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THE RELATION OF THE PROTEOLYTIC ACTIVITY OF HUMAN SEMINAL PLASMA TO VARIOUS SEMEN CHARACTERISTICS

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Summary. Proteolytic activity, acid phosphatase activity and fructose concentration were measured in seminal plasma from 205 human semen samples. Certain routine sperm characteristics were also determined. High fibrinolytic activity was found and TAME-esterase and proteinase activity was also present in all specimens. The activity of all the proteolytic enzymes correlated positively with the acid phosphatase activity suggesting the prostate gland to be the origin of the enzymes. TAME-esterase activity was inversely related to the seminal fructose content. No correlation was observed between sperm characteristics and the seminal proteolytic enzymes. The specimens with abnormal viscosity usually contained low proteinase activity.

Human seminal plasma is known to possess considerable proteolytic activity; two protein-splitting enzymes have been found, one active at an acid pH and the other having similarities to chymotrypsin (Lundquist, Thorsteinsson & Buus, 1955), Several investigators have demonstrated the presence of plasminogen activator(s) in seminal plasma (Lundquist *et al.* 1955; Rasmussen & Albrechtsen, 1960; Hisazumi, 1970). Lundquist *et al.* (1955) also discovered an enzyme system in human seminal plasma, which hydrolyses arginine esters. Suominen & Niemi (1970) have shown that this activity represents that of several different enzymes, e.g. the plasminogen activators. The function of these proteolytic enzymes is usually thought to be concerned with the liquefaction of the semen coagulum after ejaculation.

The purpose of the present study was to measure the total activity of proteinase, arginine-esterases and fibrinolytic enzymes in human seminal plasma, and to find out whether there is any correlation between these activities and certain parameters of accessory sex gland function (e.g. the activity of acid phosphatase for the prostate gland and the fructose content for the seminal vesicles) or other semen characteristics.

Semen samples were obtained from 205 patients who were being examined for infertility or for suspected disease of the accessory genital organs. The semen volume and viscosity, and the sperm density, motility, viability and morphology were analysed as described by Eliasson (1971). The following

criteria for a normal semen specimen were used: concentration of spermatozoa 50×10^6/ml or more, 50% or more of the spermatozoa having normal motility and morphology, viscosity 5 sec or less (viscosity was estimated by measuring the time taken for semen to flow through a standard glass capillary chosen so that water flowed through it in 1 sec) and semen volume 2 to 6 ml.

Cell-free seminal plasma was obtained by centrifugation at 2800 *g* for 20 min. Before the measurement of enzyme activity, the seminal plasma was diluted 1:8 with normal saline. All the measurements were made in duplicate at +37° C, 0·1 M-tris-HCl buffer was used throughout. Proteinase activity was determined using casein (10 mg/ml) as a substrate at pH 7·5, the incubation time was 2 hr. Hydrolysis products were estimated according to Folin & Ciocalteau (1927). The hydrolysis of TAME (p-toluene-sulphonyl-L-arginine methyl ester; 5 mmol/litre) was determined according to Kabakoff, Umhey, Wohlman & Avakian (1963), the incubation time was 4 hr. Fibrinolytic activity was measured using the fibrin-plate method presented by Brakman (1967),

TABLE 1

PROTEOLYTIC ACTIVITY IN NORMAL SEMEN SAMPLES

	Proteinase (*μmol/ml/hr*)*	*TAME-esterase at pH 7·0* (*μmol/ml/hr*)	*TAME-esterase at pH 8·2* (*μmol/ml/hr*)	*Plasminogen activators* (mm^2)†
Mean ± S.E.M.	3·06 ± 0·22	4·33 ± 0·34	7·50 ± 0·51	293 ± 16
Range	0·63 to 6·49	1·08 to 9·27	2·47 to 13·65	185 to 583
No.	28	28	28	28

* L-Tyrosine equivalents.
† Lysis area by 30 *μl* of the diluted seminal sample.

incubation time was 18 hr; plasminogen-rich fibrinogen (Grade B 1, Kabi, Sweden) was used and for plasmin assay the plasminogen was destroyed by heat. Acid phosphatase activity (i.u./ml) was assayed according to Sigma Bulletin No. 104 (normal values 25,000 to 60,000 i.u./ml). The fructose content of the seminal plasma (normal values >120 mg/100 ml) was determined according to the methods described by Mann (1948) and Eliasson (1965).

There was a high fibrinolytic activity in human seminal plasma (Tables 1 to 3). Arginine-esterase and proteolytic activities were also present in all specimens. On the other hand, no lysis occurred on heated fibrin plates. The variation of the enzyme activities in semen samples that were classified as normal are given in Table 1. No marked differences were observed between the characteristics of the spermatozoa and the enzymes, e.g. there was no correlation between sperm morphology and motility and seminal proteolytic activity. When the samples were classified according to acid phosphatase activity (Groups 1 to 4, Table 2), there was a significant difference ($P < 0{\cdot}001$) between the groups in proteinase and TAME-esterase activity. The correlation analysis confirmed also an interdependence between these activities and the acid phosphatase activity, the correlation coefficients were 0·70, 0·59 and 0·68, respectively. A significant increase in the activity of the plasminogen activator was noted in connection with increased acid phosphatase activity.

TABLE 2

THE RELATIONSHIP OF PROTEOLYTIC ACTIVITY OF SEMINAL PLASMA WITH PROSTATIC FUNCTION AS DETERMINED BY ACID PHOSPHATASE ACTIVITY OF SEMEN (MEAN ± S.E.M., RANGE AND NUMBER OF SAMPLES)

	Proteinase (*μmol/ml/hr*)	*TAME-esterase at pH 7·0* (*μmol/ml/hr*)	*TAME-esterase at pH 8·2* (*μmol/ml/hr*)	*Plasminogen activators* (mm^2)
Group 1 (phosphatase 0 to 9900 i.u./ml)	0·86 ± 0·11 0·12 to 1·51 17	2·65 ± 0·39 0·46 to 5·22 17	3·49 ± 0·54 0·29 to 8·34 17	244 ± 17 148 to 451 17
Group 2 (phosphatase 10,000 to 24,900 i.u./ml)	1·74 ± 0·10 0·45 to 4·11 68	3·09 ± 0·22 0·63 to 10·98 69	5·16 ± 0·32 1·66 to 15·88 69	277 ± 9 135 to 514 69
Group 3 (phosphatase 25,000 to 59,900 i.u./ml)	2·97 ± 0·12 0·63 to 6·49 79	4·16 ± 0·20 0·57 to 9·27 81	7·23 ± 0·32 2·23 to 17·99 81	307 ± 9 171 to 583 81
Group 4 (phosphatase 60,000 or more i.u./ml)	4·18 ± 0·22 2·02 to 7·28 30	8·28 ± 1·04 2·46 to 31·71 30	13·49 ± 1·17 6·00 to 33·69 30	337 ± 24 159 to 669 30

TABLE 3

THE RELATIONSHIP OF PROTEOLYTIC ACTIVITY OF SEMINAL PLASMA WITH THE FUNCTION OF THE SEMINAL VESICLES AS DETERMINED BY FRUCTOSE CONTENT OF SEMEN (MEAN ± S.E.M., RANGE AND NUMBER OF SAMPLES)

	Proteinase (*μmol/ml/hr*)	*TAME-esterase at pH 7·0* (*μmol/ml/hr*)	*TAME-esterase at pH 8·2* (*μmol/ml/hr*)	*Plasminogen activators* (*μmol/ml/hr*)
Group 5 (fructose 0 to 120 mg/100 ml)	3·34 ± 0·34 0·47 to 7·28 27	7·22 ± 1·13 0·46 to 31·71 28	11·80 ± 1·32 3·71 to 33·69 28	327 ± 23 191 to 669 28
Group 6 (fructose 130 to 440 mg/100 ml)	2·41 ± 0·10 0·12 to 6·49 151	3·96 ± 0·20 0·57 to 14·27 153	6·57 ± 0·30 0·29 to 22·44 153	292 ± 6 148 to 583 153
Group 7 (fructose 450 or more mg/100 ml)	2·53 ± 0·39 0·83 to 6·13 18	3·01 ± 0·27 1·48 to 6·08 18	5·57 ± 0·66 2·51 to 12·15 18	293 ± 25 135 to 514 18

When the semen samples were classified according to the seminal fructose content (Groups 5 to 7, Table 3), a significant difference ($P<0{\cdot}05$) in the TAME-esterase activities was noted between the groups, indicating decreased esterase activity with increased fructose content. The correlation coefficients were $-0{\cdot}37$ and $-0{\cdot}40$. The proteinase and plasminogen activator activities were negatively correlated with the seminal fructose content, the correlation being, however, clearly less than that between TAME-esterases and fructose. When the enzyme activities were compared with the semen viscosity, the proteinase activity was significantly lower in the group with abnormally high viscosity.

The prostate gland and the seminal vesicles secrete several chemical constituents into the semen, some of which can be considered as specific for the glands, e.g. acid phosphatase for the prostate and fructose for the seminal vesicles (Mann, 1964). All the enzyme activities correlated significantly with the acid phosphatase activity, which can be taken to indicate a prostatic origin for the proteases. A reverse correlation between TAME-esterase activity and fructose content and, to a lesser extent, between the other proteolytic enzymes and fructose content was noticed. This could be due to reduced enzyme activity following dilution when there was a higher proportion of seminal vesicle secretion in the semen. However, it is more likely that the seminal vesicles secrete some substance which inhibits the TAME-esterases and, to a lesser extent, the other proteolytic enzymes. It has been suggested that high viscosity of the semen might be a cause of decreased fertility (Amelar, 1966). In the present study, we observed that high semen viscosity was usually associated with low proteinase activity which may indicate that this proteolytic enzyme can be involved in human fertility.

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