THE ROLE OF SPERMINE IN THE INHIBITION OF *STAPHYLOCOCCUS AUREUS* BY HUMAN SEMEN

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(RECEIVED FOR PUBLICATION FEBRUARY 2, 1951)

The inhibitory activity of human semen on the growth of *Staphylococcus aureus* has been reported previously (Rozansky, Gurevitch, Brzezinsky, and Eckerling, 1949).

The Function of Human Semen

The investigation has now been extended using 12 additional freshly isolated strains of *Staph. aureus*.

Materials and Methods.—Seventy-five specimens of semen from 53 patients (of the male sterility clinic) between the ages of 23 and 51 years were examined. Six of these were azoospermic, 25 oligospermic (less than 60 million spermatozoa per ml.), and 22 were normospermic. Ten specimens of human blood serum, 10 of cerebrospinal fluids, 10 of fluids from ovarian cysts, eight of amniotic fluids, four of pleural exudate, three of tears, and three of ox semen* were also tested in a similar manner. Materials were only taken from patients who had not received antibiotic treatment. The specimens of human semen were kept at room temperature for four hours before examination, then tested immediately, or after 72 hours at 8° C. or 14 days' refrigeration at 8° C. Twelve specimens were also examined after heating at 90° C. for 30 minutes. In addition to the three strains of *Staph. aureus* used in the previous study, 12 freshly isolated strains from surgical cases or pyogenic skin infections were tested. All strains were actively haemolytic, coagulase-positive, and mannitol-positive. The method described in the previous study (Rozansky *et al.*, 1949) was used. On plates inoculated with the test strain of *Staph. aureus*, six cups with an external diameter of 8 mm. were placed. Each cup received one or two drops of the fluid to be tested, the plates were incubated overnight at 37° C., and the zone of inhibition measured. Wherever possible, two examinations of each specimen were made. Difco nutrient agar of pH 6.8, used throughout the previous experiments, was employed at the beginning of this series, but it was later found that a slightly alkaline medium gave better results. The remaining tests were performed on agar plates of pH 7.4.

Results.—As may be seen from Table I, of the 41 specimens of semen examined, 37 inhibited the growth of the Heatley strain of *Staph. aureus* over an area 10–25 mm. in diameter. Of these 37 specimens, 14 were normospermic, 21 oligospermic, and two azoospermic. Nineteen of these specimens were tested in duplicate and similar

* Obtained by courtesy of Dr. Y. Flesh, Jerusalem, and Mr. R. Mentzel, Israel Cattle Breeders' Association centre for artificial insemination, Kinereth.

TABLE I
INHIBITION OF THE GROWTH OF *Staphylococcus aureus* (HEATLEY STRAIN) BY
HUMAN SEMEN

	No. of Specimens Tested	Positive	Negative
Normospermic	17	14	3
Oligospermic	21	21	0
Azoospermic	3	2	1
Total ..	41	37	4

Semen tested after 4 hours' storage at room temperature.

Positive = Area of inhibition 10-25 mm. in diameter.

Negative = No inhibition.

areas of inhibition were found on both plates. Four specimens (three normospermic and one azoospermic) showed no area of inhibition when first examined, i.e., four hours after the collection of the material, but one of these retested after 72 hours produced an area of inhibition 13 mm. diameter on two plates. The other three specimens could not be examined a second time. Seven specimens heated for 30 minutes at 90° C. all retained their inhibitory effect on the growth of *Staph. aureus*.

Table II summarizes the results obtained with 21 specimens after 72 hours' cold storage. Twelve specimens were of the series already tested four hours after collection, and nine were tested for the first time after 72 hours' storage. Of the 21 specimens, 20 (eight normospermic, ten oligospermic, and two azoospermic) gave positive results, 15 of them tested in duplicate, and six on one plate only. Four of these specimens were heated at 90° C. for half an hour. The results showed no change in their inhibitory activity.

TABLE II
SEMEN TESTED AFTER 72 HOURS' STORAGE AT 8° C.

	No. of Specimens Tested	Positive	Negative
Normospermic	9	8	1
Oligospermic	10	10	0
Azoospermic	2	2	0
Total ..	21	20	1

Table III summarizes the results with 12 specimens after 14 days' storage in the refrigerator. Eight specimens (three normospermic, three oligospermic, and two azoospermic) were found to be positive, and four (one normospermic and three oligospermic) negative. Three of the specimens giving negative results had been positive when initially examined four hours after the collection of the specimens.

Table IV shows the results of the inhibitory effect of human semen on 14 different strains of *Staph. aureus*. One hundred and twenty-four tests were made with 75 specimens of semen. Strains No. 598 and No. 924, examined at the beginning of the present study, were tested with agar plates of pH 6.8 which may account for the greater number of negative results obtained.

TABLE III
SEMEN TESTED AFTER 14 DAYS' STORAGE AT 8° C.

	No. of Specimens Tested	Positive	Negative
Normospermic	4	3	1
Oligospermic	6	3	3
Azoospermic	2	2	0
Total ..	12	8	4

TABLE IV
INHIBITORY EFFICACY OF HUMAN SEMEN ON 14 FRESHLY ISOLATED STRAINS OF
Staphylococcus aureus

<i>Staph. aureus</i> Strain	No. of Specimens Tested	Positive	Negative	Media
598	9	5	4	pH 6.8
924	7	4	3	" "
1498	6	5	1	pH 7.4
1605	7	5	2	
1705	1	1	0	
1718	3	3	0	
2191	14	14	0	
2196	3	3	0	
2315	2	2	0	
2528	6	6	0	
2200	5	4	1	
750	4	4	0	
314	4	4	0	
2201	4	3	1	
Totals 14	75	63	12	

Positive = Area of inhibition 12-25 mm.

Negative = No inhibition.

Starting with *Staph. aureus* strain No. 1498, agar plates of pH 7.4 were used, and on alkalinized agar 54 specimens were positive and only five gave negative results, with areas of inhibition of the freshly isolated strains similar to those obtained with the Heatley strain. The three specimens, after cold storage for 21 days, tested with *Staph. aureus* No. 1718, gave positive results. From these results it is obvious that freshly isolated strains of *Staph. aureus* are as readily inhibited as the Heatley strain which has been used in our laboratory for several years.

Discussion

As the inhibitory effect of semen on the growth of *Staph. aureus* was observed both in normospermia and azoospermia, the active principle cannot be related to the presence of spermatozoa but is contained in the fluid portion of the semen. That the active principle is thermostable was shown by 12 specimens of semen

which, after being kept at 90° C. for half an hour, were tested with one or more strains of *Staph. aureus*; all retained their inhibitory activity.

Three specimens of ox semen were tested using four strains of *Staph. aureus*. Twelve examinations were performed, all of which gave negative results. Since the pH of ox semen is 6.6–6.8, four additional tests were made after alkalinization to a pH of 7.6–8.0 in order to approximate it to the pH of human semen. However, these alkalinized ox semen specimens also gave negative results.

Serum, tears, amniotic fluid, cerebrospinal fluid, pleural exudate, and fluid from an ovarian cyst were tested in a similar manner and did not show any signs of inhibitory activity. Four specimens of semen were tested against *Esch. coli*, *Salm. paratyphi A*, *Ps. pyocyanea*, and *Bact. alkalescens*, and, as previously reported, showed no evidence of inhibition.

The Hypothetical Function of Spermine and Spermidine

In view of the thermostability of the growth-inhibitory agent it was concluded that this agent was not a protein. It was assumed that the poly-amines, spermine and spermidine, found in human semen might be responsible for the inhibition of bacterial growth. Inhibition of the growth of *Staph. aureus* by spermine was observed by Bichowsky-Slomnitzki (1948). Synthetic spermine tetrahydrochloride and spermine phosphate extracted from human semen were therefore tested for their inhibitory effect on the growth of *Staph. aureus*.

Materials and Methods.—Spermine tetrahydrochloride (La Roche) was dissolved in distilled water in dilutions varying from 12.5 mg.% to 200 mg.%. Spermine phosphate was extracted from pooled human semen, according to the method described by Harrison (1933). Each of three pooled samples, obtained from 20 ml. of semen, yielded about 5 mg. of crystalline spermine phosphate. Since spermine phosphate is readily soluble in slightly alkaline solutions (Guggenheim, 1940), a 200 mg.% solution was made in distilled water by adding n/100 NaOH till pH 7.4 was reached.

The inhibitory activity of spermine tetrahydrochloride was tested on the Heatley strain and five freshly isolated strains of *Staph. aureus*, and spermine phosphate was tested on the Heatley strain and three of these other strains. All the strains were actively haemolytic, coagulase-positive, and mannitol-positive.

The method used was that described above, using Difco nutrient agar of pH 6.8 and the same agar alkalinized to pH 7.4 by adding a few drops of n/10 NaOH.

Results.—Using alkalinized agar (pH 7.4), spermine tetrahydrochloride (Table V) in a concentration of 200 mg.% inhibited the bacteria in all the tests. A concentration of 100 mg.% inhibited the growth in 43 of 44 tests, and a concentration of 50 mg.% inhibited the growth in 24 of 27 tests. Using a concentration of 25 mg.%, it inhibited 17 of 22 tests, but with a concentration of 12.5 mg.% it inhibited only five of 13 tests. Using the same concentration in an agar of pH 6.8, 24 of 25 tests were negative and only one test, with a concentration of 100 mg.% spermine tetrahydrochloride, was positive.

Three specimens of spermine phosphate extracted from pooled human semen were similarly tested (Table VI). Twelve tests using a concentration of 200 mg.%, and nine tests with a concentration of 100 mg.% on agar of pH 7.4 all gave positive results, the area of inhibition being 11–18 mm. in diameter. With agar of pH 6.8, of eight

TABLE V
INHIBITION OF SIX STRAINS OF *Staph. aureus* BY SPERMINE TETRAHYDROCHLORIDE
(LA ROCHE)

Concentration (mg. %)	Medium pH 7.4			Medium pH 6.8		
	No. of Tests	Positive	Negative	No. of Tests	Positive	Negative
200	10	10	0	4	0	4
100	44	43	1	8	1	7
50	27	24	3	7	0	7
25	22	17	5	4	0	4
12.5	13	5	8	2	0	2

Positive test = Area of inhibition 11–18 mm. in diameter.

Negative test = No inhibition.

examinations, six were positive (area of inhibition 10–14 mm. in diameter), and only two negative.

Discussion

The inhibitory activity of spermine tetrahydrochloride is influenced by the pH of the agar. Only very high concentrations of spermine tetrahydrochloride were able to inhibit the growth on agar of pH 6.8. A concentration of 1,000 mg.% of spermine tetrahydrochloride was tested twice, an area of inhibition up to 22 mm. being found on agar of pH 7.4, and an area of 18 mm. on agar with pH 6.8. With extracted spermine phosphate, the effect of the pH of the medium was not as striking and the results obtained resembled the findings with human semen, where greater areas of inhibition were obtained on alkaline agar of pH 7.4 than on agar of pH 6.8. As reported above, three specimens of ox semen of pH 6.6–6.8, and the same specimens alkalized to pH 7.6 and 8.0, had no inhibitory effect on the growth of *Staph. aureus*. Since ox semen does not contain spermine (Guggenheim, 1940; Harrison, 1931) this furnishes additional evidence of the role of spermine in inhibiting the growth of *Staph. aureus* by semen. The concentrations of spermine tetrachloride and spermine phosphate used in our experiments were within the range of the spermine phosphate concentrations found in human semen, i.e., 60–250 mg.% (Guggenheim, 1940; Harrison, 1933). The results of the present study seem to warrant the conclusion that spermine is the principle active in the inhibition of bacterial growth by human semen plasma.

The role of spermidine, the second poly-amine present in human semen, has not yet been examined.

Spermine tetrahydrochloride, spermine phosphate, and plasma of human semen showed no inhibitory activity when tests were performed with Bacto stock culture agar (pH 7.5).*

* A medium of a more complete nature than our standard medium. Apparently one or more of its components counteract the antibacterial effect of spermine.

TABLE VI
INHIBITION OF FOUR STRAINS OF *Staph. aureus* BY THREE SPECIMENS OF SPERMINE PHOSPHATE
EXTRACTED FROM POOLED HUMAN SEMEN

Concentration (mg. %)	Medium pH 7.4			Medium pH 6.8		
	No. of Tests	Positive	Negative	No. of Tests	Positive	Negative
200	12	12	0	5	4	1
100	9	9	0	3	2	1

Positive test = Area of inhibition 11–18 mm. in diameter.

Negative test = No inhibition.

Positive test = Area of inhibition 10–14 mm. in diameter.

Negative test = No inhibition.

Summary

Human semen, synthetic spermine tetrahydrochloride, and spermine phosphate extracted from human semen have been examined for their inhibitory effect on *Staphylococcus aureus*.

Human semen inhibited the growth of the 15 strains of *Staph. aureus* examined, the inhibition zone being larger on alkaline agar.

Spermine tetrahydrochloride, in concentrations of 100 mg.% and over, inhibited the growth of *Staph. aureus* on alkalinized media (pH 7.4) in 53 of 54 tests performed. Using the same medium and concentrations spermine phosphate inhibited the growth in all 21 tests performed. Spermine tetrahydrochloride, in concentrations up to 200 mg.%, was active only if the medium was alkaline (pH 7.4). Spermine phosphate in that concentration was active both when the medium was alkaline (pH 7.4) and also, although to a lesser degree, when the medium had a pH of 6.8. In this respect spermine phosphate resembles the plasma of human semen.

It is evident that the inhibition of the growth of *Staph. aureus* by human semen is due to its spermine content, though other substances which have not been examined may play a part.

We are indebted to B. Shapiro, Ph.D., and to E. Margoliash, M.D., for their helpful advice.

REFERENCES

- Bichowsky-Slomnitzki, L. (1948). *J. Bact.*, **55**, 27.
Guggenheim, M. (1940). *Die biogenen Amine*, 3rd ed., pp. 211–241. Basel and New York.
Harrison, G. A. (1931). *Biochem. J.*, **25**, 1885.
— (1933). *Ibid.*, **27**, 1152.
Rozansky, R., Gurevitch, J., Brzezinsky, A., and Eckerling, B. (1949). *J. Lab. clin. Med.*, **34**, 1526.