ANTI-BACTERIAL AND ANTI-SPERM EFFECT OF SEMINAL PLASMA

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SUMMARY

Bovine seminal plasma was active against a wide range of bacteria. Compounds which protected sperm from seminal plasma also reduced its anti-bacterial effect. Heating in the presence of mercaptoethanol, but not $FeSO_4$, abolished activity, indicating that disulphide bonds may be essential. Fractionation and dialysis experiments suggest that the compound is heterogeneous existing both as the monomer and various molecular weight polymers.

INTRODUCTION

Work on the effect of seminal plasma on sperm livability was summarized by Shannon (1973), who suggested that the toxic effect was due to an anti-bacterial substance. This paper reports experiments on anti-bacterial activity and anti-sperm effects of seminal plasma, and the modifying effects of protective substances and heat treatments.

MATERIALS AND METHODS

Seminal plasma from eight vasectomized bulls was pooled before use. Anti-bacterial activity was measured by the agar well technique. Four drops of any substance being tested from a pasteur pipette were placed in a 10 mm well in agar plates. Activity was measured by relative areas cleared round the well, calculated by the formula $R_1^2 - R_2^2$, where R_1 = radius from centre of the well to the edge of the cleared area, and R_2 = radius of well in mm.

Fractions of seminal plasma were obtained by sequential precipitation with increasing percentages of acetone. These fractions were re-dissolved at pH 7 and then the pH was adjusted to 4.5. At this pH a precipitate formed (with fractions up to 60% acetone) which was obtained by centrifuging. The precipitate was re-dissolved and solutions of both precipitate and supernatant were adjusted to contain the same protein level as seminal plasma at pH 7.

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RESULTS

ANTI-BACTERIAL ACTIVITY

Anti-bacterial activity was measured against *Micrococcus lacticum*, a spore-forming bacillus, *Staphylococcus pyogenes*, *Serratia* sp. and yeasts. Activity was shown against all the bacteria studied but not against yeasts. However, in subsequent studies, activity was occasionally shown against yeasts. Because of ease of culturing, the spore-forming bacillus was subsequently used as a test organism. All anti-bacterial activity was measured using this organism.

ACTIVITY OF FRACTIONS

The activity of different acetone fractions is given in Table 1. All fractions were dissolved in 2.9% sodium citrate, pH adjusted to 7.0 and protein concentration to 30 mg/ml.

TABLE 1: ACTIVITY OF ACETONE FRACTIONS AGAINST SPORE FORMING BACILLUS (AREA CLEARED)

Acetone %	*Fraction from pH Treatment at 4.5*	
	Precipitate	*Supernatant*
37.5	3.6	27.6
40.0	21.2	27.6
45.0	23.3	31.3
50.0	33.5	39.0
60.0	39.8	43.9
70.0		18.6
Final		34.3

Substantial anti-bacterial activity was found in all but one fraction. This fraction, however, accounted for less than 1% of total protein. All fractions also reduced sperm livability. A similar widespread occurrence of anti-bacterial activity was found in fractions obtained from a sephadex column.

EFFECT OF PROTECTIVE AGENTS AND HEAT

The effect of different compounds on the anti-bacterial properties of seminal plasma and heating in the presence of these compounds was tested. Results are given in Table 2.

There were significant differences between treatments. Two per cent egg yolk, 1% cocoa, 0.5% lecithin significantly reduced anti-bacterial activity ($P < 0.01$) where no heat treatment was given. Heat treatment significantly reduced anti-bacterial activity ($P < 0.01$). However, there was a significant interaction between heat treatment and compound tested ($P < 0.01$). Anti-bacterial activity was reduced when heated

TABLE 2: EFFECT OF DIFFERENT COMPOUNDS AND HEATING TO 80°C FOR 2 MIN ON ANTI-BACTERIAL ACTIVITY OF SEMINAL PLASMA (AREA CLEARED)

Compound and Level	*Heat Treatment*	
	None	*80° for 2 min*
Nil	41.1[1]	25.0
2% Egg yolk	17.3	0
0.1% Cocoa	37.1	26.7
1.0% Cocoa	10.8	5.4
0.5% Lecithin	8.1	2.6
0.25% $FeSO_4$	43.0	49.4
0.25% Mercapto-ethanol	46.2	0

[1] Four replicates for each treatment.

with either cocoa or lecithin, as well as with heat but no test compound. Activity was abolished when heated with either egg yolk or mercapto-ethanol while heating in the presence of $FeSO_4$ preserved activity. The loss of activity when heated in the presence of either mercapto-ethanol or egg yolk (which contains a large number of SH groups) suggests that activity may be dependent on disulphide bonds.

Effect of Lecithin on Sperm Livability

Because of its surfactant properties it might be expected that lecithin would be detrimental to sperm livability. This was the case, but the addition of seminal plasma modified the effect of lecithin (Table 3).

TABLE 3: EFFECT OF LECITHIN AND SEMINAL PLASMA ON PERCENTAGE SPERM SURVIVING
3 h Incubation at 37° C (% sperm surviving)

Lecithin (%)	*Seminal Plasma (%)*	
	0	*20*
0.1	15	43
0.05	23	28
0.01	57	4

Sperm survival increased with decreasing concentration of lecithin ($P < 0.01$). However, the addition of seminal plasma reversed the situation. (Interaction seminal plasma $\times$ lecithin $P < 0.01$). Additions of seminal plasma depressed livability at low levels of lecithin but increased livabilities at high lecithin levels. The results are consistent with the proposition that both substances are toxic but combine with each other to form a non-toxic combination.

EFFECT OF DIALYSIS

Semen was dialysed at pH 3 and pH 7. Considerable activity was obtained in the pH 3 dialysate, but only limited activity in the pH 7 dialysate. The dialysate obtained at pH 3 was re-dialysed at pH 3 and pH 7. Results are shown in Table 4.

TABLE 4: EFFECT OF DIALYSIS AT pH 5 AND pH 7
(Material previously dialysate obtained at pH 3)

pH	*Area Cleared*	
	Dialysed	*Dialysate*[1]
7.0	39.0	trace
3.0	26.6	9.3

[1] Four replicates.

There was a significant interaction between treatments in effect of dialysis ($P < 0.01$). Appreciable activity dialysed at pH 3 but not at pH 7.

DISCUSSION

From the evidence some postulates can be made on the nature of the compound.

(1) The fact that agents which protect sperm against seminal plasma such as egg yolk (Shannon, 1973) also inhibit anti-bacterial activity indicates that both effects are due to the same compound.

(2) The effect of lecithin is obatined by the combination of the protective agent and the seminal plasma toxin.

(3) The loss of activity when heated with mercapto-ethanol indicates activity may be dependent on disulphide bonds.

(4) The dialysis experiments suggest a possible explanation for the widespread occurrence of anti-bacterial activity. The results are consistent with the theory that the compound is a polypeptide which exists in forms ranging from the monomer to large molecular weight polymers. Maximum dissociation is obtained at pH 3.0 at which pH the small molecular weight compounds pass through the membrane.

REFERENCE

Shannon, P., 1973: *Proc. N.Z. Soc. Anim. Prod.*, 33: 40.