Studies in Semen Coagulation.* (23429)

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Most mammalian semen coagulates or gelates upon ejaculation. In a few species, such as the dog and the bull, this does not occur. Human semen at first coagulates, then liquefies, presumably as the result of fibrinolytic enzyme action. During the coagulum phase human spermatozoa are hypomotile. As liquefaction proceeds, normal motility appears.

In certain rodents (rat, guinea pig, opossum), ejaculation is immediately followed by formation of a firm coagulum which occludes the vagina. This is termed the "copulation plug" and was described by Lataste(1) who attributed coagulation to mixing of the seminal vesicle fluid with the vaginal secretions. Camus and Gley subsequently observed that the coagulum was formed by the mixing of the seminal vesicle fluid with a secretion which they aspirated from a structure just lateral to the seminal vesicles, and which they thought to be part of the prostate(2,3). These investigators were the first to recognize the enzymic nature of the reaction, and named the "prostatic" component *vesiculase*.

Walker's classical studies have provided the framework for most of the research which has been done since that time in the anatomical and kinetic aspects of rodent semen coagulation(4,5). Walker's principal contributions were identification of the coagulating glands, and of the vital role of their secretion in the clotting reaction, as well as his extensive observations on the chemistry of the clotting process. Various workers(6,7,8,9) have confirmed and extended certain of these observations. Recently Gotterer and his associates (10) have studied the kinetics of semen coagulation in greater detail. The relationship of coagulation to fertility was investigated by Blandau(11) who found that the copulation plug was essential to sperm ingress into the uterine cornua in the rat. In spite of extensive study along this line, however, the exact mechanism of coagulation still remains obscure.

In this report, a simple method will be described for the quantitation of rat seminal coagulation *in vitro*. From our studies, coagulation of normal rat semen appears to take place in 2 separate stages: 1) an initial flocculation, promptly succeeded by 2) gelation and immobilization of the flocculate. By dilution of the components of the reaction, the interval between these 2 stages can be lengthened sufficiently to permit accurate measurements of the coagulation time. Details of the method employed and a preliminary description of attempts to alter the normal rate of coagulation will be considered in separate sections.

Materials and methods. Adult male albino rats were killed by cervical fracture. The coagulating glands were carefully dissected from the seminal vesicles to avoid mixing secretions, and homogenized in 2.0 ml of isotonic saline. This homogenate was diluted 1:10 and 1:50 with saline. The seminal vesicles were excised separately and their secretion collected. Two drops of the expressed secretion were suspended in 1.0 ml of saline. One drop of diluted coagulating gland homogenate (CGH) was placed next to one drop of seminal vesicle fluid suspension (SVFS) on a glass slide, then rapidly mixed with a stirring rod. Floccules appeared almost immediately, and were suspended uniformly throughout the medium. The slide was then slowly tilted from side to side until gelation was complete, as indicated by failure of the floccules to move. The reaction was timed from the point at which mixing of the 2 secretions was begun until complete immobilization of the flocculate had occurred. The endpoint was found to be sharply defined and the elapsed time in seconds was designated as Coagulation Time (CT).

Validity of the method: The coagulation times for rats of the same size have proved reasonably constant and repro-

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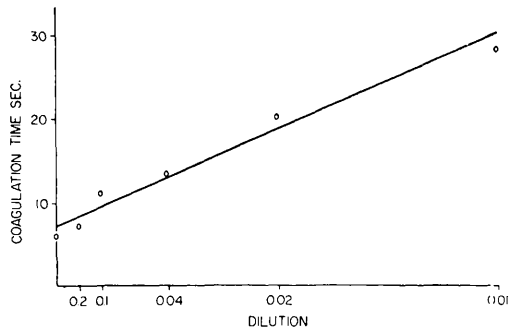

FIG. 1. Effect of dilution of coagulating gland homogenate on coagulation time (CT).

ducible. Five rats ranging between 298 g-320 g were used. Dilutions of CGH of each rat were made to 1:10 and 1:50. CT for each rat at each dilution was determined 5 times against a constant SVFS. Average CT for each rat at both dilutions fell within a narrow range of 8.0-8.8 seconds (1:10) and 20.2-21.4 seconds (1:50). In these studies, pipettes of constant bore were used, providing approximately uniform drop size.

Effect of dilution on CT: Progressive dilutions of CGH in saline were made (1:5, 1:10, 1:25, 1:50, 1:100). Coagulation times were determined using freshly prepared SVFS. The results are plotted in Fig. 1 and demonstrate that CT increases linearly with dilution.

Effect of pH on CT: One drop of CGH (1:5) was added to one drop of SVFS mixed with one drop of 0.067M phosphate buffer and CT determined. The results at different pH levels are recorded in Fig. 2. As shown, the optimum pH of the coagulation reaction is 6.8 to 8.2. This is substantially in agreement with the observation of Gotterer, *et al.* (10) (pH 6.0-8.0) using a turbidimetric method.

Effect of certain dissociated compounds on CT: The effect of certain dissociated compounds as inhibitors of coagulation has been studied. One drop of SVFS (prepared in distilled water instead of saline) was added to one drop of CGH (1:10 in distilled water) mixed with one drop of the test substance (concentration 10^{-3}M). The results are summarized in Table I. It is apparent that Zn^{++} and Cu^{++} prolong the CT, perhaps indicating

TABLE I. Effects of Various Compounds on CT

Test substance	Flocculation	Avg CT (5 determinations), sec.
Control in distilled water	+	10.6
NaCl 10^{-3}M	+	9.9
KCl 10^{-3}M	+	11.1
$MgSO_4$ 10^{-3}M	+	10.9
$ZnSO_4$ 10^{-3}M	+	31.1
$CuSO_4$ 10^{-3}M	+	21.8
NaF 10^{-3}M	+	9.7
NaCN 10^{-3}M	+	11.3

an inhibiting effect on the reaction. The other substances tested appear to have little or no effect under the conditions of the experiment, except for Na^+ which appears to shorten the CT slightly. This will be discussed in greater detail below.

Effect of blood anticoagulants on CT: Table II shows that calcium binding substances, such as sodium oxalate and sodium citrate, have no effect on the coagulation process. Heparin similarly is without effect on coagulation *per se*, but does denature the SVFS, causing flocculation before the addition of the coagulation gland component.

Sodium requirement of vesiculase: In this experiment a crude preparation of the coagulating gland substance (*vesiculase*) was made by treating the clear supernatant of centrifuged CGH with acetone (4°C). The result-

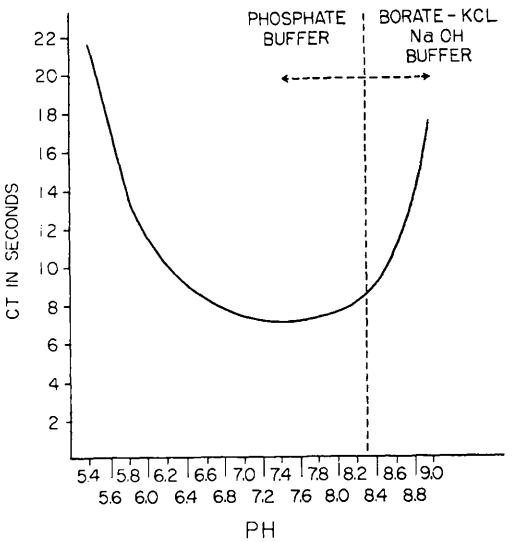

FIG. 2. Effect of pH on coagulation time (CT).

TABLE II. Effect of Blood Anticoagulants on CT.

Substance	Flocculation	Avg CT (5 determinations), sec.
Control in saline	+	8.8
Na Oxalate (100 mg/ml)	+	9.2
Na Citrate (100 ")	+	7.2
Heparin (10 ")	+	7.8

ing acetone powder was suspended in 2.0 ml of distilled water and dialyzed against running tap water for 24 hours. The dialyzate was then tested against SVF in the following diluents: distilled water, NaCl 10^{-3}M, KCl 10^{-3}M and $NaHCO_3$ 10^{-3}M. Table III shows that CT was shortened in the presence of Na^+ ion, indicating that Na^+ seems to be necessary to *vesiculase* action. The Cl^- ion apparently does not play a role in coagulation.

Effect of various physical agents on CT: Most of our experiments confirm the observations of Walker(5) and Camus and Gley(2, 3). It has been found that heating CGH to 100°C, however briefly, destroys its activity. SVFS, however, is not adversely affected by this amount of heat. Cooling of either component to 4°C does not alter the reaction. It has likewise been noted that rapid freezing does not affect coagulating activity. Slow freezing and thawing of the coagulating gland before homogenization, on the other hand, destroys activity.

TABLE III. Determination of Na^+ Requirement of Coagulation Reaction Using Dialyzate of CG Acetone Powder.

Test substance	Avg CT (5 determinations), sec.
SVFS in distilled water + dialyzate of CG acetone powder	18.0
SVFS in NaCl (10^{-3}M) + dialyzate of CG acetone powder	11.8
SVFS in KCl (10^{-3}M) + dialyzate of CG acetone powder	18.8
SVFS in $NaHCO_3$ (10^{-3}M) + dialyzate of CG acetone powder	10.1

Isolation of the coagulating gland factor (vesiculase): Using $(NH_4)_2SO_4$ and acetone precipitation, a fairly potent preparation of *vesiculase* has been obtained from CGH. This has survived unchanged for one year in the refrigerated state. Preliminary fractionation of crude *vesiculase* has yielded a substance which seems solely implicated in the flocculation phase of the reaction.

Summary and conclusions: 1. Coagulation of rat semen seems to involve a diphasic reaction consisting of 1) flocculation and 2) gelation. 2. Velocity of the coagulation reaction can be timed with reproducible results, under the experimental conditions described. 3. The reaction is linear with dilution of the component secretions. 4. Optimum pH range for the reaction lies between 6.8 and 8.2. 5. Zn^{++} and Cu^{++} appear to inhibit the coagulation reaction. Blood clotting inhibitors have no effect. Na^+ seems essential for clotting to occur. 6. Heating of the coagulating gland component destroys activity. 7. Slow freezing of the coagulating gland destroys activity, rapid freezing does not. 8. A crude preparation of the coagulating gland component has been obtained by $(NH_4)_2SO_4$ precipitation. Further work is in progress on purification of this substance.

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