The distribution of plasminogen activator in the male genital tract

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SYNOPSIS The distribution of fibrinolytic activity in the tissues of the male genital tract was studied by a histological technique. Preparations made from testis, epididymis, vas deferens, seminal vesicle, prostate, bulbo-urethral gland, and urethra showed that most activity was related to the blood vessels. However, inconsistent fibrinolytic activity related to epithelium was found in all parts of the genital tract. This epithelial activity was least in the testis, greater in the seminal vesicle and prostate gland, and was greatest in the bulbo-urethral gland and terminal urethra. No fibrinolytic activity could be demonstrated in relation to spermatozoa.

The cycle of coagulation and liquefaction of semen resembles the clotting and fibrinolysis of blood. Fibrinolytic activity appears in the blood when a pro-enzyme, plasminogen, is converted to the active protease, plasmin, by an 'activator'. This protease then digests fibrin. Similarly, when seminal fluid is ejaculated, it coagulates immediately and is then reliquefied within about 20 minutes by proteolytic activity. Human semen contains a fibrinolytic substance which Huggins and Neal (1942) thought was a protease of prostatic origin, but it was later shown that the substance was in fact an activator of the plasminogen system (von Kaulla and Shettles, 1953).

Using the histological technique of 'fibrinolysis autography' (Todd, 1959), which reveals the topography of plasminogen activator in tissues, it has been shown that activator is concentrated not only in the vascular endothelium of the prostate, but also to a lesser degree in the epithelium of the glands and ducts (Kester, 1969). In the latter studies, the amount of activator demonstrable in the prostatic epithelium seemed insufficient to be the only source of the fibrinolytic activity of human seminal fluid. In an attempt to find the source of plasminogen activator in seminal fluid, the other tissues of the genital tract have now been examined by fibrinolysis autography.

Materials and Method

Tissues were taken from the reproductive organs of 14 adult males at routine necropsies within 24 hours of death (see Table I for clinical details). Blocks from

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Case No.	Age (yr)	Cause of Death
1	68	Chronic lymphatic leukaemia
2	55	Cancer of caecum
3	46	Cerebral haemorrhage
4	58	Cancer of colon
5	82	Cardiac failure
6	77	Cancer of pancreas
7	44	Muscular dystrophy
8	60	Cerebral infarction
9	22	Multiple injuries
10	44	Myocardial infarction
11	63	Cancer of stomach
12	58	Cardiac failure
13	57	Myocardial infarction
14	61	Cancer of stomach

Table I *Details of source of human tissue*

testis, epididymis, vas deferens, seminal vesicle, prostate, bulbo-urethral (Cowper's) gland, and urethra were each placed in a small polythene bag containing Michaelis veronal buffer saline (Biggs and McFarlane, 1962), pH 7.4, ionic strength 0.16, with sodium azide 0.1% as a bacteriostatic. The samples were 'quick frozen' by plunging the bags into a dry ice-acetone mixture and stored at -30°C in the deep freeze. Tissue sections of about 8μ thickness were cut from the blocks on a cryostat microtome before making the histological preparations.

PLASMINOGEN-RICH FIBRINOGEN

A 2% solution by weight of bovine plasma fraction I (Armour)¹ was made in veronal buffer saline.

¹This preparation of bovine fibrinogen contains plasminogen as a contaminant, and 40-60% by weight of sodium citrate.

THROMBIN SOLUTION

Thrombin, topical (bovine origin) (Parke Davis): stock solution 1,000 units/ml in 50% glycerol, and this was diluted to 20 units/ml in Michaelis buffer saline to obtain a working solution.

CELLOPHANE SHEET

Cellophane PT 300-400 gauge (British Cellophane Ltd).

NEUTRAL BUFFERED FORMALIN SOLUTION (LILLIE, 1965)

Harris's haematoxylin (Harris, 1900).

The histochemical technique was that of fibrinolysis autography (Todd, 1964); a thin layer of bovine fibrin, rich in plasminogen, is applied to an unfixed dried section and incubated at 37°C (Fig. 1). Wherever the section contains activator, the plasminogen in the overlying fibrin is converted to plasmin and the fibrin is digested. When the preparation has been fixed, stained, and mounted, microscopic examination reveals the areas of digestion as pale zones in the stained fibrin background, and these can be related to structures in the overlying section.

Fig. 1 The construction of a fibrinolysis autograph.

The tissue-fibrin preparations were incubated for either 15, 30, or 60 minutes, and two samples were taken at the end of each period; thus six samples were examined from each block. In each preparation the degree of fibrinolytic activity of tissue components, ie, blood vessels, glandular epithelium, and secretion, was assessed in arbitrary units, using a semi-quantitative method (Kester, 1969) based on the magnification needed to detect the zones of lysis. An average value for the fibrinolytic activity was calculated for each type of component (Table II).

Tissue	Overall	Vascular	Epithelium	Secretion
Testis	13	13	1	0
Epididymis	16	16	6	0
Vas deferens	15	15	7	0
Seminal vesicle	16	16	12	4
Prostate	16	16	11	3
Bulbo-urethral gland	18 ¹	18 ¹	14	6

Table II Distribution of fibrinolytic activity in the male genital tract (arbitrary units)

¹Maximal activity

Results

TESTIS

The scanty blood vessels in the tunica albuginea showed little fibrinolytic activity. Zones of lysis related to the seminiferous tubules (Fig. 2) were most uncommon, and were particularly related to areas of disruption of the germinal epithelium. No fibrinolytic activity could be detected around the spermatozoa within the lumen of the tubules.

EPIDIDYMISS

Epithelial activity was infrequent, random in distribution, and of only moderate intensity (Fig. 3). Spermatozoa and secretion again seemed inactive. The blood vessels in the epididymis and the remaining accessory organs showed consistent and striking activity.

VAS DEFERENS

Scattered sites of epithelial activity of moderate intensity were seen.

SEMINAL VESICLE

The epithelial surface often exhibited striking fibrinolytic activity. Scattered within the lumen of each crypt were many disrupted cells and fragments of cellular debris; the areas of lysis were often related to these (Fig. 4). Occasionally, foci of activity seemed related only to acellular secretions. The spermatozoa again seemed inactive.

Fig. 2 *Testis: fibrinolysis related to a seminiferous tubule. 60 min $\times$ 95.*

Fig. 3 *Epididymis: digestion of fibrin by tubular epithelium. 60 min $\times$ 122.*

Fig. 4 *Seminal vesicle: an area of digestion related to disintegrating epithelial cells. 30 min $\times$ 608.*

Fig. 5 Prostate: frequent zones of lysis arising from the glandular epithelium. 60 min $\times$ 24.

Fig. 6 Cowper's gland: lysis related to several acini. 60 min $\times$ 95.

PROSTATE

Abundant zones of lysis, usually of moderate intensity, were related to the lining of the glands and ducts (Fig. 5) particularly where the epithelium was detached and presumably traumatized. Activity related to secretions was insignificant.

BULBO-URETHRAL GLAND

Of all the accessory organs, the bulbo-urethral gland showed the greatest levels of fibrinolytic activity. Figure 6 shows massive digestion of the substrate related to the glandular acini. The large ducts communicating with the urethra also exhibited conspicuous activity.

URETHRA

Figure 7 shows the vigorous fibrinolytic activity characteristic of urethral mucosa especially where there is epithelial disruption. Mild fibrinolytic

activity was related to the urethral glands of Littre (Fig. 8).

Discussion

After examining the fluid obtained by prostatic massage, Huggins and Neal (1942) presumed that the source of seminal fibrinolysin was the prostate gland. Some doubt was cast on this deduction when Harvey (1949) showed that the fibrinolytic activity of seminal plasma was independent of the proportion of the prostatic component. Ying, Day, Whitmore, and Tagnon (1956) too thought that only part of the fibrinolytic activity of semen could be ascribed to prostatic secretion, especially since such secretion crudely obtained by prostatic massage would have some contribution from vesicular and urethral secretions. Karhausen and Tagnon (1955) attributed the fibrinolytic activity of the prostate to a trypsin-

Fig. 7 Prostatic urethra: prominent activity from mucosa, especially where disrupted. 30 min $\times$ 95.

Fig. 8 Urethra: mild fibrinolytic activity arising from the glands of Littre. 30 min $\times$ 95.

like protease rather than to the presence of plasminogen activator. Although the prostate is rich in plasminogen activator (Albrechtsen, 1957; Rasmussen, Albrechtsen, and Astrup, 1958; Rasmussen and Albrechtsen, 1960), nevertheless the histological technique shows that the activator is largely confined to the vessels, making it unlikely that the prostate contributes much fibrinolytic activity to the ejaculate.

The normal secretory processes in male accessory glands are accompanied by definite changes in epithelial structure varying from desquamation to cell rupture (Mann, 1964). Analysis of the glandular fluid confirms the presence not only of whole epithelial cells but also of glandular debris. In the histological preparations, fibrinolytic activity related to such debris was conspicuous. This observation

is consistent with that of Pandolfi and Astrup (1967) that damage or disruption of corneal epithelium enhances the fibrinolytic activity of these cells.

It has been shown in the present studies that the glandular epithelium of the male accessory genital organs contains plasminogen activator in varying amounts. Although the fibrinolytic activity of blood vessels is consistently high throughout the genital tract (except in the testis), the fibrinolytic activity of epithelium increases along the genital tract and is greatest in both the epithelium and secretion of Cowper's gland. The full significance of the fibrinolysis in seminal plasma is not yet clear. Since fibrinolytic activity may be necessary to maintain the patency of the urinary tract by digesting fibrinous deposits (Astrup and Sterndorff, 1952; Ladehoff, 1960; Charlton, 1966), fibrinolysis may

similarly be necessary to maintain semen in a fluid state within the genital passages (Mann, 1964). However, the high concentration of activator in the last secretion to be added to the ejaculate—that from Cowper's glands—would suggest that the fibrinolytic activity had its main effects after ejaculation by liquefying the seminal coagulum and thus facilitating the migration of spermatozoa into the uterine cavity. It is also possible that plasmin plays a part in acrosome rupture, the process of 'sperm capacitation', and in penetration of the zona pellucida.

Huggins and Neal (1942) have shown that the fibrinolytic activity of semen was in the seminal plasma rather than in the spermatozoa; this has been confirmed using centrifugation methods checked by microscopy (Kester, 1970). In the present experiment no activator was demonstrated in spermatozoa, although Tympanidis and Astrup (1968) found that human spermatozoa in vaginal smears exhibited significant fibrinolysis. It is possible that in their material the spermatozoa were coated by seminal plasma containing activator, or that they had been activated by contact with vaginal or cervical secretions.

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