histaminase release occurs only rarely and not consistently after administration of 48/80 or polymyxin B. The amounts of histamine-destroying activity identified in this study were small: in no case was destruction more than 50% in 1½ hours' incubation. This is in contrast to values of 65 to 90%, with only rare values below these, found during anaphylaxis(2).

*Summary.* Neither sensitized nor unsensitized rats consistently released significant amounts of histaminase into the bloodstream after intravenous administration of 48/80 or polymyxin B. Only 30% of unsensitized rats given 48/80 or polymyxin B by the intraperitoneal route showed release into the blood of histamine-destroying activity, and this in small amount. It is concluded that either (1) the intraperitoneal route allows a higher concentration of drug to reach the gut and hence release histaminase or (2) 48/80 and polymyxin B do not consistently release histaminase, in contrast to the effect of anaphylactic shock in rats.

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## Enzymatic Characterization of Decapacitation Factor. (30788)

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Chang(1) has described the presence in rabbit, bull and human seminal plasma, of a substance capable of reversing capacitation. This naturally occurring anti-fertility substance has been designated "decapacitation factor" (DF). Bedford and Chang(2) described an ultracentrifugation technique for partial purification of DF. They reported that DF activity of seminal plasma was stable to heat (65°C for 30 minutes), freezing, and was non-dialyzable. Weinman and Williams (3) reported the presence of DF activity in epididymal fluid and showed that the DF pellet sedimented in the ultracentrifuge could be resuspended in buffer and passed through an ultrafilter.

This paper reports attempts to determine the chemical nature of DF by use of various hydrolytic enzymes and suggests a possible mechanism of capacitation. Portions of this work were published in a preliminary report (4).

*Materials and methods.* Initial studies were conducted with Pronase (Cal Biochemical Co.), a proteolytic enzyme attacking peptide bonds nonspecifically. Seminal plasma diluted 2.5-fold with calcium-free Krebs-Ringer phosphate (KRP) was treated with Pronase at a level of 10 μg/5 mg biuret reactive material (BRM) for 10 minutes at 37°C. Capacitated spermatozoa were then incubated with the Pronase-treated seminal

TABLE I. Effect of Pronase on DF Activity of Rabbit Seminal Plasma.

| Treatment | No. ova | No. fertile | % Fertile |
|---|---|---|---|
| Capacitated sperm control | 21 | 20 | 95.3 |
| Seminal plasma-capac. sperm | 4 | 0 | 0 |
| Pronase-capac. sperm | 10 | 10 | 100.0 |
| Pronase-seminal plasma-capac. sperm | 2 | 0 | 0 |

TABLE II. Effect of Pronase on DF Activity of Crude DF from Bull Seminal Plasma.

| Treatment | No. ova | No. fertile | % Fertile |
|---|---|---|---|
| Capacitated sperm control | 18 | 14 | 77.8 |
| Crude DF-capac. sperm | 10 | 0 | 0 |
| Crude DF-pronase-capac. sperm | 12 | 0 | 0 |
| Recapacitation of sperm decapacitated with crude DF-pronase | | | |
| Ejaculated sperm control | 8 | 7 | 87.6 |
| Decapacitated sperm | 14 | 4 | 28.6 |

TABLE III. Effect of Beta-Amylase on DF Activity in Rabbit Seminal Plasma.

| Treatment | No. ova | No. fertile | % Fertile |
|---|---|---|---|
| Capacitated sperm control | 39 | 29 | 74.4 |
| Seminal plasma-capac. sperm | 7 | 0 | 0 |
| Beta-amylase-capac. sperm | 14 | 10 | 71.4 |
| Beta-amylase-seminal plasma-capac. sperm | 15 | 8 | 53.4 |

plasma for 20 minutes at 37°C and assayed by the technique described by Williams *et al* (5) using 6-month-old, virgin New Zealand White rabbits. Similar trials were conducted with Pronase at a level of 100 μg/5 mg BRM. Finally, crude DF (with 80 units penicillin-G and 37 μg streptomycin sulfate/ml) was incubated for 12 hours at 37°C and then assayed for DF activity. Spermatozoa decapacitated in this latter trial were recapacitated(3).

In subsequent trials beta-amylase (General Biochemicals Inc.) was incubated with rabbit seminal plasma diluted 2.5-fold with KRP 10 minutes at 37°C (100 μg/5 mg BRM) and assayed for DF activity.

Uterine fluid from 6 mature does was assayed for amylase activity by the method of Fernandez and Sobel(6). In addition, an oviductal fluid collecting flask was installed in each of 3 does(7) and the fluids assayed for amylase activity. Similar assays were performed on blood serum samples obtained by heart puncture.

Twice washed ejaculated spermatozoa suspended in calcium-free KRP solution ($2.5 \times 10^5$ spermatozoa/0.25 ml) were incubated with 250 μg beta-amylase for either 8 or 12 hours *in vitro* with and without uterine fluid. These spermatozoa were then assayed to determine their ability to fertilize ova.

*Results.* As shown in Tables I and II Pronase did not destroy the DF activity of seminal plasma or the crude DF. Spermatozoa decapacitated by these preparations were capable of recapacitation and subsequent fertilization (Table II).

Treatment of seminal plasma with beta-amylase (Table III) destroyed the DF activity of the seminal plasma.

The amylase activity of reproductive tract fluids and blood serum (Table IV) indicate uterine fluids have approximately 4 times the level found in blood serum. One doe had no amylase activity in either serum or uterine fluid. A second doe showed no activity in

TABLE IV. Amylase Activity (μMoles of Maltose Equivalent Produced per Minute at 37°C) of Uterine and Oviduct Fluids.

| Fluid | No. rabbits | Amylase units | (Range) |
|---|---|---|---|
| Uterine | 4 | .156 | (.050-.278) |
| Oviduct | 3 | .023 | (.020-.027) |
| Blood serum | 3 | .039 | (.011-.077) |

the blood serum and a third had none in the uterine fluid. Data from these rabbits were omitted. The amylase activity of individual rabbit uterine fluids was quite variable and could be an indication of varying degrees of female fertility or the stage of the estrous cycle.

Attempts to capacitate sperm *in vitro* using beta-amylase with or without uterine fluid were unsuccessful.

*Discussion.* The failure to destroy DF activity with Pronase and the successful destruction with beta-amylase indicate that decapacitation factor is polysaccharide. It has previously been suggested(3) that capacitation involves the removal of DF from the spermatozoa head. The above data suggests that this action is directed against the polysaccharide components on the sperm head surface and that this action may result from the enzymatic activity of the female reproductive tract.

The finding of a high level of amylase activity in uterine fluid supports this hypothesis. The lower amylase activity of the oviductal fluid could account for the extended time required for oviductal capacitation compared to uterine capacitation(8). Based on these findings it should be possible to capacitate spermatozoa *in vitro* using beta-amylase. Since this was not accomplished, it suggests that enzymatic removal of DF from the sperm cell surface may constitute but one step of the capacitation process and that other steps may be involved such as the acrosome reaction as suggested by Austin(9).

*Summary.* The successful destruction of rabbit decapacitation factor by beta-amylase is reported. The finding of a level of amylase 4 times the level found in blood serum suggests that enzymatic removal of decapacitation factor from the surface of the spermatozoa head constitutes one step of the capacitation process. Decapacitation factor appears to be a polysaccharide since it is destroyed by amylolytic but not by proteolytic enzymes.

NOTE ADDED IN PROOF: Following the preliminary report that beta-amylase may be involved in capacitation(4), successful *in vitro* capacitation was described (Kirton, K. T., and Hafs, H. D., Science, 1965, v150, 618). Current experiments using their experimental conditions have been unsuccessful to date in obtaining *in vitro* capacitation with beta-amylase.

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