# ANTI-BACTERIAL ACTIVITY OF SEMINAL PLASMA AND OTHER TISSUES

P. SHANNON,* B. CURSON,* A. TWOMEY,† AND P. C. MOLAN‡

## SUMMARY

Anti-bacterial activity of seminal plasma and other tissues and organs was found to reside, in part at least, in a small cationic polypeptide. The peptide does not exist in the free form but complexed with large molecular weight proteins.

## INTRODUCTION

The effect of seminal plasma on sperm livability and some features of the anti-bacterial effects of seminal plasma have been reported by Shannon (1973) and Shannon *et al.* (1974). This paper reports experiments on the action of seminal plasma and compounds derived from seminal plasma and other tissues and organs on bacteria.

## MATERIALS AND METHODS

Seminal plasma was obtained from eight vasectomized bulls and pooled before use. Anti-bacterial activity was measured by the agar well technique (Shannon *et al.*, 1974).

Material from other bovine organs was obtained in the following manner. Samples of liver, lung, pancreas, spleen, and kidney from freshly slaughtered cattle were homogenized with normal saline and cell-free extracts obtained by high speed centrifuging. Saliva, intestinal mucosa and blood serum were untreated before separation procedures. Leucocytes were suspended in normal saline. Teat canals were separated into three fractions, the lower and upper teat canal and Furstenburg rosettes. The scrapings from the upper and lower teat canal and homogenized Furstenburg rosettes were suspended in normal saline.

Prior to any extraction method the samples were dialysed against five changes of distilled water at pH 7 at a ratio of 25:1 (v/v) distilled water to sample at each change for 72 h.

Fractions were obtained by the following methods:

(1) Sodium citrate was added to the dialysed sample at a concentration of 1.45% and dialysed against an equal volume of sodium citrate for 72 h.

* N.Z. Dairy Board, Newstead A.B. Centre, Hamilton.
† Waikato Dairy Laboratory, Ruakura, Hamilton.
‡ Waikato University, Hamilton.

(2) The pH of the sample was adjusted to 3 and the sample dialysed against an equal volume of distilled water adjusted to pH 3.

(3) Samples were run on a Sephadex G.50 column at pH 12.

(4) The pH of samples was adjusted to 12.8 and held at this pH for 10 minutes at 4°C. The pH was then adjusted to pH 3 at which pH a heavy precipitate formed which was removed by centrifuging. The supernatant was run on a Sephadex G.50 column at pH 12. Samples obtained by method (2) were filtered through an Amicon U.M. 10 ultrafilter and concentrated on a U.M. 2 ultrafilter at pH 3 prior to running on a Sephadex G.50 column at pH 1.7.

Studies on the mode of action of an bactericia were done by electron microscopy.

## RESULTS AND DISCUSSION

### Effect on Bacteria

The test organism was *Bacillus subtilis*. On exposure to the compounds the bacteria begins to swell, the cell wall becomes crinkled and there is a general distortion in shape. The cell membrane then parts from the cell wall and finally the cell wall bursts, discharging the contents of the cell. The action is very rapid, taking only minutes in some cases. The swelling and rupturing of the cell wall can be observed on a light microscope.

Unfractionated seminal plasma, extracts of spleen, liver, lungs, pancreas and saliva exhibited this effect prior to treatments.

### Fractionation

One problem with severe methods of fractionation is that derived compounds may in fact be artifacts. Gentle methods of dissociation have been attempted and products obtained by more severe methods compared with these.

TABLE 1: ACTIVITY OF DIALYSATES AT pH 3 AND pH 7

| *pH* | *Area Cleared Sodium Citrate* | |
|---|---|---|
| | *0* | *1.45%* |
| 3.0 | 53 | 32 |
| 7.0 | 0 | 19 |

Activity of dialysates obtained by dissociation in the presence or absence of sodium citrate at two pH's is shown in Table 1.

Greatest activity in the dialysates was obtained at pH 3 in the absence of sodium citrate. At pH 7 activity was only observed in samples dialysed in the presence of sodium citrate. The correlation between protein content and activity was +0.92 ($P<0.01$) indicating (1) that activity was due to a protein, (2) that either the dialysates relatively prue anti-bacterial compounds or that the rate of anti-bacterial to non-anti-bacterial proteins was similar in all dialysates. The mode of action of the fractions on the susceptible bacteria was the same as that of whole seminal plasma.

Crystals were obtained from acetone of this material. Crystals were redisolved at pH 3 and filtered through an Amicon U.M. 10 ultrafilter and concentrated on a U.M. 2 ultrafilter. The resultant product was run on a Sephadex G.50 column at pH 1.7.

Despite the fact that the material had dialysed and been filtered through an ultrafilter with a molecular cut-off of 10 000 running the material on a Sephadex column at low pH gave peaks with apparent molecular weights ranging from 20 000 down to approximately 3 000. Material from all the peaks exhibited anti-bacterial properties.

Electrophoresis of the peaks showed a cationic band with the same electrophoretic mobility, but varying amounts of other material.

The results indicate that, although the compound dialysed in a form less than 10 000 in molecular weight, it reaggregated or combined with anionic material to form complexes considerably in excess of a molecular size that would dialyse. The amount of small molecular weight material (*i.e.*, that about 3 000 molecular weight) constituted only a small fraction of the total material.

Active dialysates at pH 3 were obtained from all other organs and tissues except the pancreas. These fractions behaved in a similar manner to the seminal plasma extract when run on a Sephadex G.50 column.

Dissociation was greater when material was run at pH 12 on a Sephadex column. The material then separated into two main peaks, a high molecular weight peak and a low molecular peak.

The highest yield of low molecular weight material was obtained by alkali then acid denaturation followed by separation on a Sephadex G.50 column. The molecular weight peak exhibited the same anti-bacterial properties and a band with the same electrophoretic mobility as low molecular weight material obtained by

other methods. Activity has been obtained from all samples tested (spleen, pancreas, liver, lungs and seminal plasma).

The results indicate that a cationic anti-bacterial polypeptide having the same molecular weight and electrophoretic mobility is present in a wide range of tissues. The material is not dialysable at pH 7 so that previous work ascribing anti-bacterial properties to proteins may have been describing proteins to which the cationic polypeptide is attached (Hibbitt and Cole, 1968; Hibbitt *et al.*, 1969, 1971; Zeya and Spitznagel 1966).

Shannon *et al.* (1974) reported that an apparently wide range of seminal plasma proteins were anti-microbial. They showed that a dialysable fraction could be obtained by dialysis at pH 3, dialysis reducing activity of material retained within the membrane. The small molecular weight protein has since been recombined with albumin resulting in an apparently large molecular weight substance having anti-microbial properties.

The combination of the polypeptide with protein appears to confer some advantage to the anti-microbial system. This may be a result of controlled dissociation of the cation between the carrier and the microbial cell. The large molecular weight complex is less likely to be excreted and there is some evidence that this complex is less susceptible to inhibition of anti-microbial activity.

The presence in all tissues of common cationic polypeptides suggests that this is a primary non-specific anti-microbial defence mechanism. In reviewing earlier work, Skarnes and Watson (1957) considered that anti-microbial cationic tissue factors existed only in normal tissue in response to physiological changes accompanying stress. The cell-free extracts in the present study demonstrate that the activity is present in the absence of stress conditions. However, in stress conditions a decrease in pH will allow greater dissociation and possibly increase anti-bacterial activity.

## REFERENCES

Hibbit, K. G.; Brownlie, J.; Cole, C. B., 1971: *J. Hyg. Camb., 69*: 61.
Hibbitt, K. G.; Cole, C. B., 1968: *Biochem. J., 106:* 39.
Hibbitt, K. G.; Cole, C. B.; Reiter, B., 1969: *J. gen. Microbiol., 56:* 365.
Shannon, P., 1973: *Proc. N.Z. Soc. Anim. Prod., 33:* 40.
Shannon, P.; Twomey, A.; Curson, B., 1974: *Proc. N.Z. Soc. Anim. Prod., 34*: 61.
Skarnes, R. C.; Watson, D. W., 1957: *Bact. Rev., 21:* 273.
Zeya, H. I.; Spitznagel, J. K., 1966: *J. Bact., 91:* 755.