Relationship Between Human Seminal Fluid and the Fibrinolytic System. (20461)

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Activators and inhibitors as well as precursors of plasmin, the fibrinolytic enzyme of blood, have been found in demonstrable concentrations in a variety of tissues and body fluids. Although the enzymes of semen have received a good deal of attention, they have been investigated but little from this point of view. Huggins and Neal(1) showed that human and canine semen contains a fibrin-splitting enzyme in addition to another which degrades fibrinogen, both of which presumably originate in the prostate. They stated that human seminal fluid is rich in fibrinolysin and poor in fibrinogenase. Eichenberger(2) noted decomposition of fibrin flakes by semen.

Procedure and observations. Our own experiments confirm the degradation of fibrin by seminal fluid. If one part of seminal fluid is added to 10 parts of citrated plasma and if this solution is clotted, the clot is dissolved within 24 hours. There are a number of phenomena which are in contrast to those observed with plasmin alone. The latter enzyme dissolves the clot of a fibrinogen solution consistently faster than that of human plasma with a comparable fibrinogen content, since the plasma contains appreciable quantities of antifibrinolysin. If seminal fluid is used in place of plasmin, an entirely different situation prevails. It is, however, relatively easy to find a concentration of seminal fluid (the optimum is not the same for each sample of semen) in which the plasma clot is lysed faster than the fibrinogen clot, as illustrated in Table I.

Since bovine fibrinogen was used as a substrate, the findings permit 2 interpretations: (a) that the fibrinolytic enzyme of human seminal fluid is species specific and therefore attacks bovine fibrin to a lesser degree or (b) that seminal fluid contains fibrinolysokinase which activates the profibrinolysin of plasma or serum. Another characteristic to be noted, which differs from purified plasmin, is that the briefest fibrinolysis times do not correspond to the highest seminal fluid concentration but to the lower ones. This could be attributed to the presence of an inhibitor. For clarification of this, a series of experiments were performed in which the following reagents were used.

TABLE I. Fibrinolytic Action of Seminal Fluid on Fibrinogen and Plasma Clots. Fibrinogen 0.3%, 0.1 ml or plasma, 0.1 ml; aliquot seminal fluid; thrombin 200 units/ml, 0.03 ml; buffered saline ad 1.0 ml.

Seminal fluid conc. (ml)	Lysing time in min., fibrinogen exp.	Lysing time in min., plasma exp.
.5	540	300
.2	300	60
.02	180	210
.01	210	330

1) Human semen was obtained in a clinic or was sent in for investigation. Immediately after receiving, the semen was frozen and before each experiment, which was carried out within a few days, it was centrifuged until the seminal fluid was free of spermatozoa. 2) Buffered saline: one part veronal-acetate buffer, pH 7.42, plus 2 parts of saline. 3) Fibrinogen: bovine fibrinogen, Armour, 1% solution in buffered saline. 4) Plasmin: acid activated fibrinolysin 1% of von Kaulla(6) in buffered saline. 5) Prolysin: from human serum according to Lewis and Ferguson(3), dissolved in buffered saline corresponding to one-half the original amount of serum. 6) Streptokinase-Streptodornase: Lederle. Dissolved in buffered saline and diluted to 100 units/ml. 7) Thrombin: Parke, Davis Co. Dissolved in buffered saline. 8) Human citrated plasma, 1 to 4, obtained on the day of experiment.

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In all of the experiments outlined below, the same arrangement was chosen for observing fibrinolysis, *viz.*, 0.06 ml of the fibrinogen solution or 0.1 ml of citrated plasma was diluted to 1 ml with buffered saline after the addition of all other reagents, and clotting was effected by the addition of 0.03 ml of thrombin solution. This system for studying fibrinolysis was satisfactory and suitable for the small amount of material available for our experiments. This latter point is to be noted especially, for with the same arrangement, other materials could be investigated such as cervical mucus or ovarian follicular fluid, whose quantity is limited to an even greater extent. The endpoint of complete fibrinolysis was taken as the moment at which the last visible bit of fibrin net disappeared. The detection of the endpoint was easier in those tubules containing air bubbles in the fibrin net. The tubules containing the preparations were not touched during the time of observation.

The following procedure was used for the fibrinolysis test of human seminal fluid: A mixture of equal parts of seminal fluid and buffered saline was incubated at 37°C with an equal volume of prolysin for varying lengths of time. At hourly intervals, 0.1 ml of the seminal fluid and prolysin solution was taken out and added to the mixture for testing fibrinolysis and the time of dissolution of fibrin noted.

It is evident from Table II that human seminal fluid contains fibrinolysokinase which activates prolysin; the maximum activation appears to be reached after 4 to 5 hours and then a loss of activity appears probably due to inactivation of the newly produced enzyme. Seminal fluid (50%) and prolysin solution in a ratio of 1:1 appeared to be the most favorable. The yields with 5 parts of 50% seminal fluid and one part of prolysin were distinctly less; those with one part of 50% seminal fluid and 5 parts of prolysin were somewhat less favorable. Prolysin alone was not activated during the periods of the experiment. In addition to fibrinolysokinase, seminal fluid also contains small amounts of profibrinolysin, since the fibrin-splitting activity of seminal fluid can be enhanced by the addition of streptokinase. These relationships are shown in Table III.

TABLE II.
Prolysin Activation by Human Seminal Fluid.

Exp.	Incubation time (hr) 0	1	2	3	4	5	6	7	8
	Lysing time (min.)								
1	105	50	25	10	8	8	9	14	16
2	240	120	50	25	10	9	9	11	13
3	155	70	40	25	20	13	18	18	22

TABLE III. Effect of Streptokinase on Human Seminal Fluid. Fibrinolysis test mixture + 0.1 ml streptokinase 100 units/ml + aliquot seminal fluid.

	Semen No. 1	2	3	4	5	6
	Lysing time (min.) of seminal fluid with streptokinase					
(.025 ml)	12	19	20	30	25	35
(.0025 ml)	44	34	40	60	63	45
	Lysing time (min.) of seminal fluid without streptokinase					
(.025 ml)	65	160	210	400	220	230

TABLE IV. Effect of Seminal Fluid on Plasmin. Buffered saline plasmin solution 1%, 0.01 ml + aliquot part of seminal fluid incubated for 20 min., then fibrinogen added. Thrombin.

	Specimen	A	B	C
		Lysing time, min.		
Addition of seminal fluid	(.2 ml)	120	350	90
	(.1 ml)	60	111	50
	(.01 ml)	8	12	19
	(.005 ml)	10	9	14
Without seminal fluid		22	22	22

In mixtures without seminal fluid streptokinase did not induce fibrinolysis.

It is thus evident that seminal fluid profibrinolysin (or a substance closely related) occurs together with fibrinolysokinase. Antifibrinolysin is also present and in smaller concentrations than in serum. Larger amounts of seminal fluid are capable of diminishing the action of plasmin while smaller concentrations show a synergistic action with plasmin. Details are given in the examples in Table IV.

Discussion. It was possible to demonstrate that as Huggins and Neal(1) have shown seminal fluid contains a fibrin-splitting enzyme. This enzyme was found in all (50) semen samples which were investigated. Its activity, although variable from sample to

sample, does not seem to be influenced by the number of spermatozoa in that it was found in specimens with necro-, oligo-, and azospermia. The origin of this enzyme is not clear; perhaps it is formed by the prostate gland as Huggins and Neal have suggested. In cases of prostatic carcinoma, increased fibrinolysis was demonstrated in peripheral blood samples by Tagnon *et al.*(4). That this enzyme is added to the semen in a non-activated state and then becomes activated by fibrinolysokinase cannot be excluded. Fibrinolysokinase was demonstrated in all samples (14) which were investigated for its presence. Its activity, *i.e.*, concentration in various seminal fluid samples appears to be subject to some variation. The same is true for all samples containing material which was activated by streptokinase. Seminal fluid is the only body fluid which under normal circumstances contains free plasmin-like enzymes. Its full activity is produced by dilution. The significance of this enzyme system, especially in relation to fertility, is unknown. In any event, proteolytic enzymes frequently have been associated with certain fertility processes. Thus, Runnström(5) reached the conclusion that immediately after fertilization of metazoan eggs one or more proteolytic enzymes become activated. If one assumes that the spermatozoa are surrounded by an active fibrinolytic, seminal fluid layer, they encounter rather favorable conditions for maintaining this fibrinolytic activity in their passage to the ovum; the cervical mucus contains practically no anti-fibrinolysin, but small amounts of a material which is subject to activation by streptokinase; the uterus contains fibrinolysokinase as shown by Lewis and Ferguson(3), and also a fibrinolytic enzyme (Greenberg(7), Page *et al.*(8). Homogenized human fallopian tubal mucosa is also fibrinolytic and contains fibrinolysokinase. Follicular fluid usually contains large amounts of antifibrinolysin and also high concentrations of profibrinolysin. We shall report upon these findings in a later communication. It is thus possible that in order to perform its fertilizing function under normal circumstances the spermatozoon needs proteolytic enzymes which undergo, on its passage to the ovum, activation rather than inactivation. If these suppositions are correct, then one can draw the inference that some cases of sterility may be associated with disturbances of these enzymes.

Summary. Human seminal fluid contains in addition to fibrinolytic enzymes (whose identity with the enzymes of blood remains to be demonstrated) fibrinolysokinase, antifibrinolysin and a material which can be activated by streptokinase.

1. Huggins, C., and Neal, W., *J. Exp. Med.*, 1952, v76, 527; *Am. J. Physiol.*, 1943, v139, 129.

2. Eichenberger, E., *Gynaecologia*, 1946, v121, 288.

3. Lewis, J. H., and Ferguson, J. H., *J. Clin. Invest.*, 1950, v29, 1059.

4. Tagnon, H. J., Whitmore, W. F., and Shulman, N. R., *Cancer*, 1952, v5, 9.

5. Runnström, J., *Advances in Enzymology*, 1949, v9, 241.

6. von Kaulla, K. N., *Nature*, 1949, v164, 40.

7. Greenberg, E. M., *Bull. New York Acad. Med.*, 1948, v6, 397.

8. Page, E., Glendening, M. B., and Parkinson, D., *Am. J. Obstet. and Gynecol.*, 1951, v62, 1100.

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