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Studies on the Antibacterial Action of Spermine

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SUMMARY: Spermine was found to inhibit the growth of various micro-organisms. The most susceptible were *Staphylococcus aureus* and *Staph. albus*, *Neisseria gonorrhoeae* and *N. meningitidis*, and to a lesser degree *Bacillus anthracis*. The Enterobacteriaceae were relatively resistant. The action of spermine was enhanced by an increase in pH of the medium. The antibacterial effect was bactericidal; it was slowed but not prevented by a decrease in temperature from 37 to 6°. The nutrients of various media influenced the action of spermine.

Spermine, a biological polyamine, is a substance of controversial nature. It has been shown to possess growth-inhibiting properties against various micro-organisms (Bichowsky-Slomnitzki, 1948; Gurevitch, Rozansky, Weber, Brzezinsky & Eckerling 1951; Hirsch & Dubos, 1952; Rozansky, Gurevitch, Brzezinsky & Eckerling, 1949) and growth-promoting activity for *Haemophilus parainfluenzae* (Herbst & Snell, 1948). It has also been found to counteract the antibacterial effect of atebriene, propamidine and quinine on *Escherichia coli* (Silverman & Evans, 1943, 1944; Miller & Peters, 1945). Spermine is widespread in human and animal body tissue (Guggenheim, 1940; Harrison, 1933). Its role, if any, as an antibacterial agent which affords natural resistance to the body, is unknown. Further studies of this material therefore seemed worthwhile. The present investigation is concerned with the following aspects of the antibacterial action of spermine: mode of action; influence of pH; influence of temperature; sensitivity of different bacteria.

MATERIALS AND METHODS

Spermine tetrahydrochloride (Hoffmann La Roche) was used throughout the experiments. The micro-organism studied most extensively was *Staphylococcus aureus* no. 23, a haemolytic coagulase and mannitol positive strain. The other bacteria used are listed in Table 2.

Antibacterial assays of spermine were made by (a) serial dilution and (b) the cup method. Serial dilutions of spermine were made in Wassermann tubes (13 × 100 mm.) containing 2 ml. of Difco nutrient broth. Controls without spermine were included in all experiments. The cup method was the same as previously reported (Rozansky *et al.* 1949). Difco nutrient agar was used except when otherwise specified.

RESULTS

Influence of pH value of the medium on the growth inhibition of Staph. aureus by spermine

The antibacterial activity of spermine rose gradually with the increase of the pH of the medium (see Fig. 1). Change in pH value from 6.2 to 8.75 decreased

the amount of spermine required to obtain complete inhibition of *Staph. aureus* from 1000 $\mu\text{g.}/\text{ml.}$ to 2 $\mu\text{g.}/\text{ml.}$ The growth of the test organism in the control tubes, i.e. in the absence of spermine, was almost unaffected by these changes in pH value.

Fig. 1. Minimal concentration of spermine inhibiting the growth of *Staph. aureus* at various pH values of the medium. Size of inoculum *c.* 10^4 cells/ml. Medium: Difco nutrient broth. Results read after 48 hr. incubation. Inhibition i.e. no visible growth.

Bactericidal effect of spermine

In experiments with *Staph. aureus* 23, the size of inoculum did not affect appreciably the inhibitory activity of spermine. A 100- and 10,000-fold increase of the inoculum required a twofold and eightfold increase of spermine respectively.

These results suggested that the drug exerted a bactericidal effect. In order to test this, *Staph. aureus* was inoculated into flasks of Difco nutrient broth (pH 7.7) containing 25 $\mu\text{g.}/\text{ml.}$ and 50 $\mu\text{g.}/\text{ml.}$ of spermine and into control flasks without spermine. The flasks were incubated at 37° and at varying intervals of time (Table 1) samples were taken for the determination of viable counts. The pour-plate method was used, and plates were incubated for 48 hr. at 37°. All counts were performed in triplicate.

At 37° (see Table 1) 25 $\mu\text{g.}$ spermine/ml. for 3 hr. killed 70% of the staphylococci. Fifty $\mu\text{g.}$ spermine/ml. were even more effective, killing more than 99% of the bacteria in 3 hr. After 10 hr. incubation all flasks containing 25 and 50 $\mu\text{g.}$ spermine/ml. were sterile.

In order to determine whether spermine was effective against non-multiplying bacteria, broth samples containing varying concentrations of spermine were inoculated with *Staph. aureus* 23. The flasks were kept at 6° and the number of surviving bacteria was determined after 24, 72 and 96 hr. by the plate-count technique. Controls without spermine were run simultaneously.

Table 1. *Effect of spermine on Staphylococcus aureus in Difco nutrient broth, pH 7.7*

Temp. of contact (°)	Time of contact (hr.)	Concentration of spermine (μ g./ml.)											
		0			25			50			100		
		No. of colonies in thousands*	No. of colonies in thousands*	% of survivors	No. of colonies in thousands*	% of survivors	% of survivors	No. of colonies in thousands*	% of survivors	% of survivors	No. of colonies in thousands*	% of survivors	% of survivors
6	0	14	13	100	12	100	3.8	0.46	100	1.4	100	100	100
	24	14	3.3	25.3	0.3	2.3	0.4	0.05	0.18	0.1	0.03	0.2	0.2
	72	8	0.3	3.8	0.08	0.6	0.13	0.015	0.015	0.1	0	0	0
	96	7	0.08	1.1	8	100	0.6	0.015	0.13	0.1	0	0	0
37	0	14	8	100	10	100	0.6	0.06	100	—	—	—	—
	3	18	2.3	30	0.4	0.5	0	0	0.6	—	—	—	—
	10	12,000	0.04	0.5	0	0	0	0	0	—	—	—	—
	24	900,000	0	0	0	0	0	0	0	—	—	—	—

* Number of colonies developing on agar plates poured with 1 ml. samples of the broth. Counts performed after 48 hr. incubation of the plates at 37°.

It is seen from Table 1 that spermine killed the staphylococci in the cold. The action at 6° was however markedly slower than at 37°, 25 µg./ml. killed about 75 % of the staphylococci in 24 hr.; 50 µg./ml. killed 96 % of the cells in the same time; 400 µg./ml. killed 99·8 % after 24 hr.

Susceptibility of different micro-organisms to spermine

Table 2 shows that the organisms most sensitive to the inhibitory action of spermine were staphylococci, both aureus and albus types. In Difco nutrient agar at pH 7·3 and 7·5, 50 and 25 µg. of spermine, respectively, diluted in 0·1 ml. of saline were required to produce a clear zone of inhibition (not less than 12 mm. in diameter) by the cup technique. In Difco nutrient broth at the same pH values (i.e. 7·3 and 7·5) a concentration of 80 and 15 µg./ml. caused full inhibition.

Neisseria gonorrhoeae and *N. meningitidis* tested on glucose starch agar (Difco) and ascites agar were inhibited to a similar extent to that found with staphylococci.

Various Gram-negative rods of the family Enterobacteriaceae were only slightly affected by the drug. *Shigella flexneri*, however, was found to be quite sensitive at an alkaline pH. Haemolytic streptococci and *Corynebacterium hofmanni* were tested for their sensitivity to spermine in nutrient broth enriched with brain heart infusion (4 parts Difco nutrient broth and 1 part Difco brain heart infusion broth) and were found to be inhibited by 250 µg./spermine ml. (Table 2).

COMMENT

The degree of inhibition of bacterial growth by spermine depended on several factors. One of these was the pH of the medium. In Fig. 1 an almost straight line was obtained when the inhibitory activity of spermine on *Staph. aureus* 23 was plotted against the pH of the medium. It seemed, therefore, that the undissociated molecule rather than the ionized form was responsible for the antibacterial properties. The influence of the pH was less marked with *Bacillus anthracis* and *Sh. flexneri* and still less evident with the more resistant enterobacteriaceae and *Streptococcus faecalis* (Table 2). Hirsch & Dubos (1952) found that the inhibition of growth of *Mycobacterium tuberculosis*, an organism highly susceptible to spermine, was independent of the pH value of the medium between 6·3 and 7·4.

The action of spermine was primarily bactericidal and was also evident against non-multiplying organisms. Hirsch & Dubos (1952) have demonstrated the bactericidal effect of spermine for *Myc. tuberculosis*.

The activity of spermine was influenced by the medium employed. Organisms grown in brain-heart infusion were much less affected than in nutrient broth (Table 2). The spermine antagonizing effect of Bacto stock agar has been described previously (Gurevitch *et al.* 1951). It is possible that the relatively high resistance of haemolytic streptococci and *C. hofmanni* which were tested in brain-heart infusion was due to the presence of spermine antagonists in these complex media.

Table 2. *Inhibition of growth of various micro-organisms by spermine*

Strain	Medium	pH of medium	Concentration of spermine showing inhibition using		Remarks
			Dilution method μg./ml.*	Cup method μg./0.1 ml.†	
<i>Staph. aureus</i> 23	Nutrient broth or agar	6.2	1000	—	—
	Nutrient broth or agar	6.4	500	—	—
	Nutrient broth or agar	6.8	125	100	—
	Nutrient broth or agar	7.1	60	—	—
	Nutrient broth or agar	7.3	30	50	—
	Nutrient broth or agar	7.5	15	25	—
	Nutrient broth or agar	8.0	8	12	—
	Nutrient broth or agar	8.4	6	6	—
	Nutrient broth or agar	8.7	2	—	—
	4 parts nutr. br. and 1 part brain-heart broth	7.1	250	—	—
<i>Staph. albus</i> B	4 parts nutr. br. and 1 part brain-heart broth	7.9	60	—	—
<i>Staph. albus</i> H5	Nutrient broth or agar	6.8	500	—	—
	Nutrient broth or agar	8.0	4	—	—
<i>B. anthracis</i>	Nutrient broth or agar	6.8	30	—	—
	Nutrient broth or agar	8.0	1	—	—
<i>Sh. flexneri</i> 1150	Nutrient broth or agar	6.2	1000	—	—
	Nutrient broth or agar	6.8	500	100	—
	Nutrient broth or agar	7.3	250	—	—
	Nutrient broth or agar	7.9	125	—	—
	Nutrient broth or agar	8.7	60	25	—
<i>E. coli</i> 055	Nutrient broth or agar	6.4	1000	—	—
	Nutrient broth or agar	7.0	250	—	—
	Nutrient broth or agar	8.0	125	50	—
	Nutrient broth or agar	8.5	125	—	—
	Nutrient broth or agar	8.8	—	25	—
<i>Sal. typhi</i>	Nutrient broth or agar	6.8	1000	—	—
	Nutrient broth or agar	7.3	1000	—	—
<i>Str. faecalis</i>	Nutrient broth or agar	6.8	1000	—	—
	Nutrient broth or agar	7.3	1000	—	—
<i>Str. faecalis</i> 19	Nutrient broth or agar	6.8	1000	—	—
	Nutrient broth or agar	8.0	1000	—	—
<i>Myco. phlei</i>	Nutrient broth or agar	6.8	1000	—	—
	Nutrient broth or agar	7.7	1000	—	—
<i>C. hofmannii</i>	4 parts nutr. br. and 1 part brain-heart broth	7.1	250	—	—
<i>Str. haemolyticus</i>	4 parts nutr. br. and 1 part brain-heart broth	7.1	250	—	2 strains
<i>N. gonorrhoeae</i>	Dextrin starch agar	7.5	—	50	5 strains
	Ascites agar	7.6	—	50	2 strains
<i>N. meningitidis</i>	Dextrin starch agar	7.5	—	50	4 strains
	Ascites agar	7.6	—	50	2 strains

* Concentration of spermine causing inhibition of visible growth after 48 hr. incubation at 37°. Inoculum c. 10,000 organisms/ml. Twofold dilution technique.

† Concentration of spermine causing a clear inhibition zone of not less than 12 mm. in diameter, 0.1 ml. of samples containing the specified amounts were introduced into the cups.

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