

COAGULATION AND LIQUEFACTION OF SEMEN*

PROTEOLYTIC ENZYMES AND CITRATE IN PROSTATIC FLUID

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The semen of man and animals is delivered from the urethra in a liquid state but differs thereafter in its properties in three principal ways. In the guinea pig it becomes an elastic solid which is maintained in this condition both *in vitro* and in the vagina for many days. The semen of the dog remains liquid. The ejaculate of normal man solidifies but then liquefies in a few minutes at room temperature.

The solidification of the semen of the guinea pig is due to the action of an enzyme, vesiculase (1, 2) derived from a special region of the prostate (3) on the proteins of the seminal vesicle. No experiments have been reported dealing with the solid-liquid phases of human semen or with the persistence of the liquid state of canine semen, matters with which the present paper is concerned.

Our first observation was that a mixture of human semen with blood underwent coagulation but soon liquefied. Most of the present experiments deal with various phases of the effects of semen and its components on blood and its clotting constituents.

Methods

Human semen was obtained by manual ejaculation. Following liquefaction, it was centrifuged and the supernatant fluid used immediately for testing. Canine semen was obtained by pilocarpine stimulation following the prostatic isolation procedure of Huggins, Masina, Eichelberger, and Wharton (4); this fluid was passed through a Seitz filter to obtain a sterile filtrate free from cells.

Fresh blood and citrated plasma were obtained from various species. The plasma was procured by centrifugalization of blood drawn into a solution of sodium citrate, 5 per cent, of which 1 cc. was used for each 9 cc. of blood; it was recalcified with a solution of CaCl_2 , 1.5 per cent, of which 0.33 cc. was used for each cubic centimeter of plasma.

Testing for lytic activity was carried out by mixing 1 cc. of blood or plasma with 1 cc. of semen or other fluid under examination, either undiluted or diluted to 1 cc. with saline; after mixing, the tubes were stoppered and placed in a water bath at 37°C. and

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the times of coagulation and of subsequent liquefaction, when it occurred, were noted. In stating the dilutions in this paper the actual content of secretion in 1 cc. is given; saline refers to an aqueous solution of NaCl, 0.15 molar in concentration, and incubation refers to 37°C. Where citrated plasma was used the specified concentration of calcium chloride was added according to two plans; either the plasma was recalcified immediately after adding the fluid to be tested, or after a delay of minutes to hours to permit a prolonged contact, in the liquid state, of the plasma and the fluid under test.

As control, saliva, bile, cerebrospinal and spermatocele fluids were substituted for semen and the effects on the clotting and liquefaction of blood noted.

In testing the effect of the lytic factors in semen on the clotting constituents of human blood, fibrinogen, thrombin, and thromboplastin were prepared from beef plasma and lung by the method of Smith, Warner, and Brinkhous (5) and prothrombin by the technique of Seegers and colleagues (6).

Citrate analyses were made by the method of Pucher, Sherman, and Vickery (7) and throughout this paper the term, citrate, refers to the combined amount of citric acid and its salts as determined by this method. Trypsin was determined by the method of Anson (8).

The opacity developing coincident with clot formation induced by recalcification of citrated plasma, and the effect of semen upon this density were studied in an Evelyn photoelectric colorimeter. The colorimeter was mounted in an incubator at 37°C. and all of the solutions were brought to this temperature before using. A 6600 Ångstrom light filter was used. The total volume in each colorimeter tube was 13.5 cc. distributed as follows:—

5 cc. of plasma + 2 cc. of semen (saline in the control tubes) + 5 cc. of saline + 1.5 cc. of CaCl₂.

Following recalcification, readings of the galvanometer were recorded each 12 seconds until a steady state had been reached following coagulation, or until it was evident that clotting was not occurring.

RESULTS

The Phenomenon of Spontaneous Lysis of Human Semen.—The semen of normal men after ejaculation forms a solid mass and the container may be inverted without losing any of the material; within a few minutes softening and partial liquefaction is observed and within 15 minutes the ejaculate is in a liquid state save for a few sago-like particles, derived from the seminal vesicles, which remain for more than 1 hour.

The lytic phenomenon may be observed well through the microscope. In a typical example, at 3 minutes after ejaculation a thin section appeared as many interlaced bundles of long, clearly defined, parallel, refractile fibres; at 4 minutes the fibres appeared swollen; at 5 minutes the regularity had disappeared and the fibres were arranged haphazardly; at 6 minutes there was large scale liquefaction with movement visible as many of the fibres were rapidly breaking up. No fibres were seen 8 minutes after ejaculation.

In a man with hyopgonadism due to undescended testes the semen did not

clot; following intramuscular injection of testosterone propionate, 25 mg. daily for 15 days, normal clotting was observed.

The Effect of Semen and Prostatic Fluid on the Coagulation Time of Blood.—The clotting time of whole blood, 1 cc. mixed with saline, 1 cc., in 144 instances ranged from 3 to 10 minutes.

(a) *Human semen:* Two effects were observed, shortening and prolongation of the coagulation time of whole blood. Of 23 seminal specimens tested against whole human blood, 11 prolonged the coagulation time, while 12 caused a decrease of the clotting time as compared to the control saline mixtures. In the group in which a mixture of semen lessened the time of coagulation, the effect was evident with as little semen as 0.025 cc. (Table I). This

TABLE I

Effect of Semen and Other Human Secretions on the Coagulation Time of Human Blood
1 cc. of whole blood + 1 cc. of secretion, diluted secretion, or saline.

Nature of secretion	Coagulation time								
	Amount of secretion in test								
	None	1 cc.	0.5 cc.	0.1 cc.	0.05 cc.	0.033 cc.	0.025 cc.	0.0166 cc.	0.0125 cc.
	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>
Semen, group 1	8	2	3	2	2	3	3	—	5
Semen, group 2	5	30	14	6	5	5	5	5	5
Prostatic fluid	5	0	0	0	19	11	7	7	6
Prostatic fluid heated	5	0	0	0	22	—	—	—	—
Prostatic fluid and CaCl ₂ *	5	3	3	4	—	—	—	—	—
Seminal vesicle secretion	5	—	3	4	4	5	5	5	—
Hydrocele fluid	5	2½	2½	3	3	3	—	—	—
Saliva	5	3	2	3	—	—	—	—	—

* Calcium chloride, 1.5 per cent, 0.33 cc.

lessened coagulation time is due to thromboplastic substances in appreciable amounts. In the group with prolonged clotting with semen in 1 cc. amounts no, or slight clot, was observed in 30 minutes; in this group, semen in 0.2 or 0.1 cc. amounts often lengthened clotting time to, 15 or more minutes.

(b) *Human prostatic fluid:* All specimens of undiluted fluid tested (from 18 men) inhibited the clotting of human blood. With prostatic fluid 0.1 cc., clotting occurred in 14 to 50 minutes in 5 specimens, while no clot was observed in 2 hours in 13 observations. The coagulation time of blood mixed with prostatic fluid 0.02 cc. was approximately normal.

Coagulation of the blood was always induced by adding calcium chloride, 1.5 per cent, 0.33 cc. to prostatic fluid, 1 cc. In 5 cases incoagulability, or greatly prolonged clotting time, was abolished and a clotting time shorter than normal supervened; in 3 cases the coagulation time was greatly decreased

but still remained longer than the normal control. In all cases in which semen caused a prolonged clotting of admixed blood, the addition of the specified amount of calcium chloride abolished the delay and coagulation occurred more rapidly than in the control saline-blood mixture.

(c) *Dog prostatic fluid*: Of 40 specimens of human blood, 1 cc., mixed with prostatic fluid, 1 cc., the coagulation time was normal or shortened in 32 cases. In 4 cases clotting did not occur while only slight clot was observed in 13 to 20 minutes with the fluid of 4 dogs in this group. In contrast to the system, human prostatic secretion-blood, the addition of calcium ions did not initiate or hasten clotting and the delay was due to a different mechanism, namely, the disappearance of fibrinogen as it is discussed below.

Citrate Is the Cause of Prolonged Coagulation Time of Mixtures of Blood and Human Prostatic Fluid or Semen.—It was found that human prostatic fluid heated in a water bath at 100°C. for 30 minutes still delayed blood coagulation, while the delay was abolished by adding calcium ions (Table I). Large amounts of citrate in the prostatic fluid were discovered by Scherstén (9) and the finding was confirmed by Dickens (10). We likewise observed large amounts of this acid, obtaining the following values for citrate in each 100 cc.; 15 specimens of human semen, 140 to 637 mg.; 9 samples of human prostatic fluid, 480 to 2688 mg.; 20 lots of dog semen, 0. to 2.6 mg.; 2 analyses of human seminal vesicle secretion, 15 and 22 mg. While Huggins, Scott, and Heinen (11) found a high calcium content in human semen (6 millimols per liter) and in human prostatic fluid (30 mM per liter) the concentration of citrate in prostatic fluid and some seminal specimens is so high that the calcium ions are reduced preventing thrombin formation and blood coagulation. It seems reasonable to conclude that the adverse effect of human prostatic fluid on blood coagulation is a citrate effect.

Clotting Constituents of Human Semen.—The principal clotting constituents of blood were prepared from beef plasma and lung. In order to determine the presence of these or similar substances in semen, various combinations of them were placed in contact with human semen which had become liquid and the presence or absence of coagulation and the clotting time determined. Differences were observed depending on whether calcium ions were added to induce clotting immediately after adding semen or after preliminary incubation of semen with the clotting fractions of blood in the liquid state.

(a) *Immediate induction of clotting*: Human semen was found capable of substituting for fibrinogen and thromboplastin but not for prothrombin in inducing clotting (Table II, lines 1–6). The clot formed in the absence of added fibrinogen, was not firm, indicating that the fibrinogen content of semen was less than that of the prepared extract. Goldblatt (30) discovered that human semen contained thromboplastin.

(b) *Delayed induction of clotting*: Incubation of prothrombin with semen

both for 40 minutes and 18 hours decreased the coagulation time on adding fibrinogen, thromboplastin, and calcium ions (Table II, lines 7-10). Incubation of fibrinogen with semen for 18 hours abolished clot formation on adding the other principle clotting agents. Neither thromboplastin nor prothrombin were inactivated by incubation with semen for 18 hours; of the proteins implicated in blood coagulation only fibrinogen was destroyed.

TABLE II
The Effect of Human Semen on the Clotting Constituents of Beef Blood

Test	Pro-thrombin	Fibrinogen	Thromboplastin	Semen	NaCl, 0.9 per cent	CaCl ₂ , 0.5 per cent	Time of clotting	Remarks
	cc.	cc.	cc.	cc.	cc.	cc.	sec.	
1	0.5	0.5	0.5	—	0.5	0.2	44	Control—solid clot
2	0.5	0.5	0.5	0.5	—	0.2	80	Solid clot
3	—	0.5	0.5	0.5	0.5	0.2	—	No clot
4	0.5	—	0.5	0.5	0.5	0.2	300	Small amount of clot
5	0.5	0.5	—	0.5	0.5	0.2	320	Solid clot
6	—	0.5	—	0.5	1.0	0.2	—	No clot
7	0.5*	0.5	0.5	0.5*	—	0.2	22	Solid clot
8	0.5	0.5*	0.5	0.5*	—	0.2	85	“ “
9	0.5	0.5	0.5*	0.5*	—	0.2	90	“ “
10	0.5‡	0.5	0.5	0.5‡	—	0.2	22	Solid clot
11	0.5	0.5‡	0.5	0.5‡	—	0.2	—	No clot
12	0.5	0.5	0.5‡	0.5‡	—	0.2	15	A few fibres of fibrin appeared at 15 sec. Solid clot in 10 min.

The constituents designated * were incubated for 40 minutes at 37°C. and the other elements were then added.

The constituents designated ‡ were incubated for 18 hours and the other elements were then added.

Lytic Action of Human Semen on Normal Human Blood.—The blood of healthy persons when mixed with certain amounts of human semen or prostatic fluid, will still clot but subsequently liquefy. With equal concentrations of blood and semen, lysis occurs in 1 to 5 hours at room temperature, slightly more rapidly at 37°C. and not at all at 4°C. Serial dilutions of semen in saline were mixed with blood, 1 cc., and observed at 18 hours (Table III). In all instances semen, 0.03 cc., induced lysis; in 4 cases semen, 0.01 cc., liquefied the clot. Twenty samples of prostatic fluid were studied in the same way; lysis occurred in all with prostatic fluid, 0.02 cc., in 18 hours, and in one fluid 0.002 cc. induced lysis.

Human semen did not liquefy beef plasma. Lysis of dog blood and plasma

clots occurred only twice in 14 experiments and then in no less amounts of semen than 0.5 cc. and 0.1 cc. Rabbit plasma was resistant to solution.

Lytic Action of Dog Semen on Coagulated Blood of Normal and Diseased Persons.—Lysis of dog blood, clotted after mixing with dog semen, occurred regularly with semen 0.1 cc. and larger amounts. Solution of clots produced similarly with rabbit, beef, and rat plasmas occurred with dog semen 1 cc., occasionally with 0.5 cc. amounts, never with less than 0.1 cc. of dog semen.

The semen of 10 dogs was tested by serial dilution against the blood of 46 normal persons; the semen was mixed with blood, 1 cc., allowed to clot, and the experiment terminated at 18 hours. In 4 instances the blood did not coagulate, due to destruction of fibrinogen before the clotting was effected, since adding beef fibrinogen induced prompt clotting. Where clotting oc-

TABLE III

Lytic Action of Human Semen on Coagulated Blood of Various Species

1 cc. of whole blood + 1 cc. of secretion, diluted secretion, or saline.

Incubation 18 hours, 37°C.

Nature of blood	Amount of semen in test					
	None	1 cc.	0.5 cc.	0.1 cc.	0.05 cc.	0.03 cc.
Normal human	0	L	L	L	L	L
Dog	0	L	I	0	0	0
Rabbit*	0	L	0	0	0	0
Beef*	0	0	0	0	0	0

L, complete lysis; I, incomplete lysis; 0, no lysis.

* Citrated plasma, recalcified with CaCl₂, 1.5 per cent, 0.30 cc.

curred, the minimum amount of dog semen causing lysis was 0.016 cc. (Table IV). In all cases dog semen, 0.1 cc. lysed blood 1 cc., but smaller amounts were often ineffective.

In a similar manner, serial dilutions of dog prostatic fluid were tested for lytic activity against the blood of 24 patients in hospital. Most of the patients had disease of the urinary tract; 7 of them had benign or malignant tumors of the prostate gland. Clotting occurred in all of the tests. In 2 cases, both men with prostatic cancer, no lysis occurred in 18 hours with prostatic fluid, 1 cc. (Table V). In three patients with febrile illness, lysis occurred with prostatic fluid, 1 cc., but not with amounts of 0.5 cc. or less. The blood of 19 patients underwent lysis in a normal manner.

General Properties of Fibrinolysis by Semen.—When normal blood, 1 cc., was mixed with saline, 1 cc., allowed to clot, and observed 18 hours later, syneresis was always observed, and in the serum a small number (perhaps 1 per cent of the total) of erythrocytes were found lying at the bottom of the tube, detached

TABLE IV

Lytic Action of Dog Semen on Coagulated Blood of Various Species

1 cc. of whole blood + 1 cc. of secretion, diluted secretion, or saline.
Incubation 18 hours, 37°C.

Nature of blood	Amount of prostatic secretion in test					
	None	1 cc.	0.5 cc.	0.1 cc.	0.05 cc.	0.033 cc.
Human.....	0	L	L	L	I	0
Dog.....	0	L	L	L	I	0
Guinea pig.....	0	L	L	I	0	0
Rat*.....	0	L	L	0	0	0
Rabbit*.....	0	L	L	I	0	0
Beef*.....	0	L	I	0	0	0

L, complete lysis; I, incomplete lysis; 0, no lysis.

* Citrated plasma, recalcified with CaCl₂, 1.5 per cent, 0.33 cc.

TABLE V

The Lytic Action of Semen of the Dog on Human Blood Permitted to Coagulate

Total volume in test 2 cc.: 1 cc. of whole blood + 1 cc. of prostatic fluid, undiluted or diluted with saline.

Source	Amount of dog semen in test					Remarks
	1.0 cc.	0.2 cc.	0.1 cc.	0.05 cc.	0.03 cc.	
Healthy person (1)	L	L	0	0	0	
“ “ (2)	L	L	L	L	0	
“ “ (3)	L	L	L	L	L	
“ “ (4)	L	L	L	0	0	
“ “ (5)	L	L	L	0	0	
“ “ (6)	L	L	L	0	0	
“ “ (7)	L	L	L	I	0	
Patient P.M.	0	0	0	0	0	Carcinoma of prostate; febrile
“ A.Y.	0	0	0	0	0	“ “ “ afebrile
“ A.A.	L	L	L	L	0	“ “ “ “
“ F.D.	L	I	0	0	0	“ “ “ “
“ I.K.	L	0	0	0	0	Benign prostatic hypertrophy; febrile
“ A.H.	L	0	0	0	0	“ “ “ “
“ J.B.	L	L	L	0	0	“ “ “ “
“ A.W.	L	0	0	0	0	Tuberculosis of kidney; uremia, “

L, lysis; I, partial lysis; 0, no lysis.

from the clot. It is not known whether this erythrocyte detachment is due to a fibrinolysis. Syneresis is always more pronounced in the presence of erythrocytes than with plasma under the same circumstances for then it is unusual to find any clot retraction.

Fibrinolysis of whole blood by seminal fluids occurs in the absence of bacteria and of spermatozoa; the fluid component of fresh semen, free from evidence of infection and passed through a Seitz filter soon after collection, remained sterile and retained its ability to lyse blood. All samples of semen and prostatic fluids contained the enzyme. The blood of the donor of prostatic fluid was always susceptible to fibrinolysis by his fluid. After fibrinolysis the erythrocytes are intact and appear normal and the specific blood grouping is not removed.

Maintenance of seminal fluids at 60°C. for 5 minutes did not interfere with fibrinolytic action, but heating for the same period at 70°C. destroyed lytic ability. Prostatic fluid dried in air and stored for 3 weeks retained its lytic property when dissolved in quantity of saline equal to the original volume; the dried powder heated for 2 hours at 105°C. was still active. Neither toluol, sulfathiazole crystals in excess, nor merthiolate (sodium ethyl mercuri-thio-salicylate) inhibited fibrinolysis. Inhibitors of oxidation, NaF or KCN in 0.025 molar concentration, did not interfere with fibrinolysis.

The sterile supernatant fluid following fibrinolysis by semen retained its lytic activity when added to fresh blood and allowed to clot. Four such progressive transfers were made without loss of the lytic fraction.

Lytic Action of Human Body Fluids.—Fibrinolysis is not a widespread property of body fluids. As control for the lytic effect found in prostatic fluid, several human fluids were examined in a sterile state. These included; urine (10 specimens); saliva passed through a Seitz filter, 21 specimens; gall bladder bile, 6 specimens; hydrocele fluids, 2 lots; 2 spermatocele fluids; 6 normal cerebrospinal fluids; and the secretion of the seminal vesicle from three men. No lysis occurred in any.

Fibrinogenase.—When citrated plasma is recalcified, the clear plasma becomes opaque as clotting proceeds. This change in light transmission has been adapted to quantitative study of blood coagulation as a nephelometric procedure by Kugelmass (12) and especially by Nygaard (13) who devised a special apparatus for recording the changes. We investigated the opacification occurring in progressive dilutions of citrated plasma in a photoelectric colorimeter at 37°C. with plasma concentrations between 1 and 10 cc. (Fig. 1). An approximately linear relationship developed when units of density after clotting are plotted arithmetically against the quantities of fibrin present.

Human citrated blood plasma incubated at 37°C. with prostatic fluid of the dog, for short periods of time, shows progressive decrease of opacity after recalcification. In a typical experiment, 5 cc. of citrated plasma were incubated with 2 cc. of prostatic fluid and at intervals of 6 minutes, 5 cc. of saline, and 1.6 cc. of calcium chloride were added; in the control tubes prostatic fluid was replaced by saline since the principle electrolytes of dog prostatic fluid are sodium and chloride in 160 milliequivalent concentration per liter (4).

The density resulting from clot formation became progressively less with increasing incubation (Fig. 2). After incubation of 30 minutes, neither clotting nor change of density took place. The failure of clot formation was due to destruction of fibrinogen by dog prostatic fluid. However, no decrease of density or of clotting capacity occurred when human semen was added under comparable conditions to the citrated plasma. Indeed, after incubation of human semen with plasma for 4 hours only a slight decrease of density was

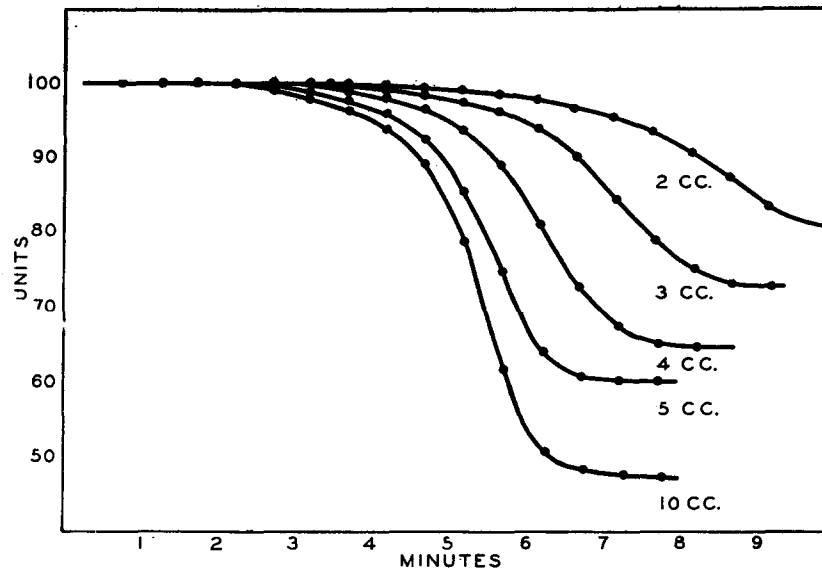


FIG. 1. A photoelectric study of the increased density occurring as clot forms in citrated plasma after recalcification. Progressive increases of density occur with increased content of plasma (cubic centimeters), the total volume of fluid in each tube being the same. Ordinates: units of galvanometric deflection. Abscissae: time in minutes after recalcification.

observed (Fig. 3). Human semen possesses only slight power of destroying fibrinogen.

Incubation of citrated plasma, 1 cc., with dog prostatic fluid, 1 cc., for 30 minutes abolished clotting on adding CaCl_2 ; prompt clotting occurred when beef fibrinogen, 0.5 cc., was subsequently added.

The differences in activity of canine and human semen on fibrinogen and fibrin were magnified by dilution of these fluids. Two series of tubes were arranged, all of which contained citrated human plasma, 1 cc., and either human or dog semen, 0.5 cc.; in series A, clotting was induced immediately, by adding calcium chloride, 0.25 per cent, 0.25 cc.; in series B, calcium chloride

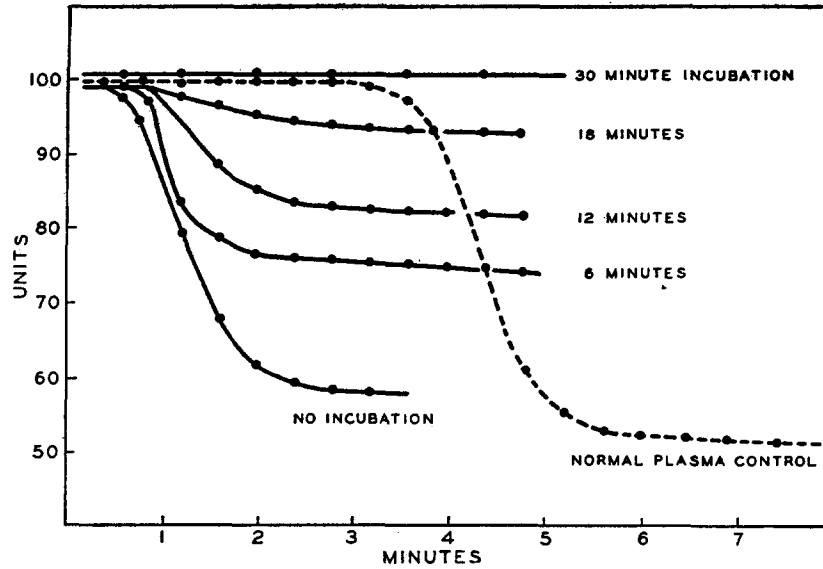


FIG. 2. Photoelectric study of the effects of incubation of dog semen with human citrated plasma on clot formation and density occurring after recalcification. A progressive decrease of clot density occurs with increased periods of incubation until contact for 30 minutes results in failure of clotting to take place. When clotting occurs, it begins in all of the tubes containing semen earlier than in the control tubes of plasma without semen, due to the thromboplastic activity of the secretion. Ordinates: units of galvanometric deflection. Abscissae: time in minutes after recalcification.

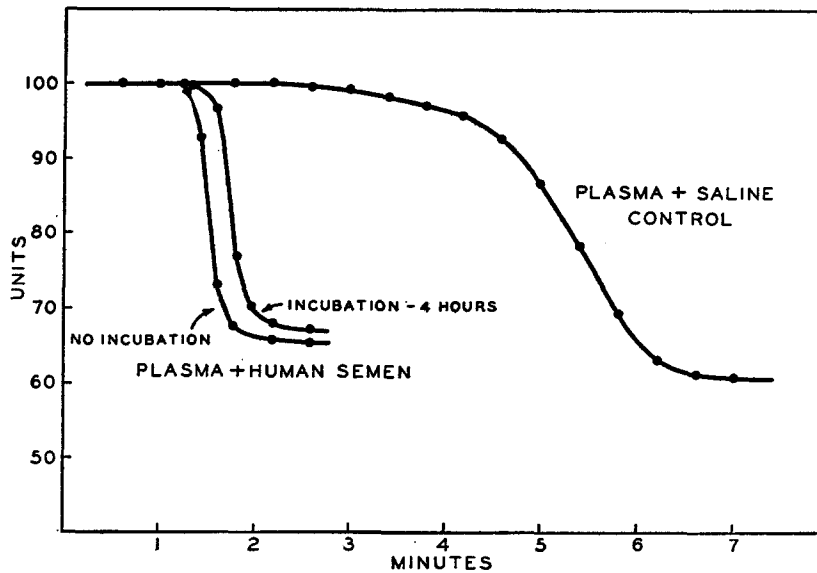


FIG. 3. A photoelectric study of the effect of incubation of human semen with citrated plasma on clot formation and density occurring after recalcification. In contrast to dog semen, only a slight decrease of density occurs after 4 hours incubation; the thromboplastic effects are evident as reflected in the increased rate of clotting over that of the control tube of plasma without semen. Ordinates: units of galvanometric deflections. Abscissae: time in minutes after recalcification.

was added after 18 hours' incubation. At 18 hours in series A, clots containing dog semen, 0.1 cc., were only slightly liquefied, and those containing 0.012 cc. were solid, while accompanying clots containing these amounts of human semen were liquefied (Table VI). At 18 hours in series B, clot could not be induced by recalcification of any of the tubes containing dog semen but the addition of calcium chloride to tubes containing less human semen than 0.1 cc. promptly formed clots which subsequently underwent lysis in

TABLE VI

The Effect of Dog and of Human Semen on Lysis of Fibrin and Fibrinogen

1 cc. of plasma + 0.5 cc. of diluted semen

Series A: 0.25 cc. of CaCl₂ added immediately.Series B: incubation of fluids for 18 hours before adding CaCl₂.

Series A Enzymatic action on clotted plasma			Series B Enzymatic action on liquid plasma		
Content of semen	Coagulation time	Extent of lysis after incubation for 18 hrs.	Coagulation time	Condition of plasma after adding CaCl ₂	Time of lysis subsequent to adding CaCl ₂
cc.	min.		min.		hrs.
Control—no semen	3½	None	4	Solid	No lysis
Dog semen					
0.1	3½	+	Did not coagulate	Liquid	
0.0125	3½	None	“ “ “	“	
Human semen					
0.1	3	++++	Did not coagulate	Liquid	
0.05	3	++++	4	Solid	2
0.025	3	++++	3	“	4½
0.0125	4	++++	4	“	No lysis

2 to 5 hours. Further, adding fibrinogen, 0.5 cc., to all tubes in series B which had not clotted after recalcification produced prompt coagulation.

Both human and dog prostatic fluids contain entities capable of destroying fibrin (fibrinolysin) and fibrinogen (fibrinogenase) but in different concentrations. Human semen contains much fibrinolysin, little fibrinogenase; dog semen contains little fibrinolysin, much fibrinogenase.

Thrombin in Semen.—The semen of certain dogs has the capacity of clotting fibrinogen, free from prothrombin, and of producing spontaneous clots in oxalated or citrated beef and rabbit plasmas. The calcium content of dog semen is about 0.3 millimols per liter (4). The thrombin activity is weak and requires 5 to 60 minutes before clot is evident. Citrated human plasma is not clotted by dog semen, obviously because human fibrinogen is destroyed by

fibrinogenase before the clot can be formed. Human semen does not contain thrombin.

To 0.2 cc. of rabbit oxalated plasma diluted with 0.8 cc. of saline, 0.5 cc. of dog semen was added and the tubes maintained at 37°C.; spontaneous clotting was observed in 18 minutes. A similar experiment using human plasma instead of rabbit plasma resulted in absence of clot at 10 minutes; the addition of 0.25 cc. of CaCl₂, 0.25 per cent did not induce clotting. Likewise, replacing dog semen with human semen, 0.5 cc., did not induce clotting in rabbit or human plasma.

To 1.0 cc. of beef fibrinogen, 0.5 cc. of dog prostatic fluid was added, clotting occurred in 20 minutes; replacing dog prostatic fluid with human semen did not result in clotting in 5 hours.

Trypsin in Semen.—The semen of both dog and man produced no visible change on coagulated egg albumin in Mett tubes when incubated overnight at 37°C. Using the method of Anson (8) small amounts of a proteolytic enzyme active at pH 7.5, trypsin, were demonstrated in 10 prostatic fluids of dogs; prostatic fluid, 1 cc. liberated chromogen equivalent to 0.029 to 0.15 mg. of tyrosin in 15 minutes. Five human semens, in 1 cc. volume liberated from 0.03 to 0.09 mg. of tyrosine, in 15 minutes; two human semens did not contain trypsin.

DISCUSSION

The mechanism by which human semen coagulates is not clear, and the problem is complicated by the difficulty of securing semen prior to clotting. The finding of fibrinogen and thromboplastin is evidence that seminal clotting resembles blood clotting; however, thrombin and prothrombin were not demonstrated after liquefaction. The abundance of citrate, which is sufficient to bind all of the calcium ions in many semens, provides an obstacle to the inference that the clotting of blood and semen is due to an identical mechanism. The presence of an active fibrinolytic agent would readily explain liquefaction, if, as seems likely, fibrin were the cause of the seminal clot.

The fibrinolysin in semen is derived from the prostate gland, and it was present in large amount in all of the prostatic fluids examined. This constant occurrence establishes a new function for the prostate. We have been unable to find previous observations relative to the action of genital secretions on fibrin except by Kurzrok and Miller (14), who were unable to demonstrate an effect of semen on fibrin, the source of which was unspecified. While all of the semens became liquid spontaneously in less than 10 minutes in our tests, the most rapid liquefaction of blood, 1 cc., by semen, 1 cc., occurred in 30 minutes. The lysis of blood clot by semen occurs less rapidly than lysis of the clotted semen itself.

Fibrinolysis without implication of the agent involved has been studied by several workers since its discovery in serum by Dastre (15). Nolf (16) re-

garded a slow aseptic fibrinolysis to be a natural sequel of plasma coagulation. Judine (17) observed that the blood of men in profound traumatic shock did not coagulate; further, that the blood of healthy persons, meeting a violent death, coagulated rapidly but within several hours became liquid and did not thereafter coagulate. Macfarlane (18) found that frequently the clotted blood of patients after surgical operations subsequently underwent complete lysis under aseptic conditions within 24 hours.

The effect of "chloroform semen" is germane to the present discussion. Howell (19) and Minot (20) discovered that clotting occurred in oxalated plasma to which chloroform had been added and Nolf (21) observed that the serum obtained from such clots is fibrinolytic. These findings have been confirmed by Tagnon (22, 23) who obtained from such serum, from which the chloroform had been removed, a globulin with marked fibrinolytic properties; the addition of this globulin to fibrinogen produced no clot but effected complete lysis of fibrinogen, while in the presence of prothrombin a clot formed which sometimes underwent fibrinolysis. The action on blood plasma and fibrinogen of this globulin resembles the effect of trypsin as described by Eagle and Harris (24). Ferguson and Erickson (25) in studying the clotting action of crystalline trypsin on citrated plasma, observed that clots so obtained undergo fibrinolysis within a few minutes; they found that trypsin, in 1 to 2 mg. amounts was optimal for the clotting of 1 cc. of citrated dog plasma.

Many points of similarity occur between the fibrinolysin of semen and that discovered by Tillett and Garner (26) in hemolytic streptococci. Both agents lyse normal human blood clot easily but act only after prolonged periods of time and in high concentration, when at all, on rabbit blood. In disease, at times, the blood of certain patients is totally resistant to lysis by semen; Tillett, Edwards, and Garner (27) got the same result with the fibrinolysin from streptococci in their patients with streptococcal infections. Further the active principle is demonstrable in dissolved fibrin even after incubation for 18 hours in both cases. Slight differences occur however. Garner and Tillett (28) found that streptococcal fibrinolysin resisted heating at 100°C. for 60 minutes, while we observed that semen is inactivated by heating to 70°C. for 5 minutes. Seminal fibrinolysin retains its activity for months in the refrigerator while that from streptococci deteriorates in several weeks.

In relating these observations to the effect of semen in the lysis of fibrin and fibrinogen, it should be stated that all of the samples of dog semen, and some human semens contained small amounts of trypsin; other human semens were free from trypsin. All samples were inactive in destroying beef fibrinogen and fibrin.

Both human and dog semens contain factors capable of inactivating fibrin and fibrinogen but in different and inverse proportions; human fluid contained fibrinolysin with greater activity than dog semen, while dog semen exerted

far greater fibrinogenase activity than human semen. It was readily possible by dilution to eliminate the weaker activity and to retain the stronger; this is evidence for the presence of two distinct proteolytic agents acting on fibrin and fibrinogen respectively. Garner and Tillet (29) observed that "solutions of human fibrinogen after brief incubation with fibrinolysin lose the capacity to form thrombin." The observed quantitative and species differences of proteolysis in semens do not fall in with the interpretation of these workers, whose observation on streptococcal filtrate with respect to fibrinogen is the same as ours on dog semen. We conclude that the agent in semen which liquefies fibrin resembles closely or is identical with the fibrinolysin of Tillet and Garner, and that the inactivation of fibrinogen is due to a separate agent, fibrinogenase.

SUMMARY

Certain specimens of human semen shorten the coagulation time of whole blood because of the presence of active thromboplastic agents, while other samples prolong its coagulation time. Human prostatic fluid in large amounts always delays or abolishes blood coagulation. The delay or absence of clotting is counteracted by adding calcium ions and is due to the large concentration of citrate in prostatic fluid and in some semens.

While most specimens of dog semen shorten the coagulation time of blood because of their thromboplastic activity, certain specimens render blood incoagulable or delay coagulation; in contrast to human semen, this adverse effect on coagulation is not overcome with calcium ions and is due to a different mechanism, the lysis of fibrinogen. The citrate content of dog prostatic fluid is small.

Human semen which has become liquefied does not contain thrombin or prothrombin, but fibrinogen and thromboplastic substances are present. Beef fibrinogen added to semen is destroyed by incubation for 18 hours, but added prothrombin and thromboplastic substances are still present after this treatment. Dog semen, in some instances, contains small amounts of thrombin.

The semens of man and dog contain a fibrinolysin for human blood which seems not to differ greatly from the fibrinolysin associated with hemolytic streptococci. The blood of the donor of prostatic fluid is susceptible to fibrinolysis by this fluid. However, the blood of persons with some diseases, is absolutely resistant to the action of seminal fibrinolysin. In how many diseases this happens has not yet been determined.

The semens of man and dog both contain an agent capable of inactivating fibrinogen, but in different amounts. This activity may be called fibrinogenase. Human semen is rich in fibrinolysin, poor in fibrinogenase; dog semen is rich in fibrinogenase, poor in fibrinolysin. These species differences, together with the fact that it is easy by appropriate dilution to retain the stronger proteolytic

agent and eliminate the weaker one, imply that fibrinolysin and fibrinogenase are different entities.

Dog semen, and less constantly human semen, contain very small amounts of trypsin.

All of these proteolytic agents derive from the prostate gland; their secretion in prostatic fluid constitutes a hitherto undescribed function for the prostate gland.

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