THE MECHANISM OF ACTION OF COWPER'S SECRETION IN COAGULATING RAT SEMEN

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In the rat, mouse and hamster, but not in the guinea-pig, Cowper's secretion reacts *in vitro* with vesicular secretion to produce a coagulant (Hart & Greenstein, 1968). Unlike vesiculase, the pH optimum of this reaction is acidic. In the rat, the active agent secreted by Cowper's glands that is responsible for coagulation is heat stable and non-dialysable. Electrophoretically the active molecule migrates only slightly at a pH 8.6 and reacts chemically as a glycoprotein.

The vesicular substrate in the rat which coagulates upon exposure to Cowper's secretion has been shown to be a protein, electrophoretically different from the substrate of vesiculase (Hart & Greenstein, 1968). Recent data suggest that vesiculase promotes vesicular coagulation by transamidase reactions (Notides & Williams-Ashman, 1967). This paper presents evidence which suggests that the mechanism by which Cowper's secretion coagulates vesicular substrate is non-enzymatic, involving, at least in part, an ionic interaction between Cowper's secretion and substrate.

Cowper's secretion was obtained from mature male rats by milking the glands into a phosphate buffered solution of pH 5.9 which contained 10% (w/v) ammonium sulphate. The solution was dialysed for 12 hr at 4° C against a 10% (w/v) ammonium sulphate solution, pH 5.9. The non-dialysable material was then dialysed for 24 hr at 4° C against several changes of phosphate buffer, pH 5.9 and centrifuged for 20 min at 4000 rev/min. The clarified supernatant of Cowper's secretion was subsequently used in studying semen coagulation. Seminal vesicle secretion was prepared as described previously (Hart & Greenstein, 1968). Protein concentration was determined spectrophotometrically (Fruton & Simmonds, 1958) and the coagulation reaction was followed photometrically at 660 m μ at room temperature (Gotterer, Ginsberg, Schulman, Banks & Williams-Ashman, 1955).

Varying concentrations of vesicular substrate were reacted *in vitro* with a constant amount of Cowper's secretion (Text-fig. 1). Regardless of the concentration of vesicular secretion used, the maximum amount of coagulation produced in each case was nearly identical. Moreover, the time required for maximum coagulation to occur did not vary as the substrate concentration increased. At 40 min after the initial coagulation reaction was complete, an identical concentration of Cowper's secretion (0.12 mg protein) was added to

each reaction tube (Text-fig. 1). In all cases, a new reaction began but, unlike the initial reaction, substrate concentration was limiting in the cases of low protein concentration and thus reduced the maximum amount of coagulation obtained. Thus, at non-limiting substrate concentrations, the amount of coagulant produced depended directly upon the concentration of Cowper's secretion.

TEXT-FIG. 1. The effect of substrate concentration on vesicular coagulation. The value next to each line represents the mg of vesicular protein per reaction tube. All tubes contained 0.12 mg of Cowper's secretion protein in a total volume of 4 ml (phosphate buffer, pH 5.9). The arrow indicates the point in the reaction where a second addition of Cowper's secretion (0.12 mg protein) was made. —, 0.06 mg; ----, 0.12 mg; - · - ·, 0.18 mg.

TEXT-FIG. 2. The effect of Cowper's secretion concentration on vesicular coagulation. The value next to each line represents the mg of Cowper's secretion protein per reaction tube. All tubes contained 2.6 mg of vesicular protein in a total volume of 4 ml (phosphate buffer, pH 5.9). —, 2.6 mg; ----, 5.2 mg; - · - ·, 7.8 mg.

Text-fig. 2 shows that there was a marked positive relationship between the amount of Cowper's secretion added to vesicular substrate and the percentage increase in the reaction maximum. These results are distinctly different from those reported for vesiculase (Gotterer *et al.*, 1955). In their report, varying the concentration of vesiculase in a reaction mixture affected only the time required for the reaction to reach maximum. The amount of coagulant obtained did not vary with enzyme concentration.

The effect of ionic strength on the coagulatory reaction is shown in Table 1. Concentrations of NaCl between 180 and 200 μ moles/ml almost completely abolished coagulation. Decreasing the concentration of NaCl below 180 μ moles/ml correspondingly increased the amount of coagulant obtained. However, at concentrations of NaCl which allowed the reaction to proceed, the time required for each reaction to reach maximum was constant and identical to the reactions illustrated in Text-fig. 1. In the case of vesiculase, ionic strength influences only the time required for the reaction to reach maximum (Gotterer *et al.*, 1955). Like vesiculase, however, Versene effectively abolished the coagulation reaction involving Cowper's secretion (Table 1). *p*-Chloromercuribenzoic acid at concentrations of 60 or 100 μ moles/ml had no effect on the ability of Cowper's secretion to coagulate vesicular protein (Table 1).

TABLE 1
THE EFFECT OF IONIC STRENGTH,
VERSENE AND *p*-CMB ON SEMEN
COAGULATION

<i>Treatment</i>	<i>Change in optical density</i>
Control	0.27
25 μ moles NaCl/ml	0.25
50 μ moles NaCl/ml	0.23
100 μ moles NaCl/ml	0.16
140 μ moles NaCl/ml	0.13
180 μ moles NaCl/ml	0.04
200 μ moles NaCl/ml	0.02
5 μ moles Versene/ml	0.27
100 μ moles Versene/ml	0.03
60 μ moles <i>p</i> -CMB/ml	0.30
100 μ moles <i>p</i> -CMB/ml	0.28

All reactions were carried out in a total volume of 4 ml (phosphate buffer, pH 5.9) which contained 8 mg vesicular protein and 0.12 mg of Cowper's secretion protein. The optical density values recorded represent the final reading made 120 min after the reactions were initiated. The control reaction lacked NaCl. *p*-Chloromercuribenzoic acid = *p*-CMB.

These data indicate that the *in vitro* interaction of Cowper's and vesicular secretion to produce a coagulant proceeds by a non-enzymatic mechanism. The effect of ionic strength on the reaction would suggest that ionic interactions are involved in coagulation. The inhibitory effect of Versene demonstrates the involvement of metal ions in the reaction, while the failure of *p*-chloromercuribenzoic acid to affect coagulation would suggest that active sulphydryl groups are not involved in the interaction (Rosemeyer & Huehns, 1967).

Despite the fact that the data presented suggest that the coagulatory mechanism of Cowper's secretion is non-enzymatic, this by no means implies the reaction lacks specificity. Cowper's secretion in the rat coagulates a specific protein fraction of vesicular secretion, but displays a sharp reduction in activity when reacted with guinea-pig vesicular secretion (Hart & Greenstein, 1968).

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