it was mixed with equal amounts of the different bis-biguanides, diluted from the stock solutions in 0·07 M phosphate buffer with a pH of 7·0. The final concentrations of the bis-biguanides were those which brought about 99·9% reduction of the bacterial cell numbers in the course of 5 min when no serum was added to the test solutions. Inactivation was achieved in a Lubrol W(ICI)-lecithin broth (3/2% w/v) and survivors in the solutions were estimated by plate counts on blood agar, incubated in a Gas-Pack anaerobic system. An agar cup diffusion method on blood agar plates was used when testing the bacteriostatic effects of the six bis-biguanides (1·1mM) on *C. acnes* NCTC 737.

The results of the first experiment showed that the two alkyl-compounds, Ic Ie, were the most effective against the bacteria tested, the Ie (MLC, $\mu g\ ml^{-1}$ 20–80) being slightly more effective than the other, Ic (MLC $\mu g\ ml^{-1}$ 40–80). Chlorhexidine (If (MLC 60–>2000) and the alicylic compound, Ib (MLC 70–600 $\mu g\ ml^{-1}$) were the least effective on the bacteria tested. Chlorhexidine did not display any effect on *C. acnes* NCTC 737 within 5 min. Neither did it show any increase in the effect against *C. acnes* NCTC 737 when the pH was raised from 5·6 to 9·0, but when extending exposure time to 30 min, chlorhexidine gave a better effect at pH 5·6 than at pH 6·2 like the other compounds. The three alkyl derivatives Ic, Id, Ie exhibited bactericidal effects on *C. acnes* NCTC 737 which increased with increasing pH values from pH 7·0 to 9·0, and the MLC values at pH 5·6 were half those at pH 6·2 for Ic and Id and half that at pH 7 for Ie.

The bactericidal effect of the Ie (20 $\mu g\ ml^{-1}$) was most influenced by serum: in the presence of 10% serum and the 13×10^7 inoculum the reduction of the effect was about 5%; it rose to about 10% with the 7×10^7 inoculum. When 50% serum was added the reduction was about 26%. The reduction of the effects of the other compounds (at concentrations from 40–250 $\mu g\ ml^{-1}$) was about half of that of the Ie with various amounts of serum added. On the blood agar Ie displayed the weakest bacteriostatic effect with growth inhibition zones of 28 mm, whereas Ib exhibited the best effect and the largest inhibition zones (67 mm). Also, Ib did not cause haemolysis seen with all of the other compounds. Chlorhexidine caused traces of precipitation in the blood agar, so too did compounds Ic and Ie. Thus, the most effective cell-reducing alkyl-derivative Ie seemed to be most influenced by blood, whereas the less effective compound, the alicyclic Ib, was least influenced. Davies (1973) reported that the bactericidal effect of chlorhexidine in acid solutions is markedly reduced. This result was not confirmed for any of the bis-biguanides when using *C. acnes* NCTC 737 as a test organism. An explanation why the effect of all compounds on this bacterium is better at pH 5·6 than at pH 6·2, might be that the former pH is near the optimum pH value for certain enzyme activities in *C. acnes* (Puhvel & Reisner, 1972). It is interesting to note that pH 5·6 is also nearly the same as the pH of the skin (Davis, 1972).

August 26, 1975

REFERENCES

CLAUSEN, O. G. (1973). *Pharm. Ind.*, **35**, 869–874.

CLAUSEN, O. G., AASGAARD, N. & SOLBERG, O. (1973). *Ann. Microbiol.* (Inst. Pasteur), **124B**, 205–216.

CUNLIFFE, W. J. (1973). *Br. med. J.*, **4**, 667–669.

DAVIES, A. (1973). *J. periodont. Res.*, **8**, Suppl. 12, 68–75.

DAVIS, J. G. (1972). *J. Soc. cosmet. Chem.*, **23**, 45–71.

HALL-SMITH, P. & MARKS, R. (1973). *Br. J. Hosp. Med.*, **10**, 410–427.

JUNGERMANN, E. & TABER, D. (1971). *J. Am. Oil Chem. Soc.*, **48**, 318–323.

KIRSCHBAUM, J. O. & KLIGMAN, A. M. (1963). *Archs Derm.*, **88**, 832–833.

MONTES, L. F. & PITTILLO, R. F. (1972). *J. invest. Derm.*, **59**, 260–263.

PUHVEL, S. M. & REISNER, R. M. (1972). *Ibid.*, **54**, 252–255.

SENIOR, N. (1973). *J. Soc. cosmet. Chem.*, **24**, 259–278.

SHEHADEH, N. H. & KLIGMAN, A. M. (1963). *Archs Derm.*, **88**, 829–831.