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Antibacterial Action of Spermine: Effect on Urinary Tract Pathogens

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The basic polyamine spermine was tested for antibacterial activity at two *p*H levels by the modified cup method against a variety of gram-positive and gram-negative organisms isolated from urine. At *p*H 6.4, with concentrations ranging from 39 to 2,500 μg per 0.1 ml, there were no clear zones of inhibition seen with any of the gram-negative test organisms, although some adverse effect on growth within the area of the cylinder was noted in 36%. Three of 17 gram-positive strains were inhibited at this *p*H. Spermine was more active at *p*H 7.4, but even at the highest concentrations only 16% of the gram-negative and 47% of the gram-positive bacteria tested showed definite zones of inhibition. It is concluded that spermine probably plays little, if any, role in natural resistance to urinary tract infections in vivo.

Spermine $H_2N—(CH_2)_3—NH—(CH_2)_4—NH—(CH_2)_3—NH_2$, a polyamine found widely distributed throughout animal tissues, has been reported to have antibacterial activity against a variety of microorganisms (5, 11). This compound, originally isolated from semen, occurs in very high concentrations in the prostate of many mammalian species (8) and is thought to be responsible for the antibacterial action of human semen (6, 9) against gram-positive organisms. In an effort to determine the role of spermine as a natural defense mechanism against urinary tract infections, we have studied the effect of spermine against a variety of microorganisms responsible for the bulk of such infections in the human.

MATERIALS AND METHODS

Organisms. The organisms employed in the study were isolated from urine cultures of patients having urinary tract infections.

Other materials. Spermine was used as the tetrahydrochloride salt and was obtained from Sigma Chemical Co., St. Louis, Mo. The Mueller-Hinton agar was obtained from Difco. The stainless-steel cylinders had an outside diameter of 9 mm and were obtained from S & L Metal Products Corp.

Assay method. The Mueller-Hinton agar was prepared fresh weekly. The medium was adjusted to *p*H 6.4 or 7.4 before autoclaving. The medium was then poured into sterile petri dishes (150 by 15 mm). After solidifying, the plates were flooded with 4 ml of a saline suspension of the test organism as described by Ericsson (2-4).

Eight hollow stainless-steel cylinders were evenly distributed on the surface of the agar after slightly warming the cylinders to ensure a proper cylinder-agar seal.

Stock aqueous solutions of spermine tetrahydrochloride (25 mg/ml) were adjusted to *p*H 6.4 and 7.4 with 1 N NaOH, frozen, and stored at −20 C until use. Serial dilutions of the stock solutions were made to final concentrations of 0.39 mg/ml. One-tenth milliliter of each dilution was then placed in each of the metal cups on the proper media with respect to *p*H. Thus, each organism was tested against a spermine solution at *p*H 6.4 and 7.4 on an agar plate at the same *p*H.

Each plate was allowed to stand at room temperature for 1 hr to permit diffusion of the samples into the agar. The plates were then incubated at 37 C for 18 hr. The zones of inhibition were measured to the nearest millimeter. Many of the spermine solutions did not produce a definite zone of inhibition (equal to or greater than 11 mm) around the cylinder but did demonstrate inhibition within the cylinder area, indicating some detrimental effect on bacterial growth. In these cases, a grading system was devised to define this activity (Fig. 1): 1+, definite diminution in colony count within the cylinder as compared to the surrounding agar; 2+, colonies inside the cylinder area were fewer and generally smaller than above; 3+, zero to five colonies within the cylinder area but no activity beyond this margin; 4+, no colonies within the cylinder area and a slight zone of inhibition around the edge of the cylinder measuring less than 11 mm in diameter.

RESULTS

Table 1 lists the effect of spermine tetrahydrochloride on the gram-negative organisms at two *p*H levels. Below the 312-μg level there was no inhibition of growth noted, except a 1+ inhibi-

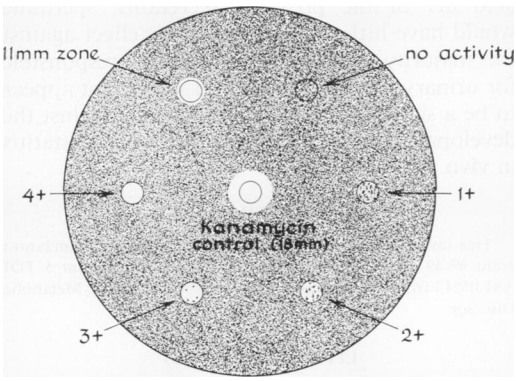

FIG. 1. *Degrees of inhibition of bacteria by spermine.*

tion of an *Escherichia coli* strain at 156 μg. At higher concentrations of the polyamine, the number of inhibited organisms increased so that, with 2,500 μg per 0.1 ml at *p*H 7.4, over 90% of the *Klebsiella* and *E. coli* had some clearing within the area of the cylinder but no zones around the cylinder.

As previously reported (10), the influence of *p*H had a marked effect on the inhibitory activity of spermine. The organisms were tested at *p*H 6.4 to simulate the conditions present in the acidic prostatic fluid and at *p*H 7.4 to approximate more closely the conditions found in the slightly alkaline human semen. None of the test organisms showed any measurable zones of inhibition at

TABLE 1. *Effect of 2,500 μg of spermine tetrahydrochloride*

Organism	No. tested	Per cent showing some inhibition in cylinder		Per cent showing measurable zone around cylinder	
		*p*H 6.4	*p*H 7.4	*p*H 6.4	*p*H 7.4
Escherichia coli	15	33	93	0	40
Klebsiella sp.	11	55	91	0	9
Pseudomonas sp.	8	13	25	0	0
Proteus sp.	5	0	0	0	0
Aerobacter sp.	5	80	80	0	0
Serratia marcescens	1	0	0	0	0
Total	45	36	67	0	16

TABLE 2. *Effect of various concentrations of spermine[a] on gram-positive organisms*

Organism	39 μg		78 μg		156 μg		312 μg		625 μg		1,250 μg		2,500 μg	
	*p*H 6.4	*p*H 7.4	*p*H 6.4	*p*H 7.4	*p*H 6.4	*p*H 7.4	*p*H 6.4	*p*H 7.4	*p*H 6.4	*p*H 7.4	*p*H 6.4	*p*H 7.4	*p*H 6.4	*p*H 7.4
Staphylococcus epidermidis	0	++[b]	0	+++	0	++++	+	12[c]	++	15	+++	17	11	21
S. epidermidis	0	0	0	+	0	++	0	+++	+	++++	++	11	+++	13
S. epidermidis	0	++++	+	11	++	17	+++	20	11	23	12	28	16	31
S. epidermidis	0	0	0	0	0	++	0	+++	0	+++	+	++++	++	13
S. epidermidis	0	0	0	+	0	++	++	++	+++	+++	+++	++++	+++	11
Streptococcus faecalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. faecalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. faecalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. faecalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. faecalis	0	0	0	0	0	0	0	0	0	0	0	0	+	+
Streptococcus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Streptococcus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Streptococcus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Streptococcus sp.	0	0	0	0	0	0	0	0	0	0	0	12	0	16
Streptococcus sp.	0	0	0	+	0	+	0	++	0	++++	0	12	+	15
Streptococcus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bacillus subtilis	0	++++	+	11	++	14	++++	18	12	21	14	24	17	26

[a] Content of spermine tetrahydrochloride is expressed as micrograms per 0.1 ml.
[b] Degree of inhibition within the cylinder in the absence of an external zone of inhibition.
[c] Diameter of zone of inhibition in millimeters.

*p*H 6.4, even in the cylinders containing 2,500 μg of the compound.

Table 2 lists the effects of spermine tetrahydrochloride in increasing concentrations on the gram-positive organisms. The most sensitive organisms were the five strains of *Staphylococcus epidermidis*. Each of the five had a definite zone of inhibition demonstrated at *p*H 7.4 at a concentration of 2,500 μg per 0.1 ml. When the *S. epidermidis* strains were tested at *p*H 6.4, only two of five showed a definite zone at the same spermine level. Eleven streptococci were also tested. Definite zones of inhibition were present against only 2 of the 11; at *p*H 6.4, only 2 of the 11 had even a minimal degree of growth inhibition.

Overall clear zones of inhibition were seen in 8 of the 17 organisms spread over agar plates at *p*H 7.4. At *p*H 6.4, only 3 of the 17 showed such inhibition.

DISCUSSION

Several authors have investigated the antimicrobial action of spermine (5–7, 9, 10). Most of these reports, however, have centered on the effect of the polyamines on gram-positive bacteria, and only a few studies of the action of spermine on gram-negative organisms have been reported. Thus, Gurevitch et al. (6) reported no inhibition of growth of various gram-negative bacteria by human semen at *p*H 7.4. Rozansky et al. (11) found some inhibition of growth of *Shigella flexneri*, *Salmonella typhosa*, *Neisseria gonorrhoeae*, *N. meningitidis*, and an 055 strain of *E. coli* with spermine by using the tube dilution method. Hirsch and Dubos (7) also reported no inhibition of growth of *Pseudomonas aeruginosa*, *K. pneumoniae*, and *Proteus vulgaris* by spermine.

Since the human prostate gland and prostatic fluid are extremely rich in spermine (12), the present study was performed as the initial step in determining what role, if any, spermine may play as a natural defense mechanism against urinary tract infections in the human male.

Our studies would indicate that, at the normal acid *p*H of the prostatic secretions, spermine would have little, if any, inhibitory effect against the majority of organisms normally responsible for urinary tract infections and would not appear to be a significant defense mechanism against the development of posterior urethritis or prostatitis in vivo.

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