THE ANTIMYCOBACTERIAL ACTIVITY OF VARIOUS AMINES RELATED TO SPERMINE IN CHEMICAL STRUCTURE

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In the course of investigations dealing with the influence of local biochemical conditions on the fate of tubercle bacilli *in vivo*, it was demonstrated that a powerful antimycobacterial action was exerted under certain conditions *in vitro* by the naturally occurring organic base spermine (1). This substance inhibited the multiplication of several strains of bovine and human tubercle bacilli, but had little or no effect on the growth of saprophytic mycobacteria or on a variety of non-acid-fast microorganisms.

The present communication presents the effect on the growth of tubercle bacilli of various biological and synthetic amines bearing structural similarity to spermine. Of the substances examined, only the closely related compound, spermidine, manifested antimycobacterial activity comparable to that of spermine.

Materials and Methods

The antimycobacterial tests were performed in liquid medium of the following composition: asparagine, 0.2 per cent; KH_2PO_4, 0.1 per cent; $Na_2HPO_4 \cdot 12\ H_2O$, 0.63 per cent; $MgSO_4 \cdot 7H_2O$, 0.001 per cent; $CaCl_2$, 0.000005 per cent; $CuSO_4$, 0.00001 per cent; $ZnSO_4$, 0.00001 per cent. No tween was included. All ingredients were dissolved in distilled water, and the medium was adjusted to pH 6.7. Aluminum-capped tubes 25 mm. in diameter containing 4 cc. of medium were sterilized by autoclaving for 15 minutes at 15 pounds pressure.

Free amines or their salts, obtained from commercial sources except as otherwise noted in Table I, were dissolved in distilled water and adjusted approximately to neutrality by the addition of normal HCl or NaOH. These solutions were then diluted to the desired strength in distilled water and 0.5 cc. was added to the tubes containing medium. Two sets of experiments were carried out; in one the amine solutions were sterilized by autoclaving with the medium, while in the other sterilization was accomplished by filtration through porcelain (Coors No. 762 P3). Since identical results were obtained using these two techniques, no distinction is made in the section which follows.

Stock cultures of an attenuated strain of tubercle bacillus (BCG-Phipps) maintained in standard Dubos tween-albumin medium served as the source of test organisms. 10 day old stock cultures were diluted 1:100 into a sterile solution containing 5 per cent glucose and 5 per cent bovine plasma fraction V (Armour and Company, Chicago). The addition of 0.5 cc. of this dilute suspension of organisms to each tube in the test produced a final concen-

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tration of 0.5 per cent glucose, 0.5 per cent albumin, and 10^{-3} of the fully grown stock culture.

The tubes were incubated at 38°C. When growth developed, the bacilli formed large clumps which could be partially dispersed by shaking. After incubation for 10 days, readings of growth were made by visual examination and comparison with appropriate control tubes containing no added amine.

RESULTS

The results are summarized in Table I. A final concentration of 3×10^{-5} M spermine or spermidine (approximately 6 μg. per cc.) inhibited the multipli-

TABLE I

The Antimycobacterial Activity of Various Amino Compounds

Growth of tubercle bacilli inhibited in a medium containing a final concentration of 3×10^{-5} M	
Spermine* $NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$	
Spermidine‡ $NH_2(CH_2)_3NH(CH_2)_4NH_2$	
Growth of tubercle bacilli not inhibited in a medium containing a final concentration of 3×10^{-4} M	
Methylamine	*N*,*N*-Dimethyl-1,3-diaminopropane
Ethylamine	Pyrrolidine
n-Propylamine	Piperidine
n-Butylamine	Piperazine
1,3-Diaminopropane	Histamine
Putrescine	2-Aminopyridine
Cadaverine	2-Aminopyrimidine
Diethylenetriamine	γ-Aminopropyl-*N*-pyrroline§
Triethylenetetramine	γ-Aminopropyl-*N*-pyrrolidine§
Tetraethylenepentamine	

* Spermine tetrahydrochloride, synthetic, obtained from Hoffmann-La Roche, Nutley, New Jersey.

‡ Spermidine phosphate, synthetic, obtained from Hoffmann-La Roche, Nutley, New Jersey.

§ Prepared from the hydrolysis of spermine with copper powder, alkali, and air after Wrede *et al.* (2, 3).

cation of tubercle bacilli under the conditions of the test. Even tenfold higher concentrations of the various other amines did not manifest similar activity. Especially noteworthy was the lack of activity of diethylenetriamine and triethylenetetramine, compounds similar in structure to spermidine and spermine except for the length of the carbon chain between nitrogen atoms. Also, no suppression of growth of tubercle bacilli resulted from the addition to the medium of various isolated portions of the spermine molecule (*n*-propylamine, 1,3-diaminopropane, *n*-butylamine, putrescine) or the products of the chemical degradation of spermine (1,3-diaminopropane, γ-aminopropyl-*N*-pyrroline, γ-aminopropyl-*N*-pyrrolidine) (2, 3). The mechanisms which may account

for the apparently specific antimycobacterial activity of spermine and spermidine are under investigation in our laboratory; these studies are reported in an accompanying communication (4).

SUMMARY

A final concentration of 3×10^{-5} M spermine or spermidine inhibited the growth of mammalian tubercle bacilli in a modified Dubos liquid medium. Various related amino compounds, including isolated portions of the spermine molecule and the products of its chemical degradation, did not manifest similar antimycobacterial activity.

BIBLIOGRAPHY

1. Hirsch, J. G., and Dubos, R. J., *J. Exp. Med.*, 1952, **95,** 191.
2. Wrede, F., Fanselow, H., and Strack, E., *Z. physiol. Chem.*, 1926, **153,** 291.
3. Wrede, F., Fanselow, H., and Strack, E., *Z. physiol. Chem.*, 1926, **161,** 66.
4. Hirsch, J. G., *J. Exp. Med.*, 1953, **97,** 327.