THE DISTRIBUTION OF PLASMINOGEN ACTIVATOR IN THE MALE GENITAL TRACT

Thesis for the Degree

of

Master of Surgery

in

The University of Cape Town

by

Ralph Charles Kester

January 1970.

To

my wife

my children

my parents

and Mark

CONTENTS

IX /

Contents

"In a priapism the blood does not coagulate,
except it threatens mortification."

John Hunter, 1793.

CHAPTER I

INTRODUCTION

INTRODUCTION

The blood of man is rich in plasminogen, the inactive precursor of plasmin, a protease (Astrup, 1956a); the most characteristic action of plasmin is the digestion of fibrin, i.e. fibrinolysis. Many tissues, including the prostate (Rasmussen and Albrechtsen, 1960a), contain substances which can activate plasminogen, and thus initiate fibrinolysis, and it has been assumed that both the excessive fibrinolysis seen in the blood of some patients with prostatic disease (Tagnon, Whitmore, Schulman and Kravitz, 1953a), and in prostatic surgery (Lombardo, 1957), is due to the release of this activator into the blood stream (Fearnley, 1965).

Human semen contains a substance which can activate the blood fibrinolytic system (von Kaulla and Shettles, 1953). Indeed, when human seminal fluid is ejaculated, it undergoes a process resembling the clotting and fibrinolysis of the blood, by coagulating then liquefying spontaneously. The coagulum is formed when a fibrinogen-like protein secreted by the seminal vesicles is acted upon by a clotting enzyme from the prostate (Mann, 1964). Coagulation is followed within about 20 minutes by liquefaction /

liquefaction of the clots by an enzyme assumed to come from the prostate (Huggins and Neal, 1942). This enzyme resembles plasmin in that it is a protease acting on a fibrin-like substrate, and that it is derived from an inactive precursor.

A great deal of knowledge has accumulated concerning the thrombosis of blood, and more recently, the blood fibrinolytic system. However, not only is the nature of the fibrinolytic system present in human semen obscure, but little is known of the source responsible for secreting such fibrinolytic substances into the seminal fluid. The exact role which such factors play in human reproduction has not been clearly decided, i.e. in the secretion and storage of seminal fluid; the cycle of coagulation and liquefaction of ejaculated semen; the interaction of such fibrinolytic substances with the secretions of the female genital tract during the ascent of motile spermatozoa; and in the fertilization of the human ovum.

The question of the origin of plasminogen activator in seminal fluid then remains open. A semen-liquefying "fibrinolysin" has certainly been found in fluid obtained by prostatic massage (Huggins and Neal, 1942), but samples obtained /

obtained by such a relatively crude method could not be purely of prostatic origin. Therefore, it seemed of interest to identify the tissue components in the male genital tract containing such activator. Using a histological technique - "fibrinolysis autography", which demonstrates fibrinolysin activator, Todd (1958, 1959) has found the activator, in most tissues, to be concentrated in the blood vessels. In the prostate, the epithelium and secretions seemed inactive, contrary to what might be anticipated from the fibrinolytic activity of prostatic secretion. In his experiments, however, postmortem tissues were used, so that the possibility of decay of the activator before testing could not be excluded.

To see whether prostatic epithelium contains any labile activator, twenty-five fresh, surgically removed prostate glands were examined (Kester, 1969), using the histo-chemical technique. The experiments confirmed the fibrinolytic activity of blood vessels, but also revealed that plasminogen activator is present in prostatic epithelium, but in amounts too small to be the entire source of the fibrinolytic activity in human semen fluid. Further studies were then carried out on samples of tissues taken /

taken at necropsy from the organs of the male genital tract, i.e. testis, epidydimis, vas deferens, seminal vesicle, prostate, bulbo-urethral gland, corpus spongiosium and corpus cavernosum. Specimens of human semen and seminal vesicular fluid were likewise tested, and the effect of various factors on the sensitivity of plasminogen activator was studied. These experiments, and those on fresh prostatic tissue have been combined to form the basis of this thesis. Altogether, over 2,000 histological preparations were examined.

CHAPTER II

THE HISTORICAL DEVELOPMENT OF FIBRINOLYSIS

PART I Of Fibrinolysis in the Blood

PART II Of Fibrinolysis by Tissues

PART III Of Fibrinolysis Related to Reproduction

PART I

THE HISTORY OF FIBRINOLYSIS IN THE BLOOD

Recorded knowledge of fibrinolysis commenced in the eighteenth century, when Morgagni (1769) was the first to observe that the blood remained fluid in cases of sudden death. He described the case of a man, who in 1725, was stabbed through the heart during a drunken brawl. He died shortly afterwards, and at the autopsy, Morgagni found the blood to be entirely fluid - "in general, more dissolv'd, and more wattery, than usual; perhaps from the quantity of fluid that had been drunk."

Morgagni's ovservation was closely followed by that of John Hunter (1793), who noted that "in many modes of destroying life the blood is deprived of its power of coagulation, as happens in sudden death produced by many kinds of fits; by anger, electricity, or lightning; or by a blow on the stomach, etc. In these cases we find the blood, after death, not only in as fluid a state as in the living vessels but it does not even coagulate when taken out of them." He commented on the postmortem fluidity of the blood in two stags, chased until they dropped /

dropped and died of exhaustion, and also on the rapid liquefaction of the blood in bats which had died after being disturbed in hibernation.

When Arnold, Berzelius (all quoted by Arthus and Dastre, in 1893) and Denis demonstrated in 1838 that fibrin was soluble in the presence of salt solutions e.g. ammonium chloride, strong interest in this characteristic was aroused. Denis postulated that a blood clot could dissolve after some time. Zimmermann (1846) found that ox fibrin left immersed in a salt solution e.g. potassium nitrate, remained intact for up to 10 days, while fibrin obtained from human blood by wet cupping dissolved within 12 - 24 hours. Limbourg showed in 1889 that fibrin was soluble in a wide range of salt solutions. Extensive experiments by Fermi (1891) revealed that many inorganic and organic acids dissolved fibrin. However he made no comment on the fact that when he left fibrin to stand in water, it dissolved.

Green's findings, in 1887, weakened the prevailing theory that the dissolution of fibrin was just a simple process. He demonstrated that fibrin decomposed in solutions /

solutions of neutral salts to form two different fibro-globulins. The decomposition was not due to bacterial putrefaction, as the reaction proceeded at temperatures just above freezing point. Arthus (1893) confirmed that the disintegration of fibrin was not due to the presence of bacteria, by using sodium fluoride to sterilize the salt solution.

Dastre, in 1893, was the first to introduce the term "fibrinolysis", after observing that clots, removed from dogs which had been exsanguinated, underwent spontaneous dissolution. He called this phenomenon "La fibrinolyse". Furthermore, he demonstrated that the end-products of fibrinolysis were peptones and not globulins (1894a), but he rejected the idea that fibrinolysis was due to a protease, as the properties of the factor seemed dissimilar to that of trypsin or pepsin, and he did not believe that the factor was present in the blood (1894b).

However, studies by Denys and Marbaix in 1889 showed that dog serum incubated with solvents such as ether, alcohol or chloroform, developed the ability to digest casein or gelatin - "autodigestion du sang additionné de chloroforme." /

chloroforme." This reaction was inhibited by the addition of a trace of untreated dog serum. They concluded that the serum contained a "digestive ferment", and an inhibitor which is normally in excess, and which is destroyed by the solvents. Delezenne and Pozerski (1903) confirmed Dastre's work, and demonstrated too, that chloroform stimulates fibrinolysis.

It was Nolf, who in 1904, observed that "la fibrinolyse" was due to a "substance sécréteé par la paroi vasculaire", and he was the first to suggest that fibrinolytic activity arose from the walls of blood vessels. By means of numerous experiments, he demonstrated that injections of peptones into dogs increased the fibrinolytic activity of blood (1904); that the liver secreted a fibrinolytic inhibitor, since extirpation of the liver resulted in increased fibrinolysis (1905); and that leucocytes have a fibrinolytic function (1908a)

Nolf postulated that the endothelial cells of blood vessels were covered by an ultra-microscopic layer of fibrin (1908b), originating the idea that fibrin formation occurs normally on certain cell surfaces in contact with the /

the blood, and that this deposition is counterbalanced by fibrinolysis. He confirmed that when chloroform was mixed with mammalian and bird plasma, fibrinolysis was induced (1921). He experimented with the coagulant and fibrinolytic properties of snake venom (1922a), and he observed that defibrination occurred when chloroform-treated serum was injected intravenously into dogs (1922b). At the same time that Nolf began his studies, Hedin (1904) had discovered that the enzyme lysing fibrin was a globulin.

That normal serum was capable of inhibiting proteolysis, had first been shown by Hildebrandt (1893) and next by Hahn (1897). Landsteiner (1900) revealed that the inhibitory factor did not reside in the serum globulin fraction, precipitated by half saturation with ammonium sulphate, but was present in the albumin portion precipitated by complete saturation with ammonium sulphate after removal of the globulin. This finding was supported by the observations of Opie and Barker (1907). Dale and Walpole (1916) examined the increased fibrinolysis in blood treated with chloroform, and showed that this was due to the destruction of an inhibitor, "anti-trypsin."

After /

After Morawitz (1906) had suggested, when noting that the blood in cases of sudden death lacked fibrinogen, that this could be due to the digestion of fibrin by an enzyme, Fidon, Gautier and Martin (1908) showed that dogs killed by drowning developed rapid lysis of their blood clots. A practical though macabre application of these observations was made by Yudin, who in 1936 reported that fluid cadaver blood from cases of sudden or violent death had been used for transfusion services. Mole (1948) demonstrated that postmortem fibrinolysis was a normal phenomenon due to fibrinolysin arising from vascular endothelium; he thought that fibrinolytic activity was the natural physiological means whereby fibrin deposits are prevented from forming on the vascular endothelium in health.

Tillett and Garner (1933) reported that filtrates of certain haemolytic streptococcal cultures exhibited marked fibrinolytic activity, due to an enzyme which they called "Fibrinolysin". Milstone (1941) elaborating further on this discovery, showed that digestion of purified fibrin was not possible, when using the streptococcal fibrinolysin; however, on addition of a small amount of normal serum /

serum, fibrinolysis proceeded. Thus, a further substance was required for the reaction, which he called the "Lytic" factor. Kaplan (1944) now found that Milstone's lytic factor was identical to the proteolytic globulin isolated from blood treated with chloroform (Tagnon, 1942).

Christensen (1945), working with serum treated with streptococcal fibrinolysin, found that this substance activated an enzyme precursor in the serum, which he called profibrinolysin. This simple presentation of the action of the streptococcal factor was later proved not to be true. Christensen and MacLeod (1945) demonstrated that the fibrinolysis brought about by chloroform treatment and that due to addition of streptococcal extract were both due to the same enzyme. They changed the name streptococcal "fibrinolysin" to "streptokinase"; from its precursor "plasminogen", was derived the proteolytic enzyme "plasmin", and its inhibitor was named "antiplasmin". They showed that the rate of change of plasminogen to plasmin was proportionate to the concentration of streptokinase.

However, Macfarlane and Pilling (1946) considered that plasmin /

plasmin and antiplasmin were associated within the albumin fraction of the serum as a complex from which plasmin can be freed by the destruction of the inhibitor by chloroform; by dilution; or be dissociated by fractionation. Geiger (1952) questioned the validity of Christensen's scheme that streptokinase activated plasminogen to active plasmin, when differing results were obtained using plasminogen derived from the blood of various animals. He suggested that a complement-like factor was necessary. Indeed, further observations by Mullertz and Lassen (1953), and confirmed later by Sherry (1954), indicated that the scheme proposed by Christensen was not sufficient. Mullertz and Lassen found that although bovine plasminogen was not activated by streptokinase, it certainly became proteolytic if a small amount of human plasma globulin was added to the reaction. Thus, according to them, human blood contains both an inactive pro-enzyme plasminogen, and its inert pro-activator. Bovine plasma was deficient in pro-activator and therefore did not become proteolytic when treated with streptokinase. The reaction proceeds as follows (Astrup, 1956a):

Pro-activator /

Pro-activator + Streptokinase - Activator

Plasminogen + Activator - Plasmin

Plasmin + Fibrin - Polypeptides

This scheme is still accepted at present, but it may be possible that these fibrinolytic components may not be independent, but rather different forms of the plasminogen molecule (Kline, 1960).

Fearnley (1953) demonstrated that labile blood activator was adsorbed to the surface of a fibrin clot, causing its lysis during incubation. A similar phenomenon of adsorption was observed by Sherry, Fletcher and Alkjaersig (1959), whereby activator was released when streptokinase entered a clot, and converted its intrinsic plasminogen to plasmin. Lysis of the clot then ensued.

Kwaan and McFadzean (1956) demonstrated that by arresting the arterial supply to a limb, fibrinolytic activity arose from the ischaemic vessel walls, mainly from capillaries and smaller veins. In 1958, Kwaan, Lo and McFadzean reported that vascular endothelium releases activator. Todd (1958, 1959, 1964) showed that the intima of blood vessels was rich in activator, veins more so /

so than arteries. Natural fibrinolysis in the blood can be demonstrated in normal persons, even at rest (Fearnley and Tweed, 1953), and it is apparent that far from being a passive system of collecting channels, the venous system has an active secretory function (Fearnley, 1965).

THE HISTORY OF TISSUE FIBRINOLYSIS

The earliest experiments studying the lysis of fibrin by tissues were conducted by Halban and Frankl(1910). Tissue fragments were placed directly on a serum substrate in a Petri dish, and the degree of fibrinolytic activity was expressed by the area of digestion of the substrate. It was shown that endometrial tissue was capable of digesting serum substrates, and they assumed that this property was due to a trypsin-like enzyme. This method was relatively inaccurate and poorly standardized, and the results were based on individual judgement. Caffier (1930) made use of similar methods.

Lambert and Hanes (1911) commented on the fibrinolysis of rat plasma media by tissue cultures of mouse sarcoma. Similarly, Carrell and Burrows (1911) were impressed by the finding that human breast cancer cells liquefied the plasma clots in which they were growing. Indeed, fibrinolytic activity displayed by malignant tumour cells in vitro, proved to be an irksome hindrance to the cultivation of permanent strains of these cells, by early tissue culture workers.

Whitehouse /

Whitehouse (1914) showed that menstrual blood was actively fibrinolytic, and he suggested that the fluidity of the blood was due to the activity of a tissue enzyme. He found that clots made from blood taken from the arm were quickly lysed by fragments of endometrium. He demonstrated that this phenomenon happened during menstruation, by observing that blood taken directly from the uterine cavity first clotted, then reliquefied. He suggested that the tissues contained a specific enzyme which digested human fibrin, since it exhibited no effect on ox fibrin.

Fleisher and Loeb (1915) investigated the fibrinolytic activity of a number of different tissues, by measuring the time taken for the lysis of a certain amount of clotted blood plasma, produced by tissue fragments in direct contact with the plasma. They placed the tissue fragments either on the clot, or the plasma was allowed to clot around the tissue, and they observed liquefaction of the clot around the tissue fragments after 24 hours. Even if the cells were first killed by chloroform, they were still able to digest the clot. They concluded that the tissues possessed a fibrinolytic enzyme.

Demuth and von Riesen (1928) studied the fibrinolytic activity of tissue cultures, and confirmed that the fibrinolytic activity of tissues was greatest at acid reaction. They suggested that the liquefaction of the plasma clot was due to dying cells liberating an "activator" of a proteolytic enzyme precursor present in the clot. Therefore, they were the first to consider that an activator was required in the fibrinolytic mechanism. These findings were confirmed several years later by Fischer (1946), while working with tissue culture media. He observed spontaneous lysis of chicken plasma clots by Rous chicken sarcoma cells; no lysis developed if the plasma was heated at 56°C. for 4 hours before incubation with the cells. He also concluded that the tumour cells released an activator which converted a precursor in the plasma to an active protease.

The Development of the "Fibrin Plate" Method

These pioneer studies on fibrin-digestion by tissues were expanded by Astrup and Permin (1947), who found that different tissues, especially those of fresh pork, contained an insoluble compound that activated profibrinolysin. They called this compound "fibrinokinase", a term later replaced /

replaced by the more acceptable "tissue activator of plasminogen". They demonstrated that the fluid expressed from fibrin clots became strongly fibrinolytic when incubated with tissue. Their attempt at aqueous extraction of this substance from tissues was unsuccessful, although suspensions of washed tissue fragments were very active.

Astrup and Permin (1948), studying the fibrinolytic activity developed by an ox plasma fraction when mixed with various tissue extracts, found that when streptokinase was used, no activation of the ox plasminogen occurred. Permin (1947) discovered that the proteolytic precursor activated by tissue extracts seemed to be identical with the plasmin formed when streptokinase activated plasminogen. The "fibrin plate method" was then developed by him to detect the presence of plasminogen activator in tissues. Here, a small amount of the tissue to be tested is placed on a thin layer of bovine fibrin, rich in plasminogen, covering the bottom of a Petri dish. After the preparation has been incubated at 37°C. for 20 hours, fibrinolysis can be seen as a clear area of liquefaction /

liquefaction around the tissue.

Now, although certain tissues are able to digest untreated fibrin, this ability is lost on heating of the fibrin (Fleisher and Loeb, 1915). Therefore, if the bovine fibrin plate was heated at 80°C. for 30 minutes, the plasminogen was destroyed (Lassen, 1952). A further modification was to use human fibrin as a substrate. These three types of fibrin plate provided both a differentiation and a qualitative estimate of fibrinolytic activity. Lysis in the plates will occur when (see Table 1):

1. Plasmin digests fibrin in all three plates.
2. The presence of plasminogen activator will lead to the digestion of only the human and the bovine unheated fibrin.
3. Kinases e.g. streptokinase, will cause digestion only of human fibrin.

Table 1

Type of Plate	Human Fibrin	Bovine Fibrin	Heated Bovine Fibrin
Factors included in Plate	Pro-activator Plasminogen Fibrin	- Plasminogen Fibrin	- - Fibrin
Streptokinase	+	-	-
Activator	+	+	-
Plasmin	+	+	+

(from Macfarlane, 1964)

By measuring the area of fibrinolysis produced by a given amount of the specimen in a given time, a quantitative estimate can be reached of the content of plasminogen activator in the specimen.

The Properties of Tissue Activator

Succeeding the development of the fibrin-plate method, the next decade was mainly concerned with elucidating the physical characteristics of the activator. Permin (1949) showed that tissue activator differed from cathepsins, in that the substance was not stimulated or inhibited /

inhibited by a variety of papainase activators and inhibitors (glutathione, ascorbic acid, cysteine, hydrocyanic acid and potassium bromate). It had been shown previously too, by Goldhaber, Cornman and Ormsbee (1947) in studies with tissue cultures, that activator differed from cathepsins. When homogenised tissue preparations were centrifuged, fibrinolytic activity was associated with the microsome fraction (Tagnon and Petermann, (1949a), whereas the inhibitors of fibrinolysis were main concentrated in the supernatant fluid.

Tissue activator is relatively heat-stable and is resistant to heating for 30 minutes at 37° - 50°C. over a broad pH range, and it is not destroyed even by heating to 70°C. at acid reaction (Astrup and Śterndorff, 1956a). It can be stored for long periods in a frozen state without loss of activity (Tagnon and Petermann, 1949b; Lewis and Ferguson, 1950). This stability contrasts with that of the precursor of plasminogen activator found in normal blood (Müllertz and Lassen, 1953), which under the influence of so-called lysokinases, is converted into a plasminogen activator which is labile, especially at acid and /

and alkaline reactions (Müllertz, 1955). Certain tissues have been studied, and have been found to contain such a lysokinase e.g. the kidney (Astrup and Sterndorff, 1956b).

Extraction of the activator from tissues is possible with distilled water (Frankl and Aschner, 1911); with ammonium sulphate solutions (Astrup and Permin, 1948); saline (Rosenmann, 1920; Astrup and Sterndorff, 1952a); but it has been found that a 2-M potassium thiocyanate solution is a specific solvent (Astrup and Stage, 1952). A tissue-activator standard can be prepared as a dried powder, which will keep its stability for at least 18 months when stored at -20°C. (Astrup, 1951). The presence of magnesium ions increases the solubility of the tissue activator (Bierstedt, 1955).

Tissue activator can withstand the action of many chemicals, viz. acids, bases, toluol, alcohol, ether and chloroform (Fleisher and Loeb, 1915), acetone (Permin, 1947), but it is destroyed by formalin (Caffier, 1930).

The Activation of Plasminogen by Tissue Activator.
(see Table 2: The Fibrinolytic Enzyme System)

Astrup (1951, 1952) has demonstrated that the amount of /

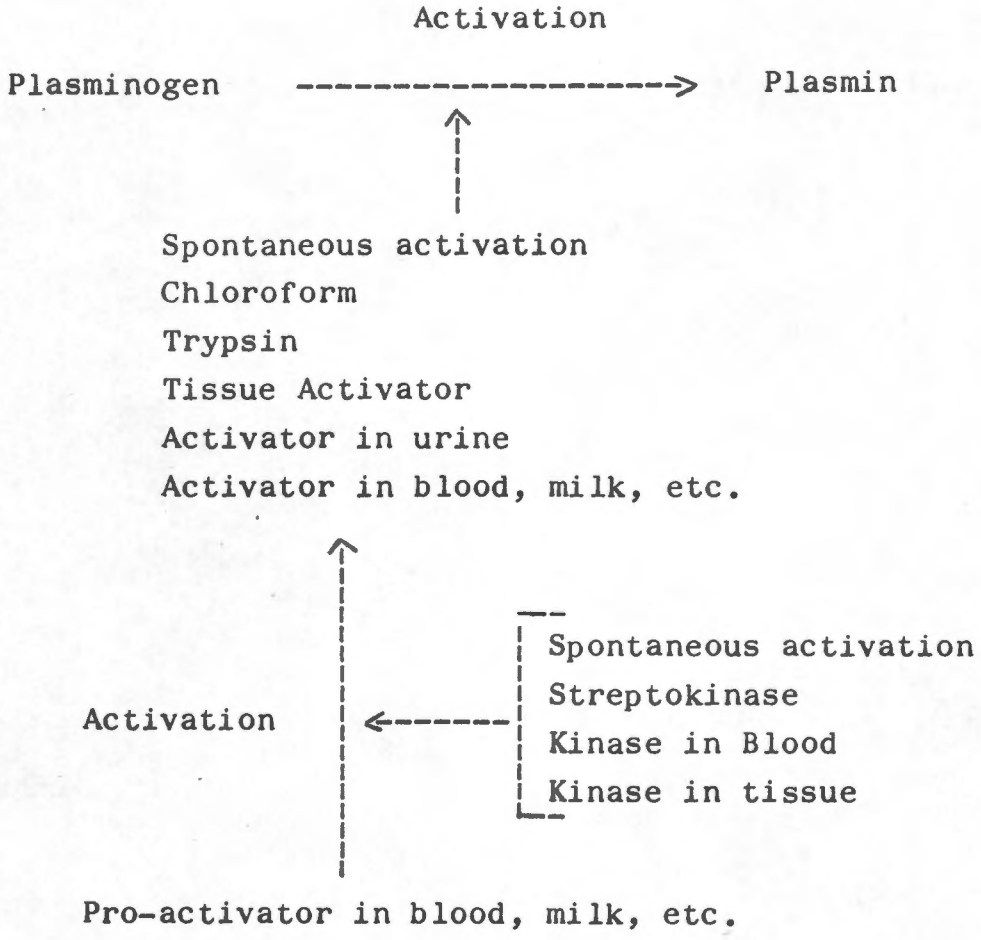


Table 2: The Fibrinolytic Enzyme System
(after Astrup, 1956a)

of plasmin produced by this process varies with the concentration of both the tissue activator and plasminogen in such a manner, that increasing amounts of plasmin are formed on addition either of increasing amounts of tissue activator to the same amounts of plasminogen, or of increasing amounts of plasminogen to the same amount of tissue activator. The reaction proceeds to equilibrium in a given time, so that complete conversion of the plasminogen in a given solution cannot be obtained even on addition of the strongest concentration of tissue activator. It has been further shown that the tissue activator inhibits the plasmin produced. It is thought that the tissue activator reversibly removes a blocking substance from plasminogen, so that this is converted into plasmin, and that the blocking substance should be attached to the activator, and that this complex should be able to inhibit the plasmin produced.

Although these studies were confirmed by Permin (1949), and Lewis and Ferguson (1950), further experiments (Tagnon and Petermann, 1949b; Tagnon and Palade, 1950) refuted some of these observations. Increasing amounts of plasmin occurred only on addition of increasing amounts of tissue activator to a given amount of plasminogen whereas/

whereas conversely, addition of increasing quantities of plasminogen to a given quantity of tissue activator did not increase the amount of plasmin produced. The process had its optimium reaction at pH 6.0-7.2. at 37°C.

Localisation of the Source of Tissue Activator

Rulot (1904), Barker (1908) and Nolf (1908a) all thought that leucocytes could cause fibrinolysis. There have been many speculations about the origin of fibrinolytic activity in blood (Nolf, 1904; Mole, 1948; Kwaan and McFadzean, 1956; Fearnley and Ferguson, 1958) who have all suggested that the active substance came from the lining of the blood vessels. Nolf (1908a) believed that the degree of fibrinolysis depended upon the amount of leucocyte and endothelial protoplasm entering into the formation of the clot.

Mole (1948), after studying fibrinolysis in cadavers, suggested that the active globulin came from the endothelium of blood vessels. Kwaan and McFadzean (1956) suggested that the capillaries and veinules were the source, after studying blood samples taken from ischaemic limbs.

A micro-modification of Permin's (1947) fibrin-

plate test was evolved by Todd, in 1958, called "fibrinolysis autography", where unfixed tissue sections were incubated on a film of plasminogen-rich bovine fibrin. From these studies, it was shown that the main source of tissue activator was the endothelial cells of veins. Arterial endothelium and serosal cells contain pro-activator (Todd, 1964); plasminogen activator was also found in the superficial endometrium at the time of menstruation; in squamous epithelium adjacent to areas of inflammation; and in thrombi and fibrinous pleural exudates.

The histo-chemical technique has been used by Warren (1964), and has been made semi-quantitative by incubating a number of preparations of the tissues under study for varying periods between 10-60 minutes (Kwaan and Astrup, 1964a, 1964b). The discovery, application and further developments of fibrinolysis autography is a major step forward in investigating the properties of a system of the body, whose potential significance is not fully understood.

Activators in Other Body Fluids

Macfarlane and Pilling reported in 1947 that human urine has fibrinolytic activity, and Ploug and Kjeldgaard (1957) later extracted urokinase in pure form.

This/

This physiological activator is being currently tested as a thrombolytic agent, as it is a non-antigenic, non-toxic activator of plasminogen.

Plasminogen activators have been demonstrated in milk (Astrup and Sterndorff, 1953), tears (Storm, 1955), saliva (Albrechtsen and Thaysen, 1955), seminal fluid (von Kaulla, 1953), and ascitic fluid (Albrechtsen, Storm and Claassen, 1958). Pro-activators have been found in amniotic fluid (Albrechtsen and Trolle, 1955), in cerebro-spinal fluid; fluid from synovial cavities, hydroceles and blisters; lymph (Albrechtsen, Storm and Claassen, 1958). These substances are the precursors of an activator similar to the one formed in blood treated with streptokinase i.e. the activator is relatively labile, and is destroyed at acid reaction, and at temperatures of 70 - 100°C.

PART III

THE HISTORY OF FIBRINOLYSIS RELATED TO REPRODUCTION

In most animal species, semen is ejaculated in a liquid or a semi-liquid state. In some species, e.g. the bull and the dog, the ejaculate remains fluid, but in others it is discharged either in a partly gelified state, or it becomes a gel or a firm coagulum soom afterwards (Mann, 1964). Human semen clots immediately after ejaculation, but it liquefies within about 20 minutes.

Thoughtful observations were made as early as 1786 by John Hunter, about the consistency of the secretions of the male genital apparatus. He did not agree with the current opinion that the glands called the "vesiculae seminales", situated between the rectum and the bladder, were "reservoirs for the semen, secreted by the testicles, in the same manner as the gall-bladder is supposed to be a reservoir for the bile", but were glands producing a characteristic mucous secretion "of a lightish whey colour, having nothing of the smell of semen; and in so fluid a state as to run out on cutting them". He observed that in rats the seminal vesicles contained "a thick /

thick ash-coloured mucus, nearly of the consistence of soft cheese, very different from what is found in the vasa deferentia of the same animal, with which they do not communicate." A similar occurrence was found in the seminal vesicles of the guinea-pig, where the contents of the seminal vesicle were fluid near the fundus, but of cheese-like consistency near the ejaculatory ducts.

In rodents, e.g. rats, mice and guinea-pigs, a vaginal plug or "bouchon vaginal" forms after mating, when emitted semen coagulates, and the coagulum serves to prevent the loss of seminal fluid from the vagina. The vaginal plug consists of protein material secreted by the seminal vesicles (Bischoff, 1852; Bergmann and Leuckart, 1852; Stockard and Papanicolaou, 1919). It was discovered by Camus and Gley (1896, 1922) that the coagulating factor was an enzyme, which they called "vesiculase". The source of this enzyme was pinpointed to the so-called coagulating gland, adjacent to the seminal vesicle (Walker, 1910). It was only during ejaculation that the enzyme came into contact with the protein substrate and coagulation then proceeded.

If the normal mechanism of ejaculation is upset, clotting may occur within the male passages, e.g. semen may/

may be ejaculated retrogradely into the urinary bladder of male rats to form a "soft calculus" here (Vulpé, Usher and Leblond, 1956); or cheese-like lumps may form in the urinary bladder of bilaterally adrenalectomized guinea-pigs, and these may frequently block the urethra (Clayton, Hammant and Hawkins, 1956).

The coagulation of semen in rodents occurs in two stages (Mann, 1965):

Procoagulase (seminal vesicles)	+	Vesiculase (coagulating gland)	-	Coagulase (clotting enzyme)
Coagulinogen (seminal vesicles)	+	Coagulase	-	Coagulated protein

Investigating the properties of human semen, Huggins and Neal (1942) observed that the liquefaction of semen was due to a protease which they called "Fibrinolysin", because of its ability to digest fibrin clots obtained from the blood, and its resemblance to the fibrinolytic factor found in the filtrates of haemolytic streptococcal cultures (Tillett and Garner, 1933). They suggested that this enzyme was of prostatic origin. It was further observed that although dog prostatic fluid was rich in an enzyme digesting fibrinogen - "fibrinogenase", the content of this protease in human seminal fluid was quite/

quite low.

Harvey (1949) extended these experiments performed by Huggins and his co-workers. She measured the time taken for blood clots to liquefy when a constant volume of oxalated blood was mixed with varying amounts of semen, and the blood was next clotted with calcium chloride. No connection was found between the level of fibrinolytic activity and the viscosity of the semen. Extremely viscous specimens of seminal fluid, however, did appear to have low fibrinolytic activity.

During an analysis of human seminal fluid (von Kaulla, 1953; von Kaulla and Shettles, 1953), the samples were found to contain plasmin; plasmin inhibitors; "fibrino-lysokinase" (plasminogen activator) which activated "prolysin" or "profibrinolysin" (plasminogen); and a precursor activated by streptokinase. It was noted that the fibrinolytic activity of seminal fluid was enhanced by dilution (of the inhibitor). There was no relationship between the number of spermatozoa and the fibrinolytic activity of the seminal fluid, because fibrinolysis occurred equally well in specimens of necro-, oligo-, and azo-spermia. Von Kaulla and Shettles concluded that human seminal fluid was the only body fluid to contain a free plasmin-like enzyme/

enzyme under normal circumstances.

Lundquist, Thorsteinsson and Buus (1955), in trying to isolate the various proteolytic components in semen, discovered an amino-peptidase which hydrolysed dipeptides and tripeptides; a kinase; an arginine-esterhydrolysing enzyme, which digested benzoyl-l-arginine ethyl ester; and a proteinase resembling trypsin. The latter enzyme could digest casein, haemoglobin, and human and bovine fibrin. Huggins and his co-workers (1942, 1943), too, had demonstrated the similarity between the proteolytic properties of prostatic secretion and those of pancreatic trypsin.

A further method of determining the fibrinolytic activity of seminal fluid was described by Ying, Day, Whitmore and Tagnon (1956), who measured the liberation of soluble radio-iodine from a substrate consisting of fibrin tagged with I^{131} isotope. Using the fibrin-plate method, Rasmussen and Albrechtsen (1960b) confirmed von Kaulla's discoveries, by demonstrating in seminal plasma the presence of small amounts of plasmin, which could digest heated bovine fibrin; plasminogen activator; trypsin inhibitors; and small amounts of pro-activator (converted/

(converted by streptokinase). The plasminogen activator was of the stable tissue type, similar to the thermostable activator found in homogenized prostatic tissue.

In 1952, Lundquist isolated a "seminal fibrin" from human semen, which was readily digested by seminal and prostatic proteolytic enzymes to yield the same free amino acids found normally in liquefied semen.

The mechanism of coagulation-liquefaction in human semen was summarised as follows (Mann, 1964):

1. Formation of a fibrin-like clot by the action of a clotting enzyme produced in the prostate, on a fibrinogen-like protein substance secreted by the seminal vesicles.

2. Fibrinolysis occurred next, as the result of the action of a plasmin-like enzyme of prostatic origin, formed from an inactive precursor, due to the presence of plasminogen activator.

3. Further proteolysis of the lysed fibrin to form free amino-acids and ammonia.

In 1954, von Kaulla and Shettles suggested that the fibrinolytic activity of semen may influence the power of spermatozoa to penetrate the zona pellucida of the ovum. The mechanism whereby a spermatozoon enters into the ovum has been studied mainly in invertebrates, rather than in mammals. Consideration has been paid to the part played/

played by so-called lytic agents in the process of ovum fertilisation, and by the influence of what was previously called the "ovulase" activity of spermatozoa.

Tyler (1939) was the first to show that the lytic agents were enzymic in nature, when he identified an "egg-membrane lysin", a protease, in the sperm of the key-hole limpet and the abalone. Similar proteolytic substances were detected in sea-urchin spermatozoa by Tyler and O'Melveny (1941) which they called the "jelly-coat dissolving or precipitating protein-factor"; and an "egg-surface liquefying agent" or "sperm lysin" (Runnström, Lindvall and Tiselius, 1944) was found in sea-urchin and salmon spermatozoa.

It was noted that in invertebrates (Jean Dan, 1952, 1956), contact of a special nature was established between the acrosome of the spermatozoon's head and the egg, before the rest of the spermatozoon could pass across the jelly-coat. This phenomenon was called the "acrosome reaction". Further observations by Colwin and Colwin (1960, 1961) supported the presence of this reaction, when studying the fertilisation process in the annelid Hydroides hexagonus. During fertilisation, the sperm-atozoon/

spermatozoon bores a hole through the vitelline membrane of the egg by means of a lytic enzyme called the "vitelline-membrane lysin".

Austin (1959) added to the speculation concerning the fertilisation process by suggesting that the perforatorium, a structure normally located between the acrosome and the sperm-nucleus, may possess a lytic enzyme which he provisionally named "zona lysin". The digestive action of this enzyme would lead to the penetration of the zona pellucida by the spermatozoon. It may be likely that the "zona lysin" could be a plasmin-like enzyme formed during capacitation i.e., the process undergone by spermatozoa in the female genital tract prior to fertilisation of the ovum.

CHAPTER III

THE SIGNIFICANCE OF FIBRINOLYSIS

THE SIGNIFICANCE OF FIBRINOLYSIS

It was Nolf (1904) who first introduced the concept that fibrin deposition and removal are in a state of dynamic equilibrium, and that fibrinolysis was the natural sequence of blood coagulation. The idea that blood coagulation was a continuous physiological phenomenon within the vascular system (Allen, 1951; Jensen, 1956; Copley, 1957; Roos, 1957) was supported by Astrup (1955a, 1956b), who postulated that the process of fibrin formation was continuous. The fibrinolytic reaction was co-existent with coagulation, and so removed excess fibrin deposits or blood clots.

Circumstantial evidence favours the concept that continuous fibrin formation is balanced by fibrinolysis. Copley (1954), Roos (1957) and Woolf (1961) have attempted to prove that a thin layer of fibrin forms the lining of blood vessels. Salmon (1961) and Das, Allan, Woodfield and Cash (1967) have detected the presence of fibrin degradation products in normal blood, indicating that fibrinolysis may be a continuous phenomenon.

Todd (1969b) believes that blood fibrinolytic activity may be a spill-over by-product, influenced by dilution in the blood stream, of a process occurring locally. He suggests that the fibrin film does not occur in close juxtaposition to the endothelial surface, but that it exists as a gel, forming a boundary zone between fibrinogen-rich plasma in the plasmatic zone of flowing blood, and a zone of plasminogen activator which is in immediate contact with the endothelial cells. The fibrin layer may become wider due to the increase of thromboplastic substances, or due to a decrease in fibrinolytic activity, and this may lead to thrombosis. Alternatively, if the secretion of activator increases, then the fibrin gel layer will diminish, allowing the escape of fibrinogen-rich plasma into the vessel wall, and leading to the fibrinous vasculosis described by Lendrum (1961, 1964).

There have been a number of reports where thrombosis has accompanied a decrease in fibrinolysis below that normally found in blood.

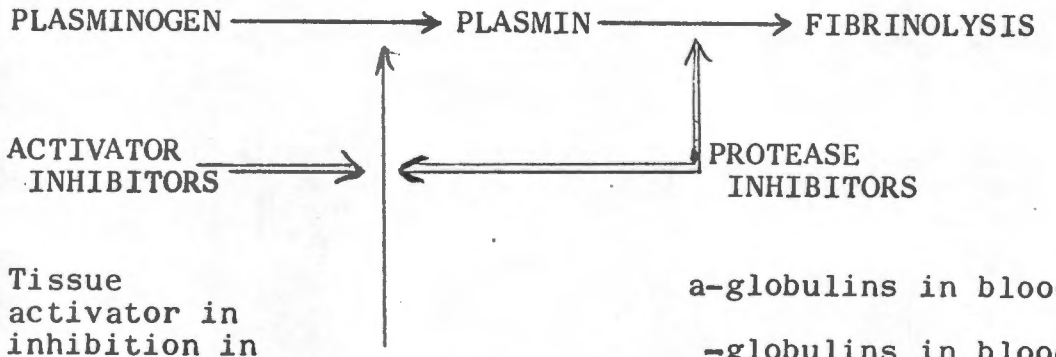
Nilsson, Krook, Sternby, Söderberg and Söderström (1961) /

(1961) described the presence of recurrent thrombosis in a patient with a normal plasminogen level, but with increased inhibition of its activation. Similarly, Nilsson, Niléhn, Cronberg and Nordén (1966) reported increased inhibition of a normal content of plasminogen, in a patient who succumbed after developing fibrin deposits and thrombosis in many arteries and veins. Brakman, Mohler and Astrup (1966) found increased inhibition of tissue activator in several patients who developed thrombotic episodes. A more recent report, providing further evidence of an abnormal coagulation-fibrinolysis equilibrium in favour of thrombosis, has described the appearance of peripheral symmetrical gangrene in African children, associated with an abnormally low level of plasminogen activator and increased amounts of fibrinolytic inhibitor (Turpie, Forbes and McNicol, 1967). The administration of a specific plasminogen inhibitor viz. epsilon aminocaproic acid has reduced the fibrinolytic activity of circulating blood, resulting in widespread intravascular thrombosis (Naeye, 1962; Sharp, 1964).

In many/

In many unrelated clinical syndromes, disseminated intravascular coagulation or defibrination occurs, due to the release into the circulation of large amounts of thromboplastin from damaged tissues (Vreeken, 1965; Hardisty and Ingram, 1965; McKay, 1965), e.g. transfusion reactions, obstetrical accidents, major surgery, and in neoplastic disease. Pathologically increased fibrinolysis occurs as a secondary phenomenon, leading to a serious haemorrhagic condition. Indeed, McKay demonstrated fibrin deposits in many pathological conditions. Hardaway (1966) similarly found that disseminated intravascular coagulation, accompanied by secondary fibrinolysis, occurred in the seriously shocked patient.

The degradation of fibrinogen and fibrin leads to the formation of split products which are polypeptides. These fibrin degradation products have been shown to have an anticoagulant behaviour by Niewiarowski and Kowalski (1957) who confirmed the finding by Gratia (1921) that lysed fibrin prolonged coagulation in normal plasma. This is attributed to a disorder of fibrin polymerisation so that a clot is formed which is structurally defective (Fletcher, Alkjaersig and Sherry, 1962). (See Table 3 for Scheme of activation and inhibition of Fibrinolysis).



Tissue activator in inhibition in blood		a-globulins in blood
		-globulins in blood
	ACTIVATORS	
		Mingin in urine
Urokinase inhibition in blood	Tissue activator	Pulmin in ox lung
	Urine activator	Kunitz pancreas inhibitor
	Blood activator	
	Staphylokinase	Kallikrein inhibitor in parotid
	Pro-activator & streptokinase	Epsilon amino caproic acid
	Pro-activator & lysokinase	Inhibitors in legumes (soya bean, peanuts)

Table 3: A Scheme of Activation and Inhibition of the Fibrinolytic System

(from Brakman, 1967).

Fibrinolysis and Wound Healing

Minor injuries to tissues continually occur, and the damaged tissues release thromboplastins, leading to fibrin formation. This extravascular coagulation is part of normal tissue repair processes (Astrup, 1956b, 1958); the fibrinolytic system is a very powerful factor in the restoration of normal conditions in traumatized tissue (Astrup, 1955b; Astrup and Sterndorff, 1956a; Albrechtsen, 1957). It is suggested that the most frequent cause of fibrinolytic activity in the blood is due to tissue injuries (Stefanini, 1952), due to the liberation of plasminogen activator from damaged tissues (Tagnon, 1953)

Fleisher and Loeb (1915) and Rosenmann (1920) suggested that the fibrinolytic activity of the tissues might prevent the formation of connective tissue, by digesting fibrin and so destroying the growth substrate of connective tissue cells. Extravascular coagulation provides a scaffolding for phagocytes and fibroblasts, and is important in the formation of connective tissue. Proteases and cathepsins from phagocytes and disintegrating tissues were believed to remove excess fibrin. However,/

However, Kwaan and Astrup (1964a, 1964b) have shown that it is the fibrinolytic mechanism which produces fibrin dissolution in the healing wound. Increased or diminished fibrinolysis will interfere with the proper function of fibrin deposits in tissue repair. For instance, in patients with congenital deficiency of fibrin stabilizing factor (Factor XIII), excessive scar tissue forms after trivial injuries (Duckert, Jung and Shmerling, 1961).

In addition to evidence revealing the intravascular presence of fibrin, interstitial fibrin formation has been demonstrated in the extravascular space by Lendrum (1961, 1964). The presence of fibrinogen occurring outwith the blood vessels has been shown by McFarlane, Todd and Cromwell (1964) and by Freeman (1964).

Fibrinolysis and Inflammation

Fibrinolysis is closely associated with inflammation, since fibrin is a factor common to both. Dissolution of the fibrinous exudate in lobar pneumonia has been attributed partly to fibrinolysis, since the lung is rich in activator. Studies on leucocytes trapped in thrombi have/

have shown them to possess fibrinolytic activity (Todd and Nunn, 1967). Low fibrinolytic activity in patients with rheumatoid arthritis was corrected by corticosteroid therapy (Chakrabarti, Fearnley and Hocking, 1964). Fibrinolytic enzymes have accordingly been used topically to assist in "physiological debridement" (Cliffton, 1960) of fibrinous exudates. The idea that certain chronic diseases e.g. hyaline membrane disease, kwashiokor, cystic fibrosis of the pancreas and chronic relapsing pancreatitis, may be mediated by defective fibrinolysis, has been prompted by Innerfield (1960).

Phillips and Skrodelis (1958) have detected diminished fibrinolytic activity in the blood of infants with hyaline membrane disease of the lungs. Further studies have revealed that there is a deficiency of tissue activator of plasminogen, due to the presence of an inhibitor in the lungs of such infants (Lieberman, 1961). Treatment of the disease has been attempted with plasmin because of the discovery of a reduced level of blood plasminogen (Ambrus, Weintraub, Dunphy, Dowd, Pickren, Niswander, and Ambrus, 1963).

Fibrinolysis and Vascular Disease

Labile activator is adsorbed to the surface of a fibrin clot from the circulating blood (Fearnley, 1953). In static blood, inhibition outweighs fibrinolytic activity (Fearnley, 1961), so that whilst in a mural or retracted thrombus, activator is available for adsorption and concentration from circulating blood, yet in occlusive thrombosis, the circulation ceases and activator is not available.

The endothelium adjacent to thrombi in veins, pulmonary arteries, cardiac atria and coronary arteries contains activator (Todd and Nunn, 1967; Todd, 1969a). In cases where the thrombus has been loosened, activator can be detected upon the fibrin surface. Activator can also be detected within the thrombus, apparently trapped by retraction or re-thrombosis. These workers therefore express the suspicion that plasminogen activator plays a major part in the loosening of thrombus and the detachment of emboli.

Although the endothelium of veins and pulmonary arteries are rich in activator, arteries on the other hand, are/

are deficient in activator and contain pro-activator (Todd, 1959, 1964). The adventitia of arteries have been shown to be rich in activator, but the substance is absent from the intima and media (Astrup and Claassen, 1958). Naimi, Goldstein and Proger (1963) have shown that the fibrinolytic activity of arterial blood was significantly lower than that of venous blood.

Reviving von Rokitansky's (1852) "encrustation theory" of atheroma formation, Duguid formulated in 1946 his thrombogenic theory of atherosclerosis. By carrying out comparative histological studies, he observed that fibrin deposits on the arterial intima became organized and changed into atherosclerotic plaques. There is evidence to indicate that low fibrinolytic activity occurs in occlusive vascular disease (Nestel, 1959; Fearnley, 1965); in diabetes mellitus (Fearnley, Chakrabarti and Avis, 1963); and in myocardial ischaemia (Chakrabarti, Fearnley, Hocking, Delitheos and Clarke, 1966).

Thrombolytic Therapy
(see Table 4 for Pathophysiology of Thrombolysis)

The presence of activator is necessary for the dissolution of thrombi. Sherry and his co-workers (1959) have/

PLASMINOGEN–PLASMIN SYSTEM

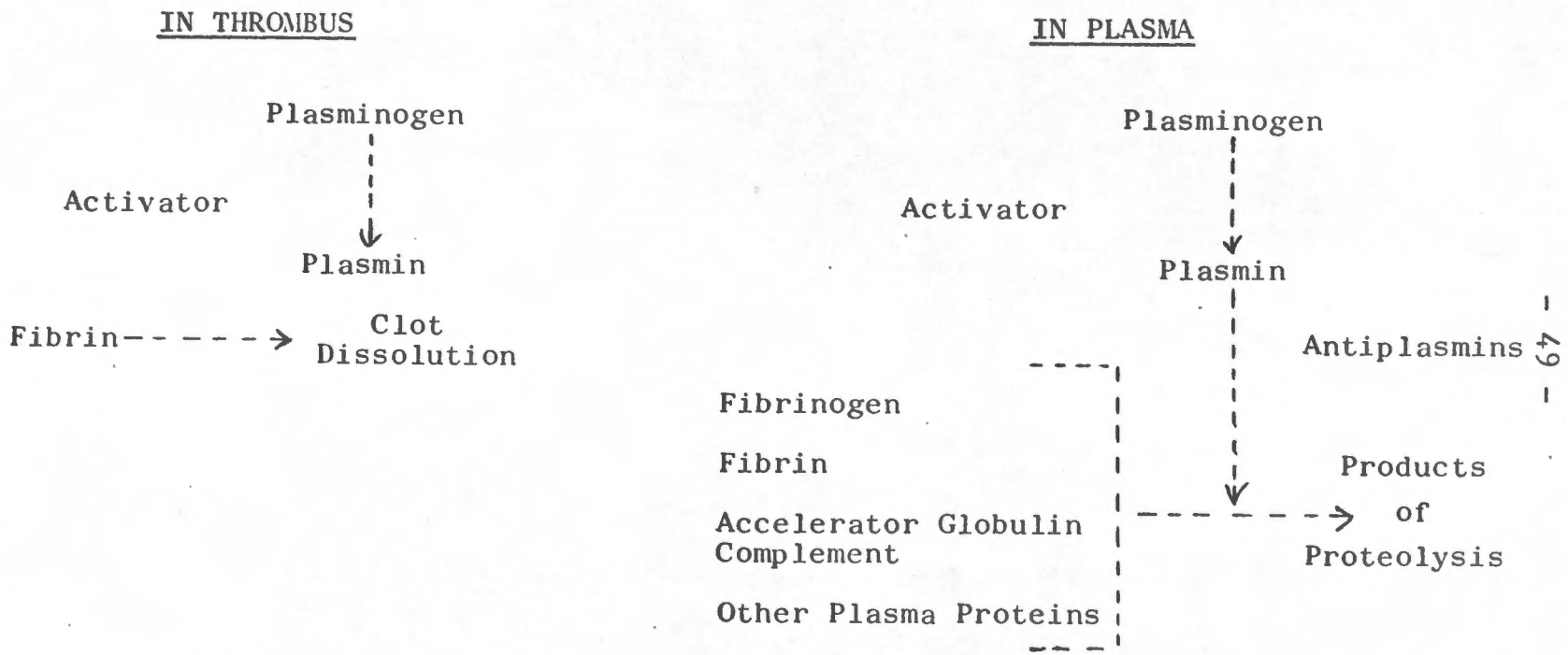


Table 4: Pathophysiology of Thrombolysis
(after A.P. Fletcher, 1960)

have demonstrated that activator diffuses into a thrombus and converts the intrinsic plasminogen of the thrombus to plasmin, thereby causing its lysis. Thrombolytic therapy has developed mainly along two paths. Firstly, over the short term, by the treatment of established venous or arterial thrombosis, or pulmonary embolism, with powerful exogenous activators of plasminogen e.g. streptokinase (Verstraete, Vermylen, Amery and Vermylen, 1966; Bose, Brose and Hartley, 1969; Kakkar, Howe, O'Shea, Flute and Clarke, 1969) and urokinase (Mavor and Galloway, 1969; Prentice, McNicol and Douglas, 1969).

Secondly, by the method of long-term activation for the prevention of intravascular fibrin deposition. Meaningful results have been achieved by the use of several pharmacological agents to enhance blood fibrinolytic activity viz.:

Nicotinic acid (Weiner, Redisch and Steele, 1958).

Sulphonyl-ureas (Fearnley, Chakrabarti and Vincent, 1960).

Testosterone and Nandrolone (Fearnley and Chakrabarti, 1962).

Phenformin and Clofibrate (Fearnley and Chakrabarti, 1964).

Prednisolone, Cortisone and ACTH
(Chakrabarti, Fearnley and Hocking, 1964).

The combination of phenformin and ethyloestrenol would seem to be suitable as a prophylactic measure in arteriopathic patients, because of the sustained and favourable effect by increasing the fibrinolytic activity of the blood and reducing the plasma fibrinogen, the stickiness of platelets and the serum cholesterol. (Fearnley and Chakrabarti, 1969).

PATHOLOGICAL FIBRINOLYSIS

Since Jürgens and Trautwein (1930) first described a haemorrhagic diathesis due to fibrinolysis, occurring in a patient with disseminated carcinoma of the prostate, over 100 similar cases have been described (see Table 5 for list of cases recorded). Metastatic lesions, especially in the bones, seemed to be necessary for the development of pathological fibrinolysis, and extracts of both primary tumours, and of their metastases were rich in fibrinolytic activity (Tagnon and others, 1953a).

In association with such fibrinolysis, deficiencies of /

TABLE 5

Cases of Prostatic Cancer with Hyperfibrinolysis and a Haemorrhagic Diathesis

Reference	No. of Cases.
Aboulker, Soulier, Larrieu and Wartelle (1955)	2
Aggeler and Seale (1951)	1
Andersson (1963a)	12
Balfour (1959)	6
Bergen and Schilling (1958)	1
Boros, Tarnoky, Vereczkey and Rāk (1962)	1
Bredt (1962)	1
Brown, Campbell and Thompson (1962)	1
Burghele, Papadopol, Hozoc and Burchi (1961)	1
Cibert, Favre-Gilly, Traeger and Durand (1952)	2
Cosgriff and Leifer (1952)	1
Cottier, Leupold and Scheitlin (1955)	1
Crane, Ware and Hamilton (1955)	2
Croizat, Revol, Favre-Gilly and Thouverez (1955)	1
Dolaz, Dolehide and Friedman (1955)	1
Dossogne (1959)	1
Fiehrer, Bordas, Migault and Renard (1957)	1
Foret, Bounameaux and Lecarte (1959)	1
Frick/	

Reference	No. of Cases.
Frick (1956)	1
Galeone and Pelocchino (1956)	1
Hadorn (1953)	1
Hayashi, Yano, Sakurai, Murakami, Kadota and Kusakari (1962)	1
(Japanese authors: quoted by Hayashi and others, 1962), 1961	1
Herbst (1942)	2
Jürgens and Trautwein (1930)	1
Kellock and Gallagher (1958)	2
Ledent and De Geeter (1959)	1
Lee (1962)	2
Leng-Levy, David-Chausse, Julien and Riviere (1959)	1
Lombardo (1958)	3
Malcolm and O'Connor (1963)	1
Manrique and Horder (1965)	1
Marchal, Duhamel, Samama and Flandrin (1963)	1
Marchal, Escalier, Duhamel, Le Roux, Nivet and Samama (1959)	2
Marder, Weiner, Shulman and Shapiro (1949)	1
Meuwissen and Zaman (1956)	1
Miller, Meisel, Jackson and Collier (1959)	1
Naeye /	

Reference	No. of Cases
Naeye (1962)	1
Neft, Dugdale, Biggs and Raines (1961)	4
Nour-Eldin and Draisey (1963)	1
Ohler, Fischer, Enders and Kikillus (1966)	3
Pellman, Ridlon and Phillips (1966)	1
Phillips, Skrodelis and Furey (1959)	1
Plauchu, Potton, Brizard and Rouchon (1955)	1
Prout, Siegel, Cliffton and Whitmore (1956)	1
Ramioul (1960)	1
Rapaport and Chapman (1959)	1
Rasmussen and Schwartz (1957)	2
Salomon and Stefanini (1954)	3
Scott (1956)	1
Seale, Jampolis and Bargen (1951)	1
Serre, Izarn and Simon (1963)	1
Sigstad (1961)	1
Stefan, Chrobák, Nerad and Groh (1959)	2
Stefanini and Dameshek (1955)	1
Stefanini and Gendel (1953)	1
Stocker and Maier (1964)	1
Surós and Foz (1965)	1
Swan /	

Reference	No. of Cases
Swan and Kerridge (1965)	2
Swan, Wood and Daniel (1957)	2
Tagnon, Whitmore, Schulman and Kravitz (1953a)	6
Vlaardingerbroek (1960)	1
Watson, Schultz and Wikoff (1947)	1
Zeh and Oft (1954)	1
Total No. of Cases extracted from the literature	105

of fibrinogen and blood clotting factors occurred, together with a prolonged prothrombin time. The development of such a haemorrhagic state was not related to the level of the serum acid phosphatase. The presence of sex hormones influenced the fibrinolytic activity of the tumour, since the administration of oestrogens was followed by the disappearance of pathological fibrinolytic activity, and a rise in the plasma fibrinogen level, whereas the giving of testosterone produced a fall in the plasma fibrinogen level, accompanied by the reappearance of fibrinolysis (Tagnon, Schulman, Whitmore and Leone, 1953b).

An increased level of fibrinolytic activity in the blood stream occurring after different types of prostatectomy, has been noted (Scott, Matthews, Butterworth and Frommeyer, 1954; Lombardo, 1957; Rasmussen and Ladehoff, 1959; Urlus, 1962). Severe blood loss from the prostatic bed has been attributed to the digestion of haemostatic blood clot by local tissue activators released from damaged tissues (Andersson and Nilsson, 1961; Koller, 1963), and to the urokinase content of the urine (McNicol, Fletcher, Alkjaersig and Sherry, 1961a, 1961b).

Such /

Such postoperative blood loss was effectively reduced by the administration of antifibrinolytic agents, e.g. epsilon aminocaproic acid and Trasylol (McNicol and others 1961a, 1961b; Andersson and Nilsson, 1961; Sack, Spaet, Gentile and Hudson, 1962; Fürstenberg; 1962, Beck, Schmutzler and Duckert, 1963; Schmutzler and Fürstenberg, 1966). It has been shown that there is a rough correlation between the extent of lysis of plasma clots by tissue cultures of hyperplastic prostates and the severity of postoperative haematuria (Källén and Röhl, 1960).

Significantly raised levels of fibrinolytic activity have been recorded in neoplastic disease (Dillard and Chanutin, 1949; Stefanini and Dameshek, 1955; Zucker, 1964). A fatal bleeding state, due to a tremendous hyperplasminaemia in association with an operation on a case of carcinoma of the pancreas, was recorded by Ratnoff (1952). Other similar instances of a fatal bleeding condition have been noted in metastasizing cancer of the pancreas (McKay, Mansell and Hertig, 1953; Frick, 1956), and in cancer of the Ampulla of Vater (Crehan, 1957). Increased fibrinolytic activity in the blood associated with a bleeding diathesis has been seen in cases of disseminated /

disseminated cancer of the bladder (Lombardo, 1958; Stefanini, 1958).

A haemorrhagic syndrome accompanied by hypofibrinogenaemia, is most commonly associated with an adenocarcinoma which has metastasized to the bone marrow, viz. stomach (Fleischhacker, 1940; Frick, 1956; Boomgaard and Moers, 1963; Welborn, Brennan and Hathaway, 1964); bronchus (Schmid, 1951; Cohen and Kupfer, 1958); gallbladder (Braun and Horányi, 1951); uterus (Bennike and Mülleritz, 1952).

It is likely that many of these cases are examples of the syndrome of disseminated intravascular coagulation or defibrination (Hardisty and Ingram, 1965; McKay, 1965), following the release of thromboplastic substances from the tumour into the bloodstream. Clotting factors and platelets are seriously depleted by their consumption in this wide-spread clotting process, leading to a dangerous bleeding state. The situation is aggravated by secondary fibrinolysis, which as a defence mechanism, removes these fibrin deposits within the small vessels.

It has been shown that the lung is rich in tissue activator (Albrechtsen, 1957), and that activator is concentrated more in the pulmonary arteries rather than in the pulmonary veins (Todd, 1959). The liberation of activator from the lung leads to a higher level of fibrinolytic activity in the blood in the pulmonary vein than in the pulmonary artery (Mathey, Daumet, Soulier, Le Bolloch and Fayet, 1950; Lincoln, Moorman and Schultz, 1957). It is then not surprising that operations on the lung have incurred a haemorrhagic diathesis (Mathey and others, 1950; Chalnot, Michon and Lochard, 1952; Soulier, Mathey, Le Bolloch, Daumet and Fayet, 1952; Penn and Walker, 1954).

Abnormal fibrinolysis has been reported in patients undergoing extracorporeal circulation during intra-cardiac surgery (von Kaulla and Swan, 1958; Andersen and Mendelow, 1963; Tice, Reed, Clauss and Worth, 1963). Thyroid operations have similarly been accompanied by increased fibrinolysis (Lhoiry and Fayet, 1954). Obstetrical complications e.g. pre-eclamptic tozaemia, premature separation of the placenta, amniotic fluid embolism or infusion, incur the hazard of disseminated intravascular coagulation. /

coagulation.

A link between cirrhosis of the liver and excessive fibrinolysis leading to a bleeding state was first established by Goodpasture (1914). Nolf (1905) had earlier demonstrated that the fibrinolytic activity of blood increased when the liver was excluded from the circulation, and he surmised that this effect was due to the production of a fibrinolytic inhibitor by the liver. His assumption was supported by the finding that the blood in the hepatic veins was much lower in fibrinolytic activity than the blood in the systemic veins (Kwaan, McFadzean and Cook, 1957).

Normal liver has been found to have a negligible activator content (Albrechtsen, 1957) whereas patients with cirrhosis have an increased fibrinolytic activity (Kwaan, and others, 1957). Todd (1961) has shown that the scar tissue of cirrhotic liver has a high content of activator. Indeed, there is a greatly reduced incidence of myocardial infarction in cases with cirrhosis (Howell and Manion, 1960). Severe bleeding due to fibrinolysis may follow portocaval shunt operations (Marchal, Frileux, Bilsky-Pasquier, /

Pasquier, Weiss, Cornet, Samama and Larrieu, 1959; Ende and Auditore, 1963). Bleeding from oesophageal varices has been influenced not only by the deficiency of clotting factors, but also by increased blood fibrinolysis when the damaged liver parenchyma fails to produce anti-fibrinolysin (Tocantins, Reid, Silver and Kazal, 1964).

Therapeutic Inhibitors of Pathological Fibrinolysis

Even though it has been demonstrated that the amount of plasmin inhibitors in the plasma is thirty-fold the content of plasmin (Norman, 1958), a bleeding diathesis may still occur when excess plasmin appears in the blood stream. Treatment with fibrinolytic inhibitors is then necessary.

Epsilon aminocaproic acid (EACA) has been most commonly used as a fibrinolytic inhibitor (Okamoto, 1954; Sato, Ishibashi, Endo, Watanabe and Nakajima, 1959) since its original use in animal experiments (Thomas and Goerne, 1914). It inhibits plasmin at a concentration of 5 X 10^{-2}M or above, while at a concentration of 10^{-4}M, it inhibits the activation of plasminogen (Ablondi, Hagan, Philips and de Renzo, 1959; Alkjaersig, Fletcher and Sherry /

Sherry, 1959).

EACA has been used to reduce postprostatectomy bleeding by inhibiting urokinase activity in the urine (McNicol and others, 1961a, 1961b); in the treatment of haemorrhagic blood fibrinolysis in cases of prostatic disease (Andersson, 1963b), and to reduce the postoperative blood loss after open-heart surgery (Sterns and Lillehei, 1967). In contrast, EACA has been administered to haemophiliacs, resulting in an appreciable decrease in the number of spontaneous bleeding episodes (Mainwaring and Keidan, 1965; Gordon, McNicol, Dubber, McDonald and Douglas, 1965). It was postulated that the clinical improvement resulted from an alteration produced in the normal dynamic equilibrium between fibrin formation and fibrin digestion, in favour of a delay in fibrinolysis.

The substance Trasylol (Aprotinin), derived from bovine parotid also inhibits activation of plasminogen (Marx, Clemente, Werle and Appel, 1959; Steichele and Herschlein, 1961), and when combined with EACA, the blood loss after prostatectomy was effectively reduced by over five-fold (Schmutzler and Fürstenberg, 1966). A further compound, "AMCHA" (Trans-aminomethylcyclohexane carboxylic acid), has proved to be even more potent as a fibrinolytic inhibitor than EACA (Okamoto and Okamoto, 1962; Maki and Beller, 1966).

CHAPTER IV

MATERIALS AND METHODS

MATERIALS AND METHODS

MATERIALS

Human tissues: Blocks of tissue were taken from prostate glands immediately after enucleation prostatectomy; specimens of testis, epidydimis, vas deferens, seminal vesicle, seminal vesicular fluid, prostate, bulbo-urethral gland, corpus spongiosium and corpus cavernosum were obtained at routine postmortem examinations performed on 14 adult males within 12 - 24 hours after death. Tissues were only obtained from those males below the age of 65 years, where possible. Further details concerning the sources of postmortem tissues are given in Table 6. Samples of human seminal fluid were obtained from the Infertility Clinic for Men, Dundee Royal Infirmary.

Preservation media: Tissue culture medium No. "199" (Morgan, Morton and Parker, 1950) (Glaxo Labs., Ltd.). Michaelis Veronal buffer-saline (Biggs and Macfarlane, 1963), pH 7.4, ionic strength 0.16, with sodium azide 0.1% as a bacteriostatic. The buffer-saline was made by dissolving the reagents listed below in distilled water, and making up the total volume to 2.5 litres:

Sodium /

TABLE 6: Details of Source of Human Tissues

Case No.	Age	Postmortem No.	Cause of Death
1	68	6307	Chronic Lymphatic Leukaemia
2	55	6330	Cancer of Caecum
3	46	6358	Cerebral Haemorrhage
* 4	43	105064	Orchidectomy
5	58	6771	Cancer of Colon
6	82	6770	Cardiac Failure
7	77	6774	Cancer of Pancreas
8	44	6773	Muscular Dystrophy
9	60	E337/68	Cerebral Infarction
10	22	Forensic	Multiple Injuries
11	44	E344/68	Myocardial Infarction
12	63	6793	Cancer of Stomach
13	58	6907	Cardiac Failure
14	57	E76/69	Myocardial Infarction
15	61	6918	Cancer of Stomach

* The testicular tissue was obtained from a normal organ removed during a herniorrhaphy.

Sodium acetate	9.714G
Sodium barbitone	14.714G
Sodium chloride	17.0G
0.1 N. Hydrochloric acid	500 ml.
Sodium azide	2.5G

Plasminogen-rich fibrinogen: A 2% solution by weight of Bovine Plasma Fraction I (Armour)* was made in veronal buffer-saline, i.e. 0.2G dissolved in 10 ml. buffer-saline, and this solution was filtered through cotton wool.

* This preparation of bovine fibrinogen contains plasminogen 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, stored at 4°C. for a period not exceeding 6 months. A working solution of 20 units/ml. in Michaelis buffer-saline was made, i.e., by dissolving 0.1 ml. stock solution in 5 ml. buffer-saline, and this was stored at 4°C. and discarded after one week.

Cellophane sheet: "Cellophane PT", 300-400 gauge (British Cellophane Ltd.).

Neutral buffered formalin solution: (Lillie, 1965).

Harris's haematoxylin: (Harris, 1900). No acetic acid should be added, otherwise the fibrin will not stain well.

Special apparatus: A sheet of 6mm. thick plate glass, about /

about 60cm. X 45cm. in size, with three levelling screws. A Perspex frame, 20cm. X 14cm. X 0.2cm., with a central oblong aperture 16.3cm. X 6.0cm. Engineer's spirit level. Plastic boxes, approximately 32mm. X 22mm., containing a square of damp plastic sponge, utilized as incubation chambers. (Messrs. Chance's coverslip boxes are ideal).

METHOD

Storage of tissues: Samples of tissues from the male genital tract taken at necropsy were immersed in buffer-saline. Blocks of prostate, taken immediately from the gland after prostatectomy, were immersed in tissue culture No. "199" , and also in buffer-saline, to provide a comparison between veronal buffer and tissue culture medium as preservative. These blocks of tissue were stored at 4^{o}C. for 1-12 hours until an opportunity arose to "quick-freeze" the tissues. This was done by placing them in a small polythene bag, then plunging the bag into dry ice-acetone mixture. When frozen, they were stored in the deep freeze at -30^{o}C. for periods of up to two months. If sections were to be made /

made immediately, the tissues were frozen on the cryostat microtome chuck in the usual way.

Tissue sections of 7-8u were cut on a Pearse cryostat microtome, and were applied to 32 x 22mm. coverslips. The sections were allowed to dry at room temperature for about 30 minutes before application of the fibrin.

Preparation of Autographs: The glass plate was levelled by means of the screws and the spirit level. The perspex frame was applied to the glass using a little buffer-saline to make it stick. A sheet of cellophane, pre-soaked in buffer-saline, was laid over the frame, and smoothed out over the glass exposed in the central space. Into the shallow bath so formed, a clotting mixture consisting of 3.5ml. fibrinogen solution and 0.2ml. of thrombin. This was allowed to clot, and the cellophane-backed fibrin sheet, which was approximately 0.4mm. thick, was then cut into squares. A fibrin square was applied to each section. The tissue-fibrin preparations were then placed in separate incubation boxes, and incubated at 37°C. for either 15, 30 or 60 minutes. Two samples were taken from each period; thus 6 samples were examined from each block.

Fibrinolytic/

Fibrinolytic activity was arrested by immersing the autographs in buffered formal-saline. After about 1 minute in fixative, the cellophane sheet was stripped off, leaving the fibrin and the section adhering to the coverslip. The preparations were next stained (see below) and mounted for microscopy. Whenever the section contained activator, the plasminogen in the overlying fibrin was converted to plasmin, and the fibrin was digested. Microscopic examination of these preparations now reveals the areas of digestion as pale gaps in the fibrin background, and these may be related topographically to structures in the overlying section (see Fig. 1).

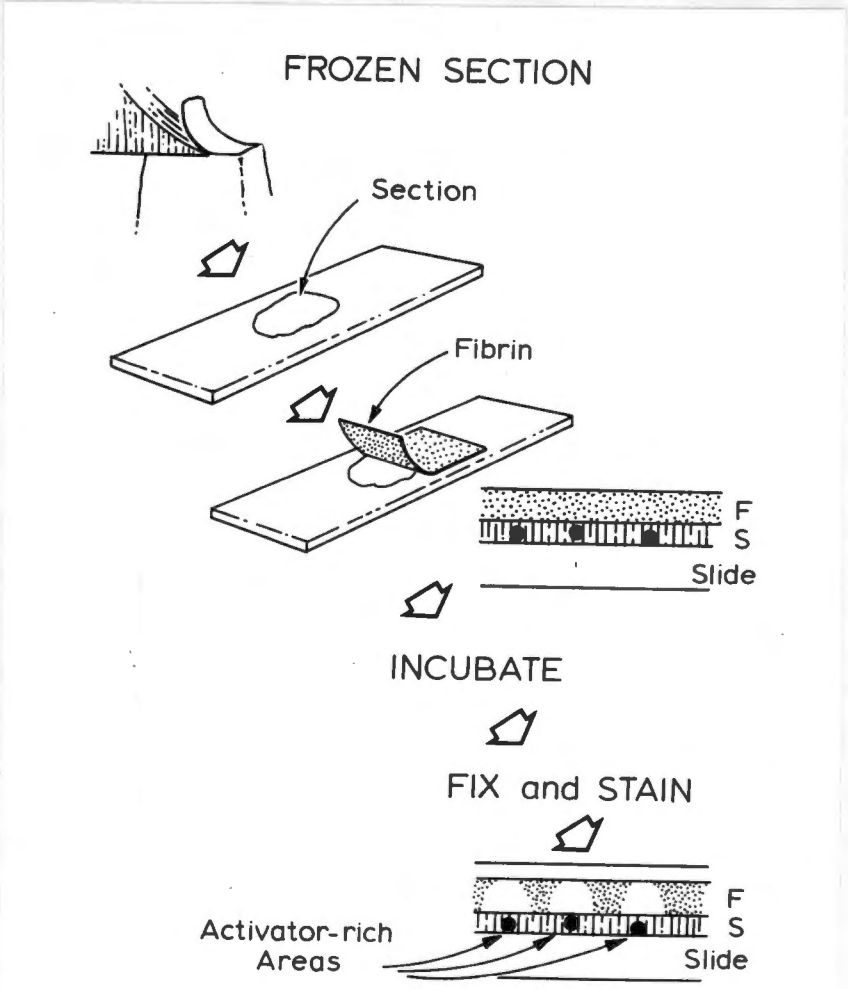
The degree of fibrinolytic activity of blood vessels, glands, ducts and secretion at 15, 30 and 60 minutes was assessed, using a system of grading modified from that of Pandolfi, Nilsson, Robertson and Isacson (1967).

The activity was scored for each preparation as follows:

activity detectable with naked eye	 3
activity only detectable using a 10 X magnifier	 2
activity only detectable microscopically at magnification X 100	 1

For/

Fig. 1: The Construction of a Fibrinolysis Autograph



For each tissue component, the arbitrary units derived from the 6 samples were summed, and this value represents the fibrinolytic activity for that component.

Adjacent frozen sections taken at the commencement and completion of sectioning the tissue block, were placed on clean glass slides, allowed to dry, and then stained with/

with haematoxylin and eosin (see below); the appearances of these were compared with the autographs to ensure that a representative sample had been tested. The appearances of those sections made from the enucleated prostate glands were compared with the surgical pathologist's routine report made on the remainder of the gland.

Method of Staining Fibrinolysis Autographs

1. The fibrin-slide preparations were left overnight in formal-saline. (Messrs. Chance's coverslip boxes make ideal containers). They were then:
2. Washed in distilled water.
3. Stained in Harris's haematoxylin for 20-30 minutes.
4. Differentiated overnight in gently running tap water.
5. Washed with distilled water.
6. Dehydrated in 70-80% Ethyl Alcohol for 30 minutes.
7. Placed in Methylated Spirits for 30 minutes.
8. Placed in Absolute Alcohol I overnight.
9. Placed in Absolute Alcohol II for 30 minutes.
10. Cleared in Xylene I for 30 minutes.
11. Placed in Xylene II for 30 minutes.
12. Mounted in D.P.X.

The/

The scheme was later shortened and modified, without any apparent change in the quality of the stain. After the autographs had been left overnight in fixative, they were:

1. Placed into running tap water for 10 minutes.
2. Harris's haematoxylin for 20-30 minutes.
3. Running tap water - 15 minutes.
4. Methylated Spirits - 15 minutes.
5. Ammoniated Absolute Alcohol - overnight.
6. Absolute Alcohol - 3 hours.
7. Xylene for 30-60 minutes.
8. Mounted in D.P.X.

Routine histological slides of the tissues were prepared in the following manner:

1. Sections were fixed in 5% Acetic Acid in Absolute Alcohol for 5-10 minutes.
2. Dipped into 70% Ethanol.
3. Stained in Harris's haematoxylin for 30-60 seconds.
4. Excess stain washed off in running tap water.
5. "Blued" in Ammoniated Alcohol for 15-30 seconds.
6. Washed with Absolute Alcohol.
7. Counter stained with Eosin for 30-60 seconds.
8. Washed/

8. Washed with Absolute Alcohol.

9. Washed with Xylol.

10. Mounted in D.P.X.

Some pitfalls encountered when preparing Fibrinolysis Autographs:

During Cryotomy of the tissue block*

(1) The tissue block may detach itself from the microtome chuck, because insufficient water has been used in mounting the block, or because the surface of the chuck is dirty or greasy.

(2) Because the tissue block is too cold, causing it to become brittle, horizontal cracks may appear in the section, or the tissue may shatter during cutting These difficulties may be overcome by blowing a few puffs of warm air through a glass tube on to the face of the block.

(3) Vertical scores or lines may be produced in the section by nicks in the cutting edge of the knife; by ice or frozen tissue adhering to the edge or face of the guide plate; or by hard calcified particles in the tissue block.

*(For fullest details concerning fault diagnosis, see page/

page 68 of the working manual for the "Pearse" Cold Microtome (Cryostat) Type H, second edition, published by the South London Electrical Equipment Co. Ltd. 1964.).

During Preparation of the Fibrin film

(1) After the fibrinogen solution has been allowed to clot within the Perspex frame, the ideal result is to obtain an opaque fibrin film. Inspection of the progress of fibrinolysis during incubation will reveal the areas of lysis as translucent zones in the layer of fibrin. If the fibrin film remains transparent, this may be avoided by altering the ionic strength of the buffer-saline used to dissolve the fibrinogen, viz. add 1-2ml. distilled water to the fibrinogen, and make up to 10ml. with the buffer-saline. If this device is unsuccessful, an alternative ruse is to use only O.1ml. Thrombin solution to clot the fibrinogen.

(2) The cellophane-backed fibrin film should be immersed only for a moment in the formal-saline, otherwise it will adhere to the fibrin too firmly to be peeled off. If this should happen, the fibrin-slide can still be stained in the usual way, without removing the cellophane.

During Staining of the Autograph/

During Staining of the Autograph

After fixation of the section, the formal-saline should be thoroughly washed out of the fibrin-slide, otherwise, because of the acidity of the fixative, a reddish rather than a blue colour may occur, after staining with haematoxylin. The final preparation may contain opaque areas due to insufficient dehydration by alcohol, or insufficient clearing by Xylol; it should then be thoroughly washed with alcohol, before undergoing further dehydration in spirits.

Artefacts in the Preparation (from Todd, 1961)

Areas of lysis in the autograph may be unrelated to overlying structures in the tissues. These may be due to:

(1) If the Armour fibrinogen is not filtered, undissolved particles of fibrinogen will cause focal spontaneous lysis of the fibrin film.

(2) Detached cells and loosely connected tissues, after digesting the fibrin, may float away during the subsequent manipulations.

(3) The active parts of the section tend to sink below/

below the level of the rest, and appear out of focus under the microscope.

(4) Fat globules from the section may coat areas of the fibrin film and make these areas resistant to penetration by aqueous haematoxylin. These zones are due to failure of staining rather than to fibrinolysis. This artefact can be abolished if the preparation is defatted by immersion in spirits and then treated with Xylene, before staining.

(N.B. The last two situations only apply to the method where the section is placed on the fibrin film, rather than on the coverslip.)

CHAPTER V

THE DISTRIBUTION OF PLASMINOGEN ACTIVATOR IN THE PROSTATE

PLASMINOGEN ACTIVATOR IN FRESH PROSTATIC TISSUE

METHOD

Blocks of tissue were taken from 25 glands immediately after enucleation prostatectomy, and immersed in tissue culture medium No. "199". In 14 cases, blocks were also immersed in Michaelis buffer-saline, to provide a comparison between veronal buffer and tissue culture medium as a preservative. In 3 cases there was a carcinoma; the remaining 22 glands showed benign fibromuscular glandulo-cystic hyperplasia.

RESULTS

The degree of fibrinolytic activity of blood vessels, glands, ducts and secretions at 15, 30 and 60 minutes (with 2 preparations for each period) was summed, and this figure represents the fibrinolytic activity in arbitrary units for each tissue component (see Table 7). It is seen that the blood vessels have the greatest concentration of plasminogen activator, whilst moderate fibrinolysis arises from the epithelium of glands and ducts./

ducts. The activity of the secretions was insignificant.

No difference was detected between the activity of adenomatous and carcinomatous sections. In the tissues immersed in veronal-buffer, the fibrinolytic activity was comparable to that seen in the tissues stored in tissue culture medium (see Table 8). The potency of plasminogen activator was unaffected by storage of the tissue blocks for periods up to 17 days (see Table 9).

As before, fibrinolytic activity was always seen in relation to the blood vessels (see Figs. 2 - 5). Vascular activity was most striking in the fibromuscular stroma surrounding groups of glands (see Figs. 6,7). The adenomatous nodules were consistently ringed by foci of activity, where the vessels were sinuses often consisting only of endothelium.

Fibrinolysis of a lesser degree than that around vessels, was seen in relation to the glands (see Figs. 8,9), and ducts (see Fig. 10). The activity from these structures was infrequent and of haphazard distribution, most of the glands and ducts being inactive. The lysis was most pronounced where the epithelial lining was disrupted/

disrupted, and was proportional to the number of detached cells (see Figs. 11 - 13).

Secretion within the glands (see Figs. 8,14) or ducts was seldom active and lysis from it was seen in only 3 autographs. In a unique instance, activity was related to an inspissated mass of secretion (see Fig. 15).

There was often increased activity at the edges of the section, where the tissue was most damaged. No fibrinolysis was seen related to collections of polymorphs.

Case No.	Overall Activity	Vascular	Glands	Ducts	Secretion
1*	12	12	4	4	0
2	6	6	0	0	0
3	10	10	4	2	0
4	12	12	4	0	0
5	16	16	10	0	0
6*	12	12	4	0	0
7	16	16	0	0	0
8	16	16	0	0	0
9	16	16	12	12	0
10	16	16	8	8	0
11	16	16	8	8	6
12	14	14	8	4	0
13	16	16	10	6	0
14	12	12	4	0	0
15	18	18	8	0	2
16	16	16	4	0	0
17*	8	8	6	0	0
18	16	16	8	0	0
19	16	16	8	8	0
20	16	16	10	4	0
21	16	16	8	0	0
22	6	6	2	0	0
23	10	10	0	0	0
24	16	16	4	4	0
25	10	10	2	0	0

*Denotes Carcinoma

Table 7: Distribution of Fibrinolytic Activity from Prostatic Tissue Components (arbitrary units)
(Immersion in Tissue Culture medium No. "199")

Case No.	Overall Activity	Vascular	Glands	Ducts	Secretion
3	5	5	1	1	0
4	6	6	2	0	0
5	9	9	3	0	0
6*	6	6	1	0	0
7	5	3	2	2	0
9	8	8	4	5	0
10	8	8	4	4	0
11	7	7	3	3	0
12	7	7	6	2	0
13	8	8	5	5	1
14	6	6	0	0	0
15	9	9	0	0	0
16	8	8	4	0	0
17*	4	4	3	0	0

*Denotes Carcinoma

Table 8: Distribution of Fibrinolytic Activity from Prostatic Tissue Components (arbitrary units)
(Immersion in veronal buffer-saline)

Case No.	Overall Activity	Duration of Storage	Case No.	Overall Activity	Duration of Storage
1	12	2 hours	14	12	17 days
2	6	2 "	15	18	16 "
3	10	2 "	16	16	2 hours
4	12	6 "	17	8	24 "
5	16	3 "	18	16	5 days
6	12	3 "	19	16	24 hours
7	16	5 days	20	16	3 days
8	16	2 hours	21	16	3 "
9	16	2 "	22	6	24 hours
10	16	3 "	23	10	3 "
11	16	5 "	24	16	9 days
12	14	5 "	15	10	7 "
13	16	2 "			

Table 9: Relationship of Fibrinolytic Activity to Duration of Storage

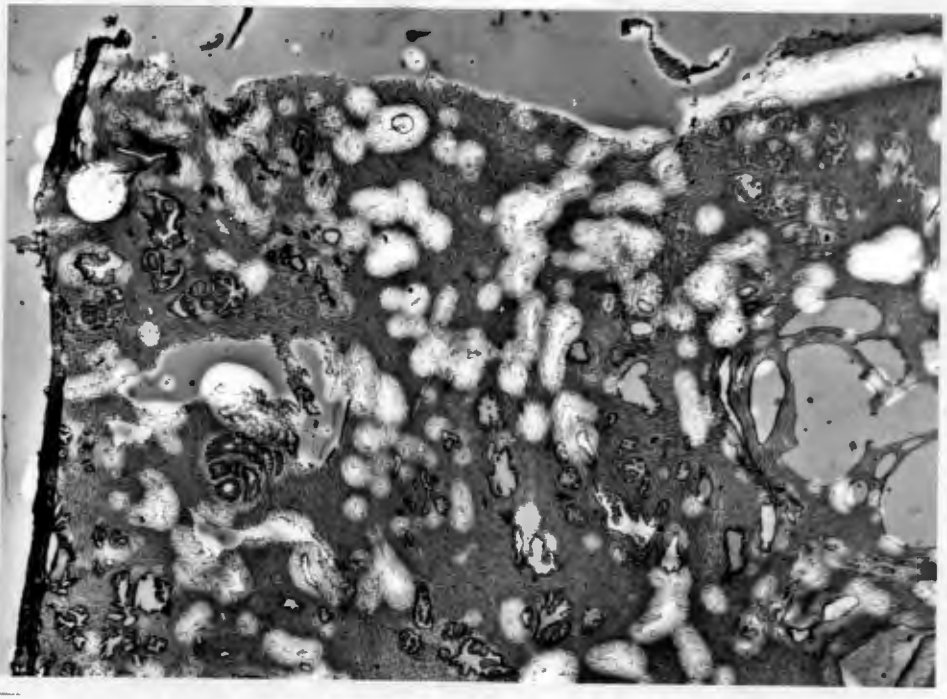


Fig. 2
X 15
30 mins.

Figs. 2-5: Striking fibrinolytic activity consistently related to the blood vessels of the prostate. Note the areas of lysis occurring at the edges of the sections. (Final magnification and incubation period indicated).

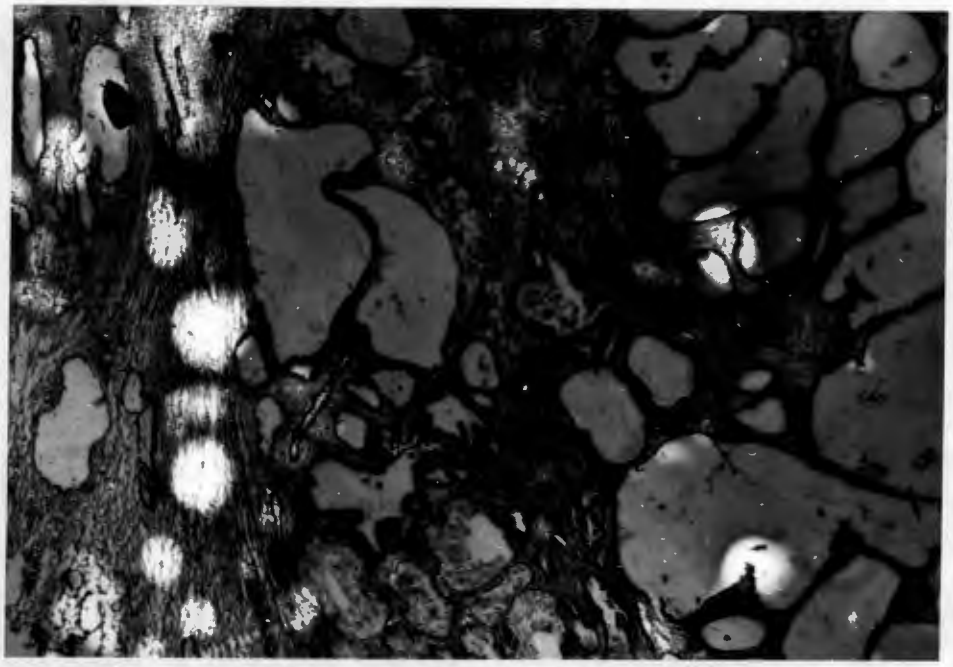


Fig. 3
X 24
60 mins.

Fig. 4: 60 mins. X 20

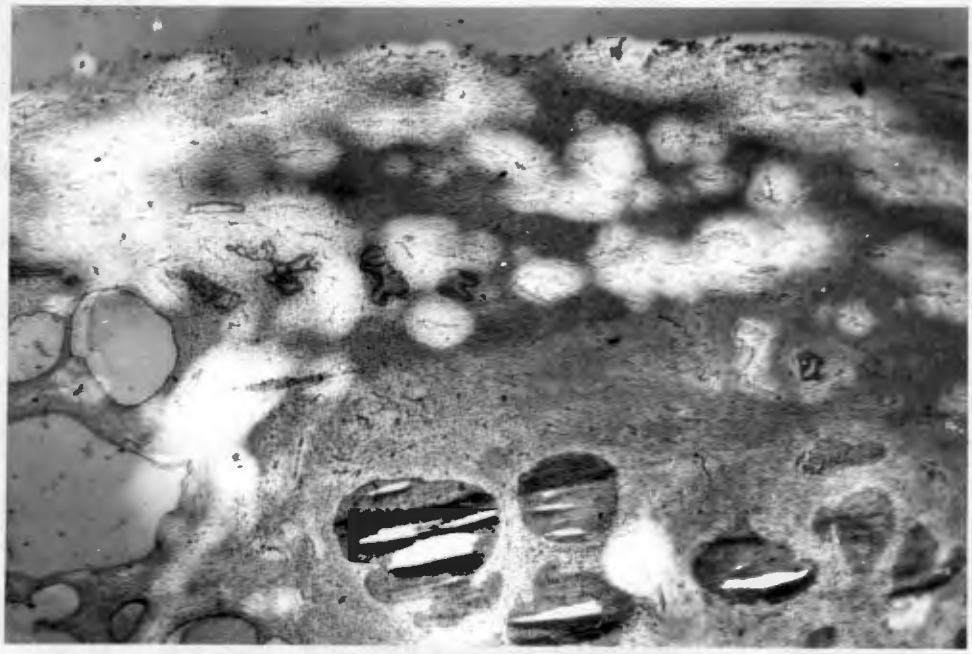
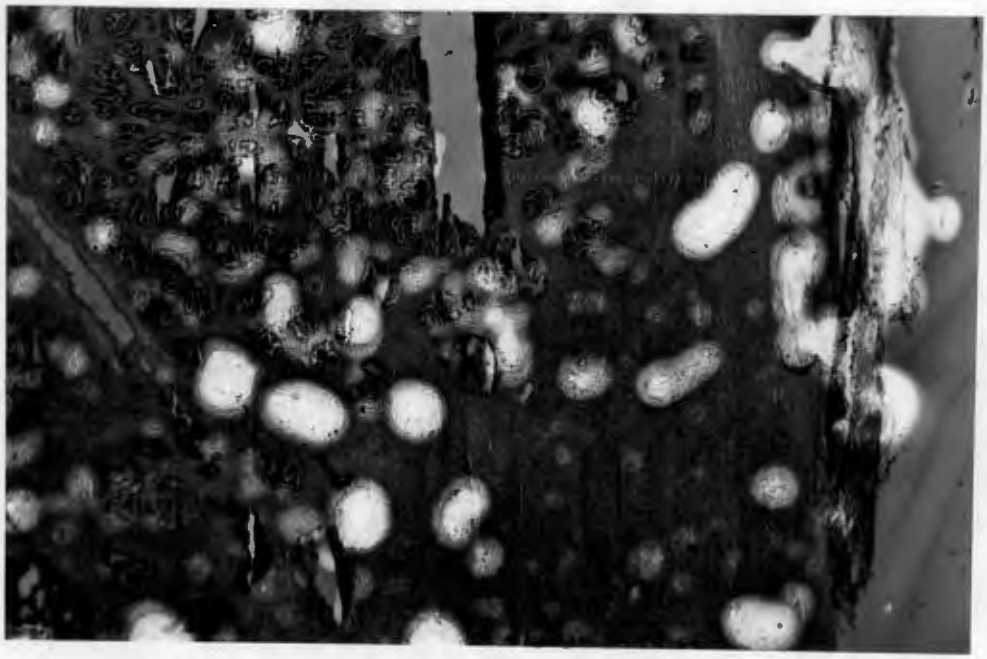


Fig. 5: 60 mins. X 15

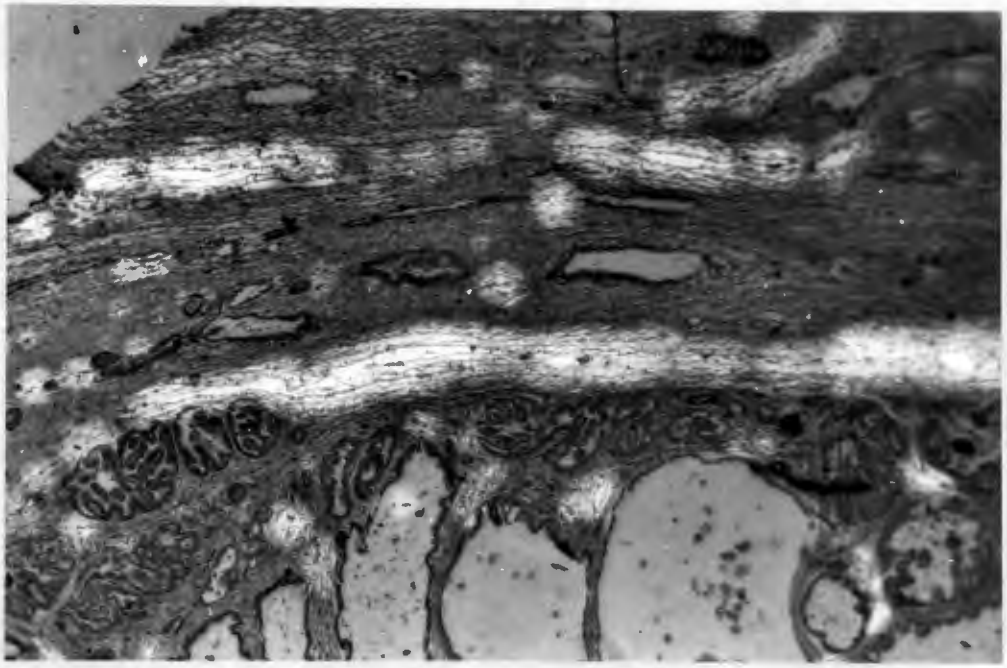


Figs. 4,5: Prostate - impressive activity from blood vessels.

Fig. 6: 30 mins. X 15

Zones of lysis arising from the blood vessels in the fibro-muscular stroma surrounding groups of prostatic glands.

Fig. 7: 30 mins X 24



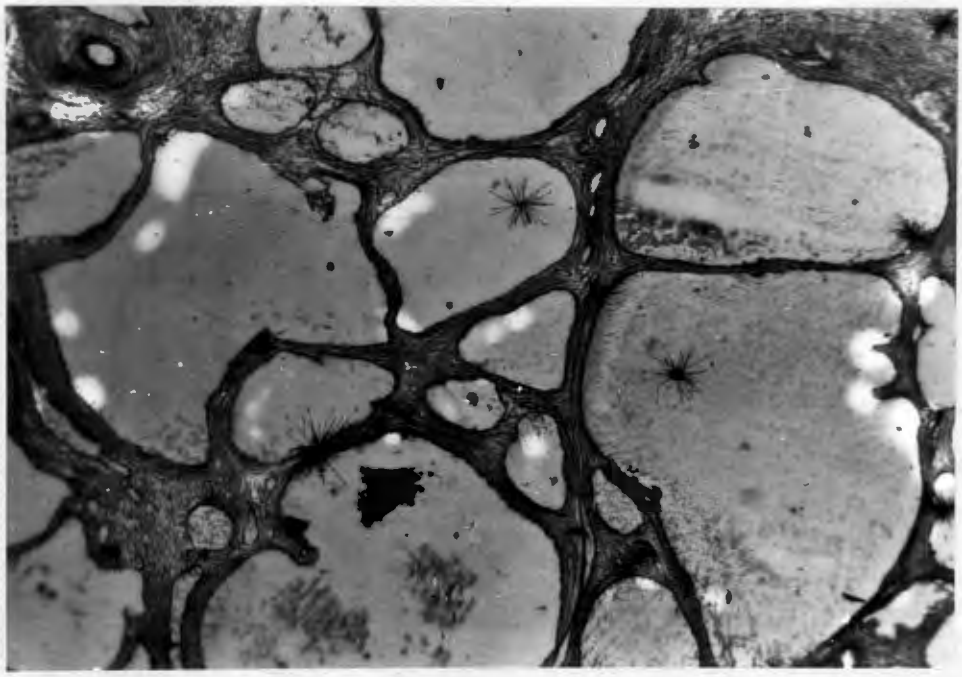


Fig. 8
X 24
30 mins.

Areas of digestion arising haphazardly at various sites from the glandular epithelium. Several isolated areas of fibrinolysis occur within the secretions.

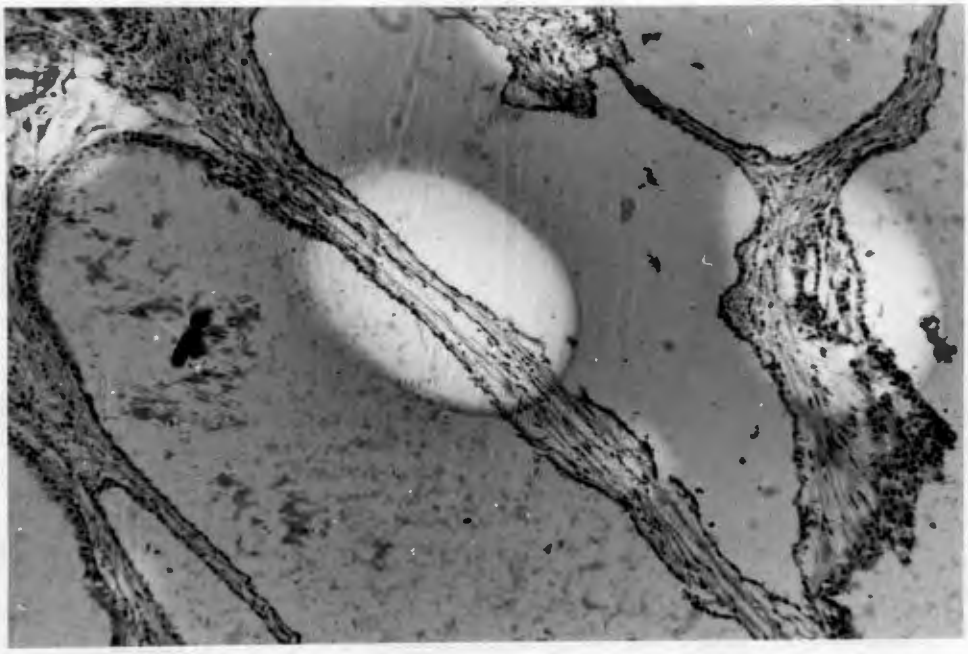


Fig. 9
X 95
30 mins.

Higher magnification of an area of epithelial activity.

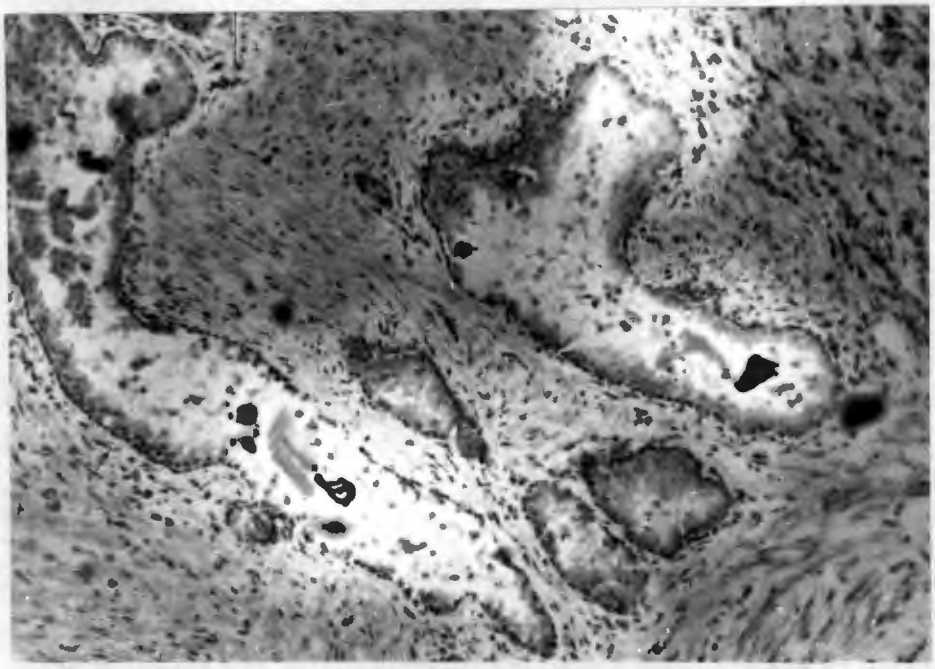


Fig.10
X 95
60 mins.

Fibrinolysis related to a group of ducts.

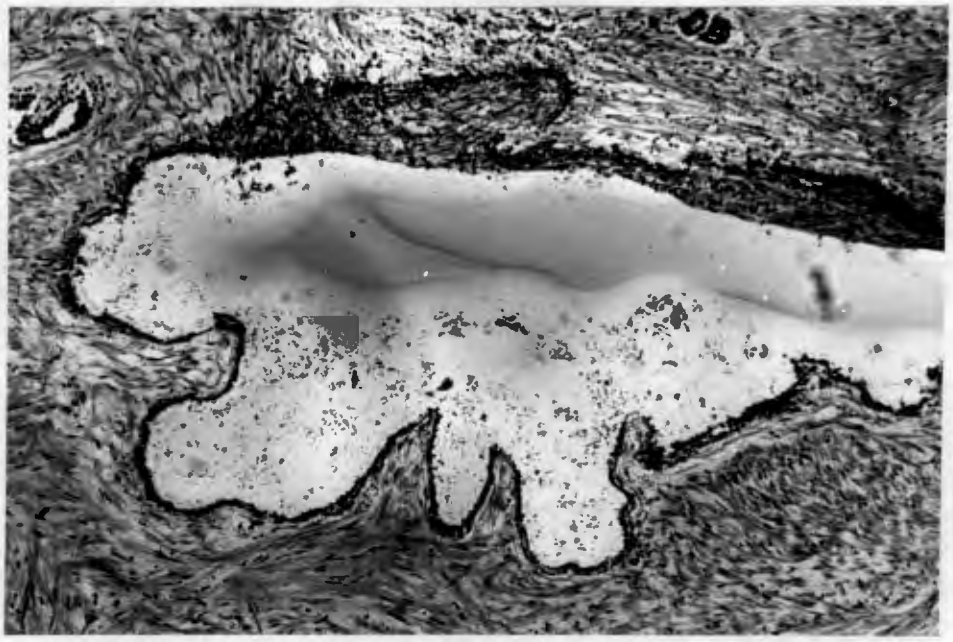


Fig.11
X 95
30 mins.

An active prostatic duct, with maximum digestion related to detached epithelial cells.

Fig.12
X 95
30 mins.

Areas of digestion concentrated mainly around shed epithelial cells within the ducts.

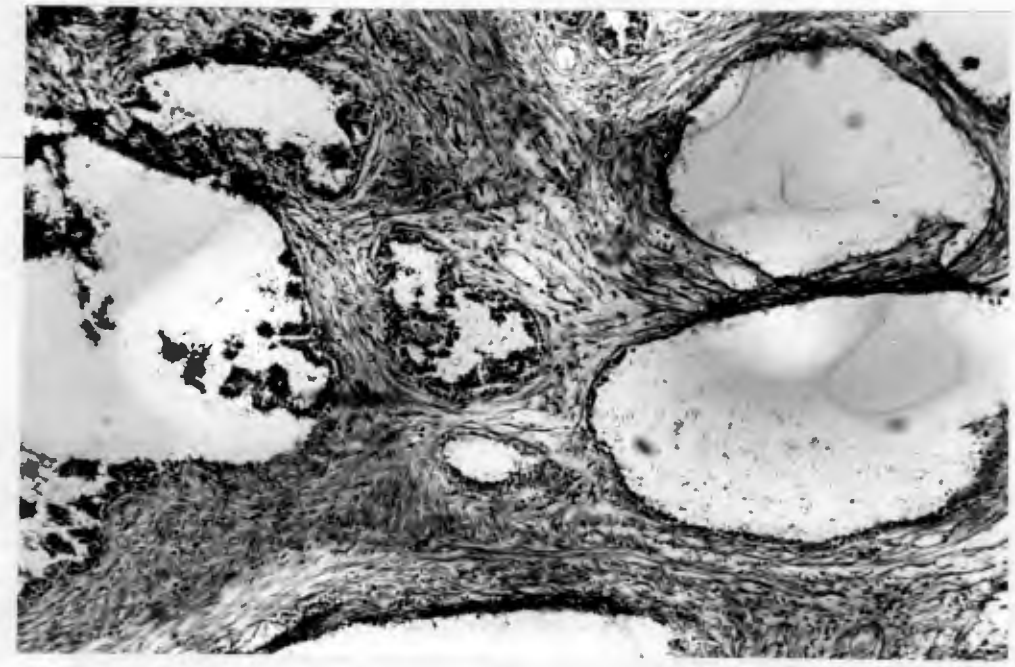


Fig.13
X 95
30 mins.

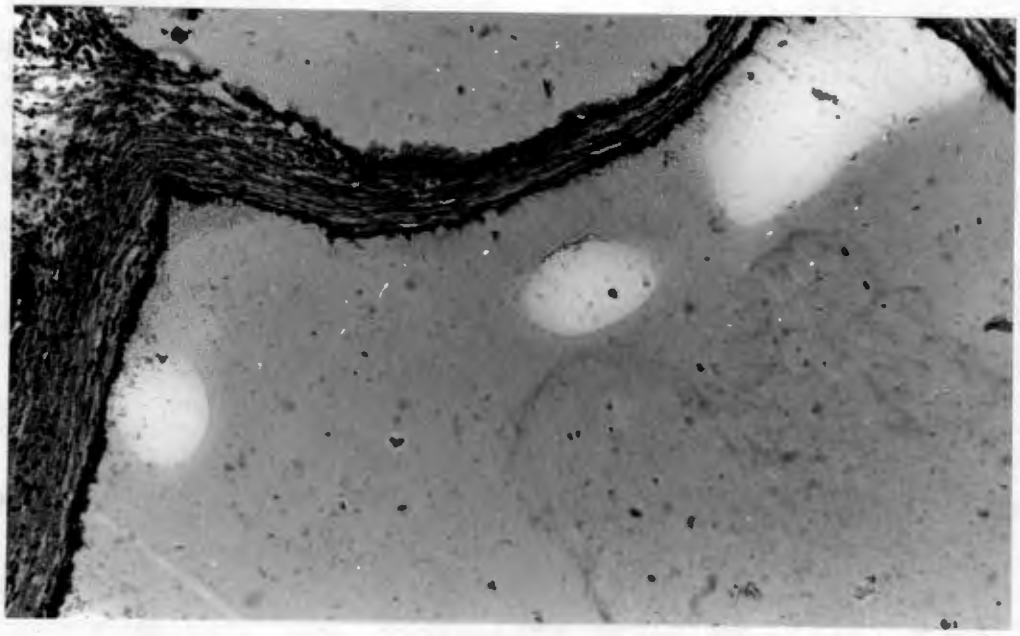


Fig.14
X 95
30 mins.

Areas of fibrinolysis related to the epithelium and secretion of a gland.

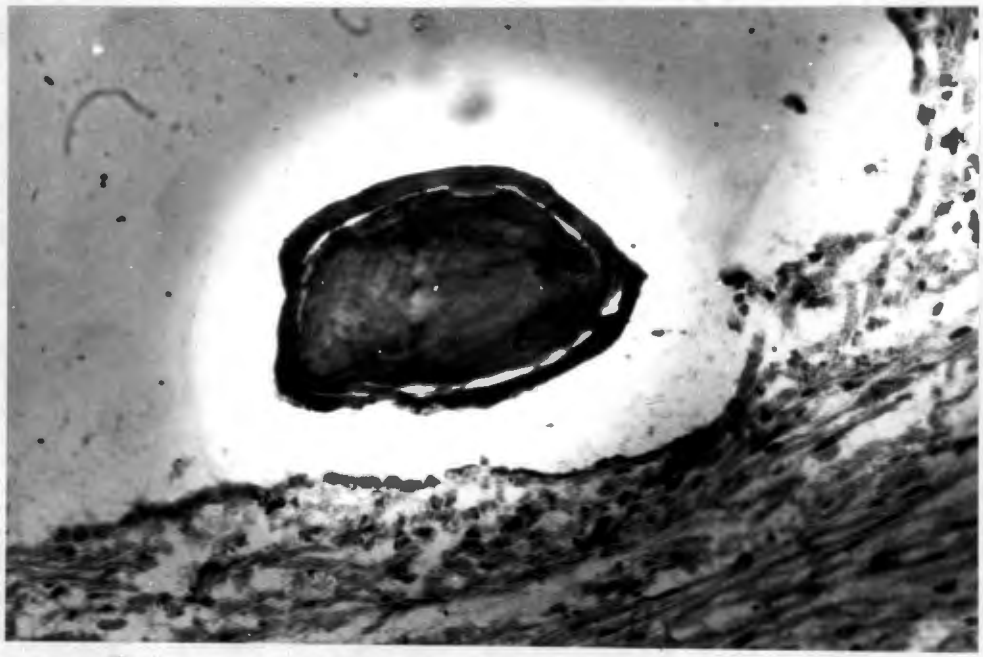


Fig.15
X 240
30 mins.

A prostatic concretion or amylaceous body surrounded by a zone of fibrinolysis.

DISCUSSION

Local tissue fibrinolysis may be enhanced by factors related to the surgical removal of the prostate. Indeed, increased fibrinolysis in the blood has been related to pre-operative anxiety (Macfarlane and Biggs, 1946); during general anaesthesia (von Kaulla, 1947); after trauma and ischaemia (Kwaan and McFadzean, 1956) ; during surgical procedures (Lynn Evans, 1964); and in the post-operative period (Macfarlane, 1937). Wendt, Perlick and Seyffarth (1957) detected a greater level of fibrinolytic activity after prostatectomy when controlled hypotension was used, than when this technique was not employed.

Although earlier studies have demonstrated a direct relationship between the number of leucocytes in the prostatic secretion from patients with chronic prostatitis, and the fibrinolytic activity of the fluid (Huggins and McDonald, 1944), in the autographs examined, no such topographical relationship to leucocytes was seen.

It has been shown by Astrup and Permin (1947) that the fibrinolytic activity in human tissues is due to their ability to activate plasminogen. Prostatic tissue /

tissue is rich in substances which can activate plasminogen (Rasmussen and Albrechtsen, 1960a), and it has been assumed that the increased fibrinolysis associated with abnormal bleeding in patients with disseminated prostatic cancer (Jürgens and Trautwein, 1930; Tagnon and others, 1952a, 1953a), or with benign prostatic hypertrophy (Andersson and Nilsson, 1961), and also in prostatic surgery (Scott and others, 1954; Lombardo, 1957; Baurys and Jacobson, 1959; Urlus, 1962), is due to the release of this activator into the blood stream (Fearnley, 1965). It has also been assumed that this activator is of epithelial origin and identical with the semen-liquefying protease (Huggins and Neal, 1942).

In the light of these and other observations (Huggins and Vail, 1943; Tagnon and others, 1952b, 1953b; Scott, 1956), it is surprising that, with the histological technique, prostatic epithelium shows only slight ability to activate plasminogen. The present experiments do not support the view that fibrinolysis in prostatic disease could come from the glandular/

glandular epithelium, and confirm the previous finding (Todd, 1961), that the bulk of the fibrinolytic activity of normal and carcinomatous prostatic tissue arises from the blood vessels. However, none of the cases in the present series showed a bleeding tendency, and until tissue from such haemorrhagic cases can be examined by fibrinolysis autography, the possibility remains that some types of prostatic epithelium, especially in carcinoma, may have a greatly increased activator content.

It is equally likely that the bleeding in cases of metastatic prostatic cancer is a manifestation of disseminated intravascular coagulation (Seale, Jampolis and Bargen, 1951; Swan, Wood and Daniel, 1957). Rapaport and Chapman demonstrated in 1959 the presence of hypercoagulability and hypofibrinogenaemia in a case of disseminated prostatic carcinoma. Intravascular clots have been shown at the autopsy of a similar case by Aggeler and Seale (quoted by Seale and others, 1951). It is known that the prostate is rich in thromboplastin (Seale and others, 1951; Swan and others, 1957; Benzer, Blümel and Piza, 1962), which may/

may be released into the circulation when the cancer or its metastases are damaged or necrotic. Fibrinolysis would then follow as a secondary phenomenon.

It has been shown that the level of circulating fibrinolysin in the blood of patients with cancer of the prostate, is significantly elevated above that detected in other urinary tract diseases, or in normal cases (Anglesio, Pelocchino and Badà, 1959; Mairano and Rigotti, 1960; Andersson, 1963a). Indeed, Tagnon and his colleagues (1953a) collected 48 cases of metastatic cancer of the prostate, of which 6 had a bleeding tendency due to abnormal fibrinolysis. Swan and Kerridge (1965) added to such controversy by finding no abnormal fibrinolytic activity in the blood of 68 patients with cancer of the prostate (of which 22 had metastases), when these cases were compared with those in a control group.

The results also cast doubt on previous assumptions about the source of plasminogen activator in semen. Immediately after ejaculation, human semen coagulates, and this is followed within 20 minutes by liquefaction of the clots by a plasmin-like enzyme said to come from the /

the prostate (Huggins and Neal, 1942), although there is no direct evidence that this enzyme has come from prostatic epithelium. A semen-liquefying "fibrinolysin" has certainly been found in fluid obtained by prostatic massage (Huggins and Neal, 1942) but the reason for supposing that it came from the gland, rather than from the seminal vesicles, is not clear. There is evidence in the autographs to suggest that epithelium is more active when damaged and desquamated, so that prostatic massage may enhance the fibrinolytic activity of the secretory apparatus by the trauma it causes.

The minor degree of fibrinolytic activity detected in prostatic epithelium seemed insufficient to account either for the fibrinolytic activity of semen, or for the haemorrhagic blood fibrinolysis seen in some cases of prostatic disease. It may be that either damage or stimulation of the epithelium is necessary to release the activator, or that seminal "activator" comes from other sites in the male genital apparatus, in addition to that secreted by prostatic epithelium.

CHAPTER VI

FURTHER STUDIES ON ACTIVATOR IN THE PROSTATE

PART I Regional Fibrinolytic Activity

PART II The Activity of Corpora Amylacea

PART III Fibrinolysis by Prostatic Imprints

PART I

REGIONAL FIBRINOLYTIC ACTIVITY

On superficial inspection, the human prostate may appear uniform, but its various regions or so-called lobes are quite diverse in function (Mann, 1964). For instance, the predilection of prostatic carcinoma for the posterior region of the gland is well known (Luck, 1965). The histological structure varies regionally, in that fibro-muscular stroma predominates in the anterior region, the prostatic glands are situated chiefly in the lateral and posterior regions or lobes, and the excretory prostatic ducts are numerous deep to the urethral crest (vera montanum).

The initial studies of the distribution of activator were on samples from adenomas or carcinomas which had been shelled out of the enclosing capsule. Because of the sampling from haphazard sites, and bearing in mind the regional histological variation, it seemed necessary to determine whether the distribution of activator could differ from region to region, by examining specimens taken from selected sites in the whole gland.

METHOD /

METHOD

Prostate glands were obtained from 10 necropsies, and blocks of tissue were taken from the anterior and posterior regions of the glands, and from the area of the vera montanum. Fibrin-slide preparations were made, and the degree of activity was assessed (see Tables 10, 11 and 12).

RESULTS

No obvious regional difference in the overall intensity of fibrinolytic activity was detected in the samples taken from the anterior or posterior lobes, or from the vera montanum. However, the scores for the epithelial activity within the anterior lobe and the vera montanum were significantly higher than that attained by the glands and ducts in the posterior lobe.

As before, it can be seen that the maximum activity was related to the blood vessels (Figs. 16, 17). Inconstant activity was related to the epithelium of glands (Figs. 17, 18 and 19) and ducts (Figs. 20, 21), and also to secretions and corpora amylacea (Fig. 22). In the sections of vera montanum, zones of lysis were common/

common around the excretory and ejaculatory ducts (Fig. 23) approaching the urethral lumen, especially where the epithelium had been desquamated. In one particular prostate, taken from necropsy No. 13, the excretory ducts had been packed with amyloid bodies and calculi. A broad zone of lysis was related to the lining of one remarkably dilated duct (Fig. 24).

When present, the urethral mucosa exhibited striking activity (Figs. 24, 25 and 26). In such samples obtained at necropsies, striated muscle fibres were often seen in the sections, and occasionally, these were related to small areas of lysis (Fig. 27). Damage or disruption enhanced the activity of all the tissue components.

TABLE 10: Fibrinolytic Activity of Tissue Components in the Posterior Lobe of the Prostate

Necropsy No.	Overall Activity	Vascular	Glands	Ducts	Secretion
5	16	16	4	6	0
6	14	14	6	6	2
7	12	12	2	2	0
8	16	16	10	6	0
9	16	16	10	10	0
10	16	16	9	7	2
11	18	18	16	16	8
12	16	16	10	10	2
14	16	16	10	10	0
Average	16	16	9	8	2

TABLE 11: Distribution of Activity in the Anterior Lobe of the Prostate

Necropsy No.	Overall Activity	Vascular	Glands	Ducts	Secretion
5	16	16	0	2	0
8	16	16	16	16	0
9	18	18	16	16	4
11	16	16	16	16	16
13	18	18	16	16	0
14	16	16	6	6	0
15	16	16	4	4	0
Average	17	17	11	11	3

TABLE 12: Activity of Tissue Components in the Vera Montanum

Necropsy No.	Overall Activity	Blood Vessels	Glands	Ducts	Secretion
5	12	12	4	8	0
6	16	16	10	10	0
7	16	16	10	10	0
8	16	16	6	6	0
9	16	16	16	16	0
10	16	16	6	6	0
11	16	16	16	16	12
13	16	16	16	16	4
15	18	18	14	14	0
Average	16	16	11	11	2

Fig. 16: Anterior lobe of prostate. Areas of lysis related to the blood vessels. 30 mins. X 19

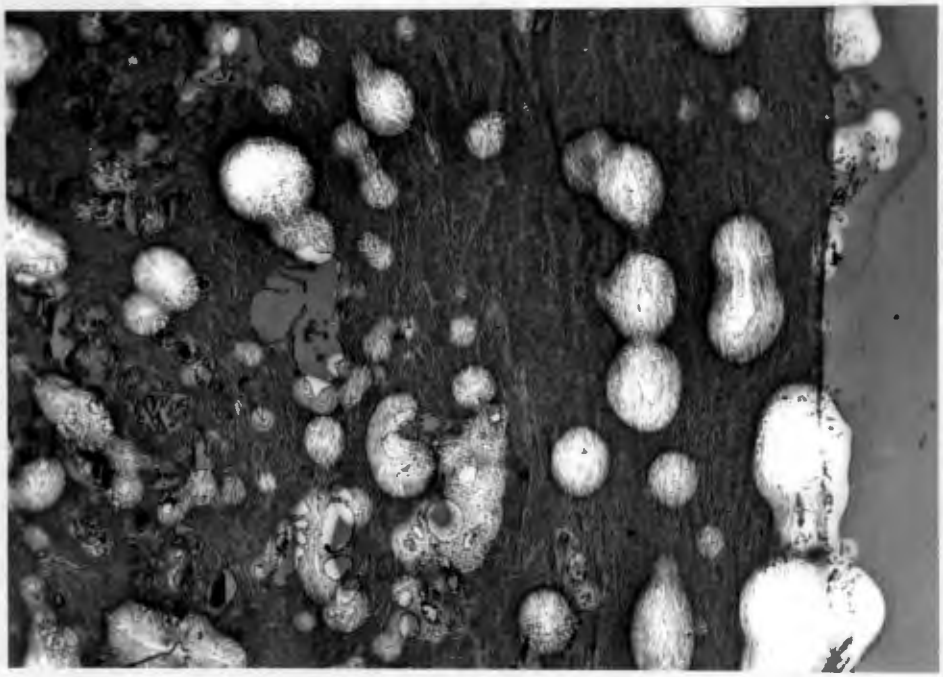


Fig. 17: Vera montanum. Fibrinolytic activity arising from blood vessels, and also from the epithelium of large glands. 30 mins. X 24

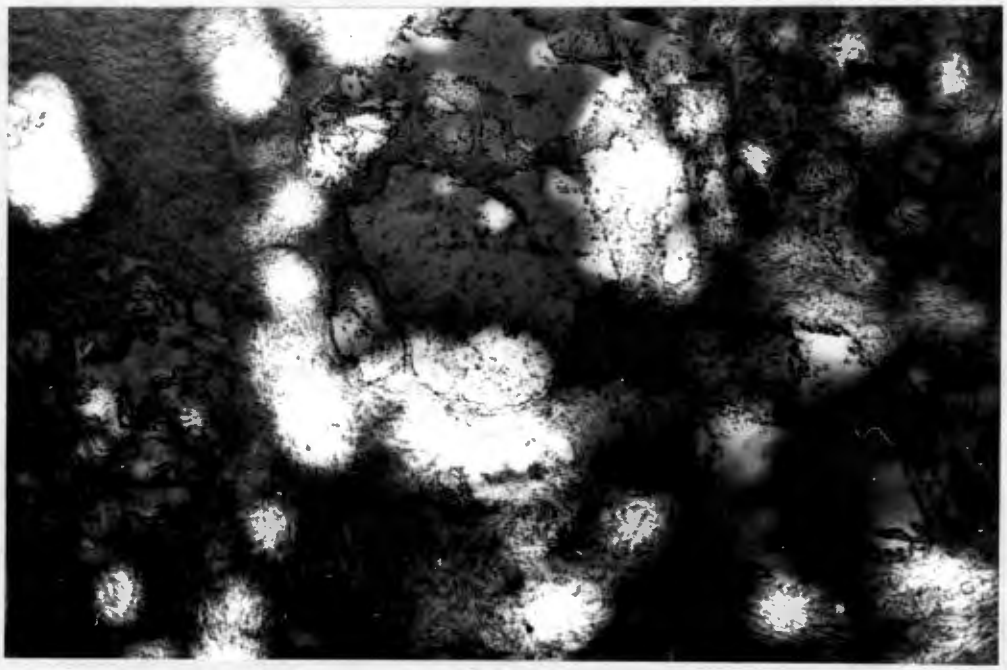
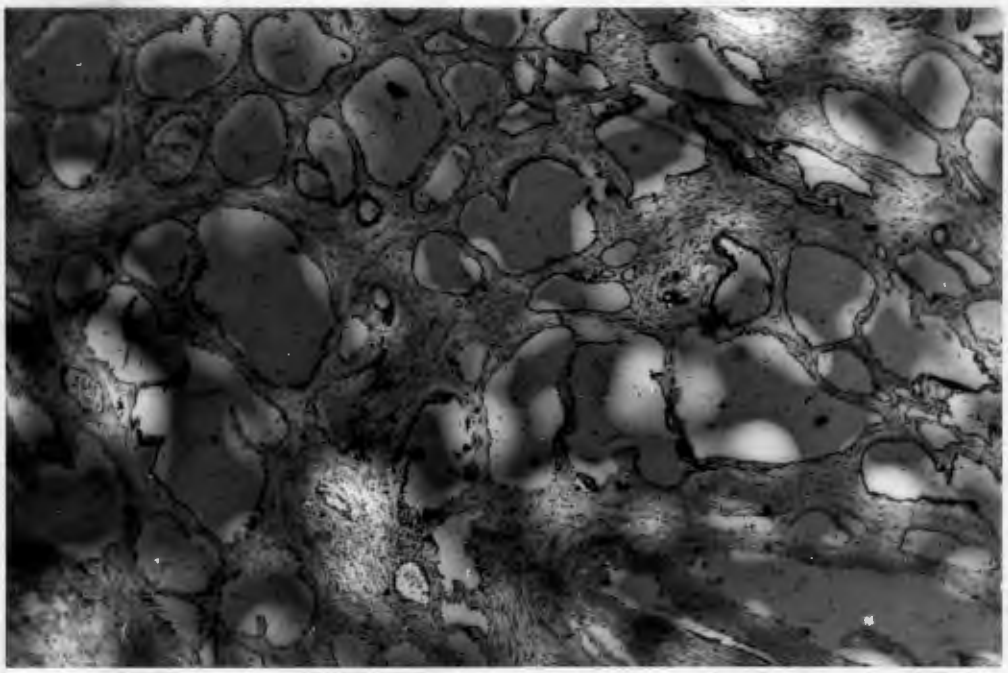


Fig. 18: Anterior lobe. 60 mins.X 24



The glands show prominent zones of digestion throughout the sections.

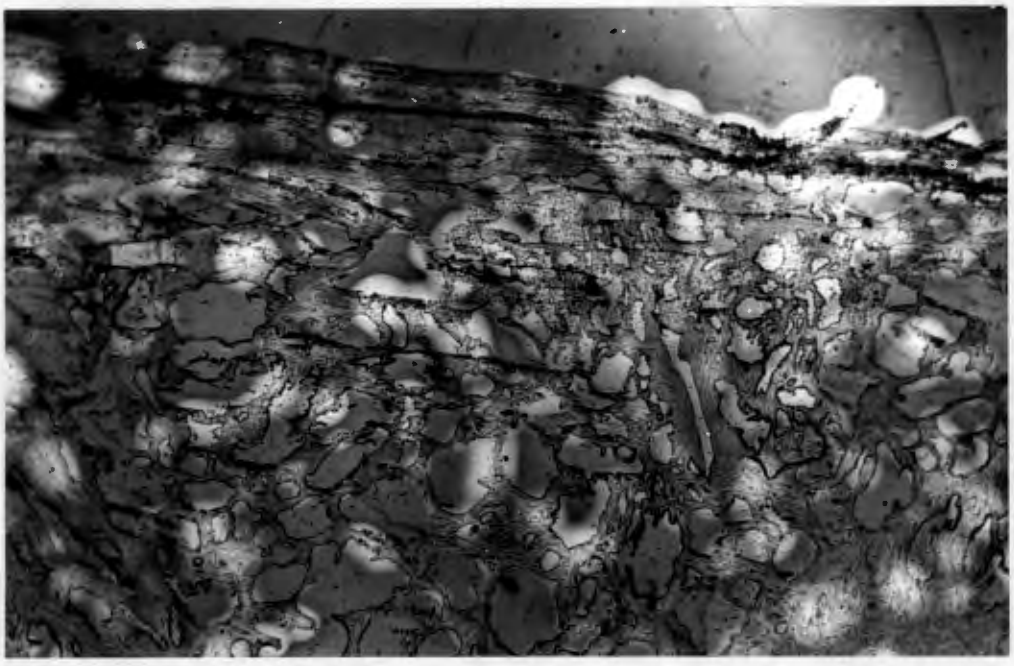


Fig. 19: Vera montanum. 30 mins. X 15

Fig. 20: 30 mins. X 122

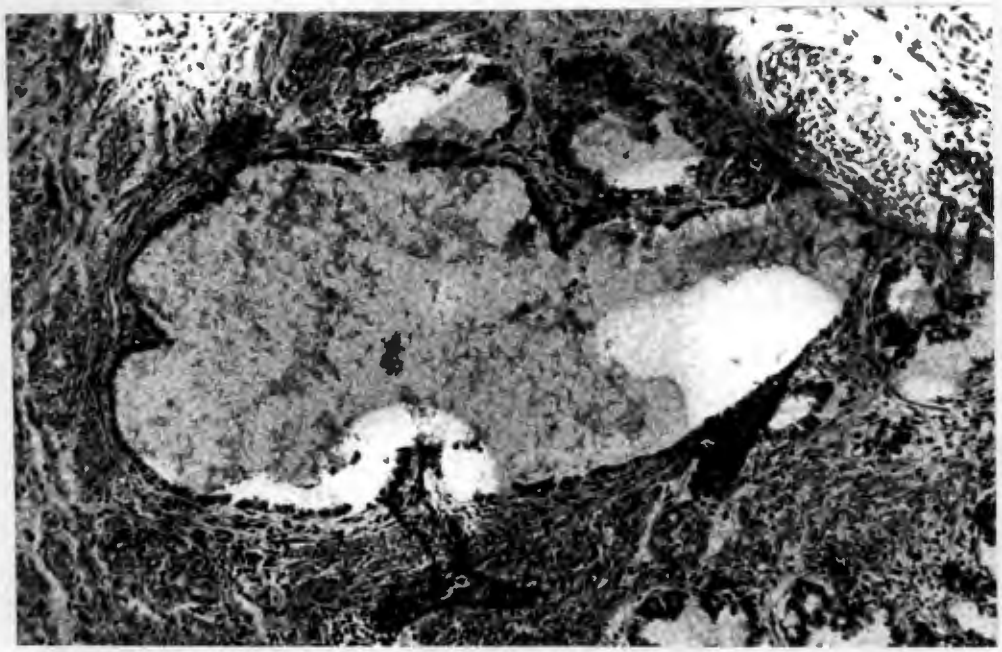
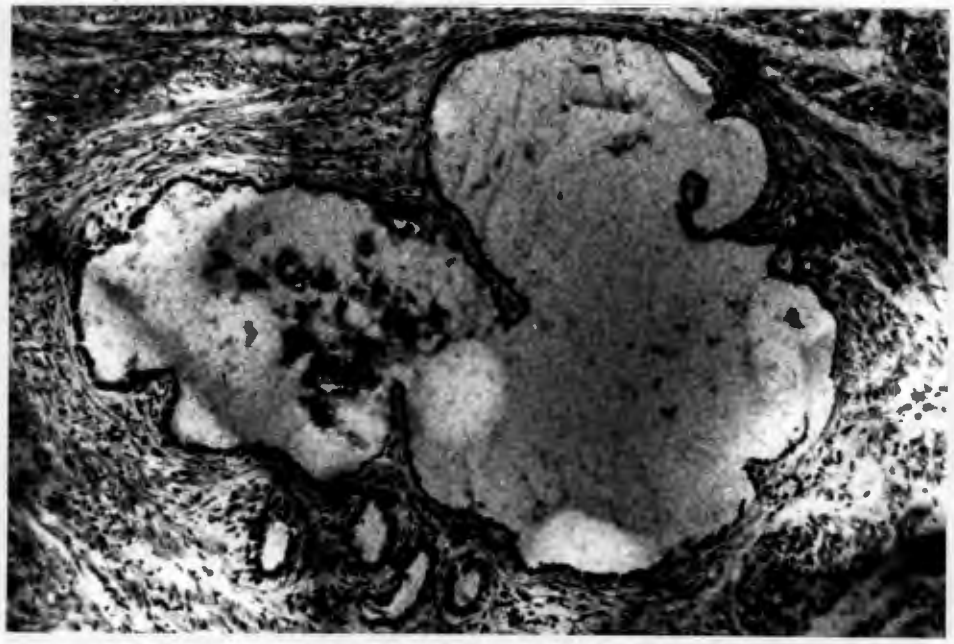


Fig. 21: 30 mins. X 95



Figs. 20,21: Ducts in the anterior lobe of prostate exhibiting epithelial activity.

Fig. 22: Vera montanum. Fibrinolysis arising from the secretions and inspissated material. 30 mins. X15

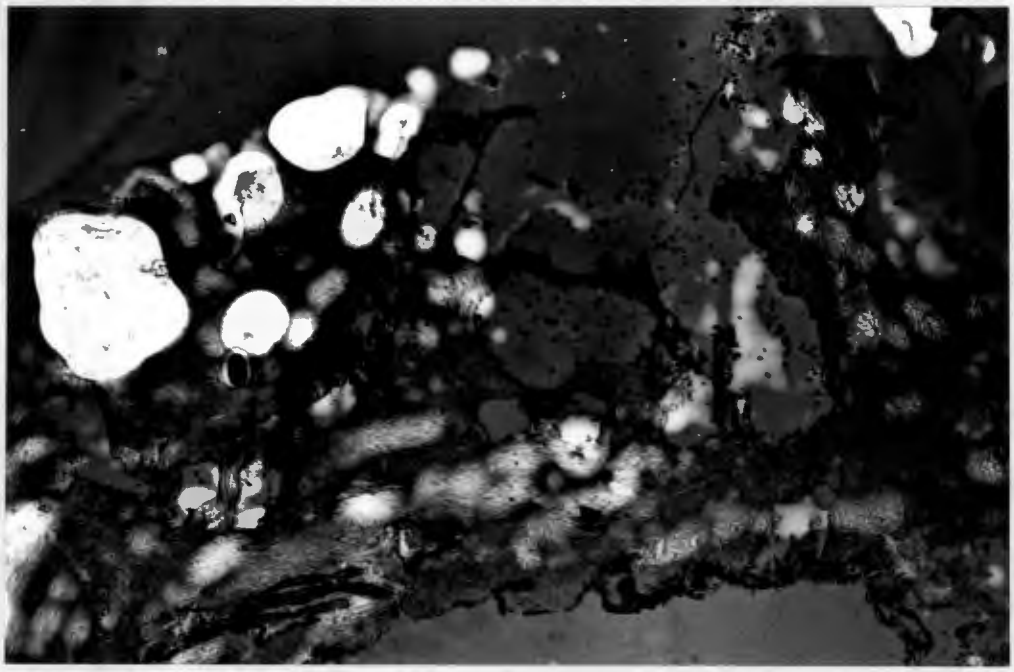


Fig. 23: Vera montanum. Epithelium of an ejaculatory duct surrounded by a wide area of digestion. 30 mins. X 95

Fig. 24: Vera montanum. A ring of digestion arising from the glandular epithelium. Fibrinolysis also related to amylaceous material. 30 mins. X 15

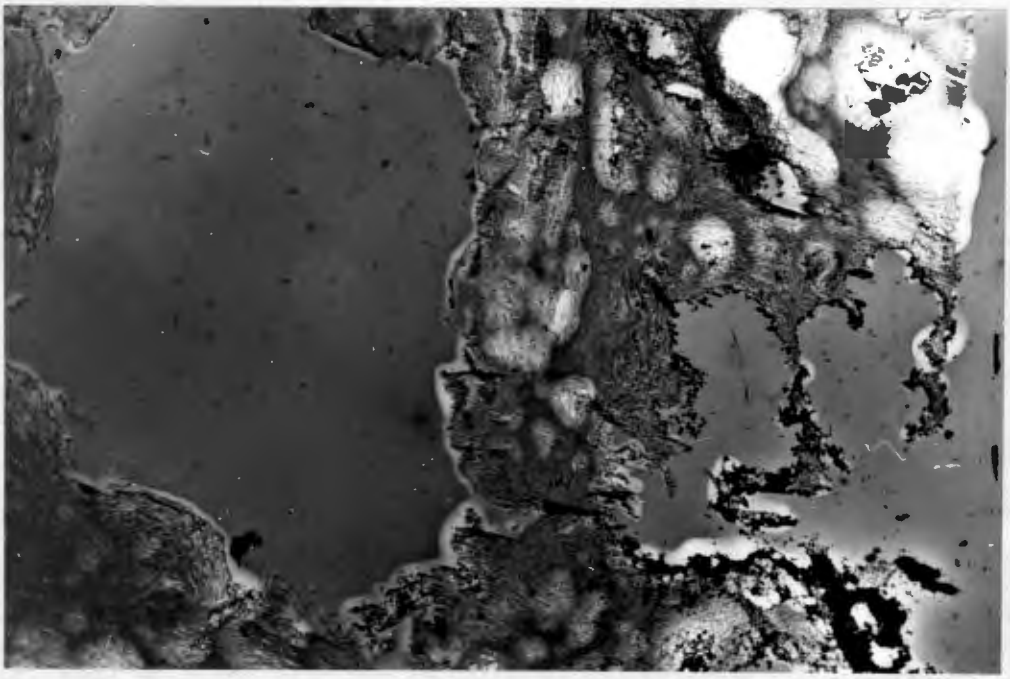


Fig. 25: Urethral mucosa with a wide band of digestion. 60 Mins. X 122

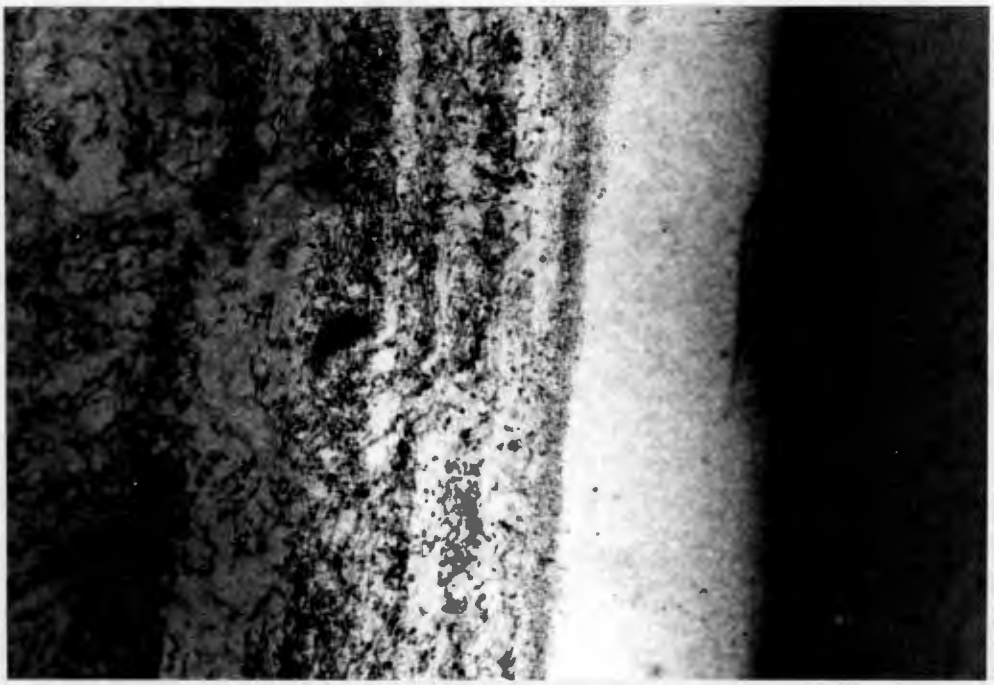
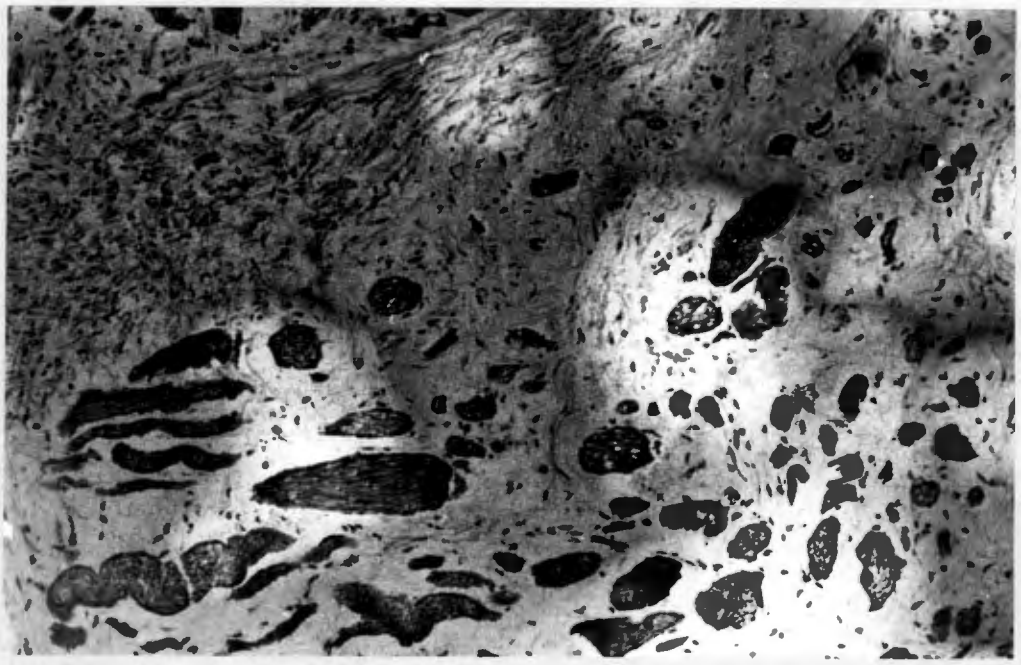


Fig. 26: Vera montanum. 30 mins. X 95

Prominent activity arising from urethral mucosa, especially where disrupted.

Fig. 27: Anterior lobe of prostate. 30 mins. X 24

Fig. 28: Anterior lobe of prostate, with areas of digestion around striated muscle fibres. 30 mins. X 95



PART II

THE ACTIVITY OF CORPORA AMYLACEA

On many occasions in these studies, prostatic concretions, corpora amylacea or amylaceous bodies had shown themselves to contain plasminogen activator. These bodies were round or oval in shape, and often consisted of concentric layers. A favourable opportunity arose to carry out further experiments on these bodies, when section of the prostate of necropsy No. 13 revealed that the excretory ducts leading to the urethra were packed with hundreds of corpora amylacea or calculi of all sizes, the largest being 1-2 mm. across.

METHODS AND RESULTS

Corpora amylacea and calculi were washed twice with Michaelis buffer-saline, and allowed to dry on filter paper. They were next divided into 3 lots, and from one portion, samples were placed separately on a layer of fibrin, and incubated using a low temperature technique developed by Todd and Nunn (1967). In this method, the sensitivity /

sensitivity of the standard autograph can be greatly improved by exposure at 4°C. for 24-48 hours, followed either by immediate fixation, or incubation for 15-30 minutes at 37°C. The fibrin-slide is next fixed. The more intense foci are damped down, while small foci, undetectable in the standard autograph, are strikingly demonstrated. The explanation for this result may be that activation of plasminogen in the fibrinolysis autograph proceeds at a similar rate at 4°C. or 37°C., while digestion of fibrin is rapid at 37°C. Naturally, during such a long exposure, preservation of the tissue is better at 4°C. than exposure at room temperature or 37°C.

TABLE 13: Fibrinolytic Activity of Corpora Amylacea

Slide No.	No. of Active Corpora	Total No. of Corpora
1	6	7
2	13	19
3	6	10
4	7	8
5	11	16
6	8	17
7	7	8
8	18	20
9	52	58
10	5	7
Total	133	170

The/

The corpora amylacea were exposed overnight (17 hours) at 4°C., followed by incubation for 60 minutes at 37°C. Most of these bodies were active (Fig. 29), and when counted, these formed 78% of the total (Table 13).

The bodies and calculi of the second portion were ground to a fine powder, and suspended in 5 ml. compound Ringer's lactate solution to form a thick suspension.

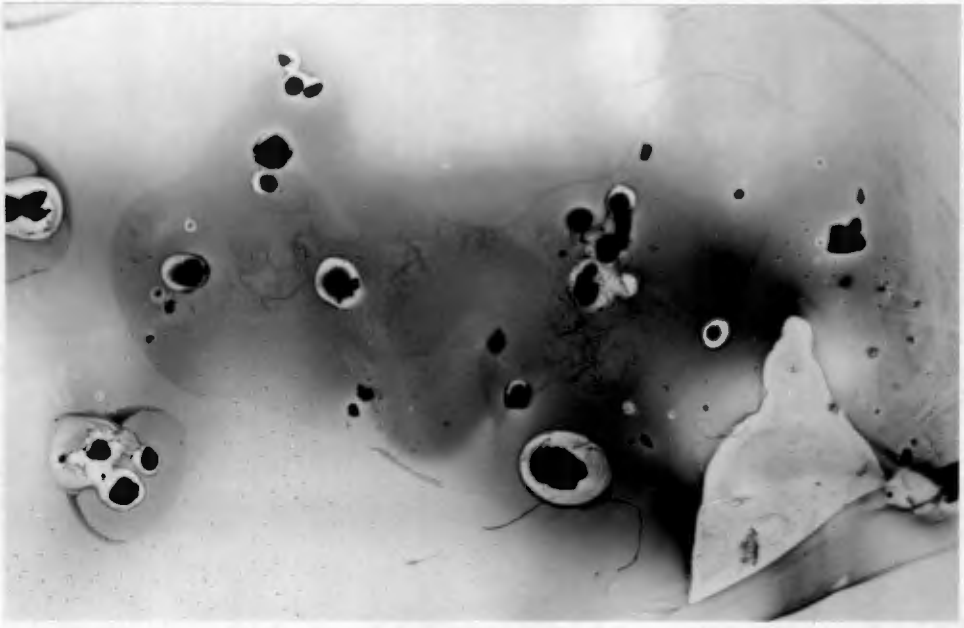


Fig. 29: Digestion of fibrin by corpora amylacea. Exposure overnight at 4°C. X 5

A drop of the suspension was spread out on a coverslip, and overlaid by a square of fibrin. Eight autographs were prepared - all of these showed zones of digestion surrounding/

surrounding the powdered amylaceous material (Fig. 30) after overnight exposure (17 hours) at 4°C., succeeded by one hour at 37°C.

The third lot of corpora amylacea was also ground to a fine powder, and portions weighing 0.2G. each were placed into equal volumes (2.0 ml.) of physiological saline, veronal-acetate buffer and 2M potassium thiocyanate. The mixtures were thoroughly stirred for several minutes, centrifuged, and the supernatant fluid was reserved.

A 2M potassium thiocyanate solution by itself produces a marked zone of apparent "fibrinolysis", in the presence of citrate (or phosphate) ions. This "citrate ion effect" (Mullertz, 1952) can be prevented by the addition of calcium ions, which bind citrate. However, the enhancing effect of citrate ions on plasminogen activator is thereby removed (Astrup and Sterndorff, 1960). Therefore, to neutralize the effect of potassium thiocyanate, about 0.4 ml. of M/10 calcium chloride solution was added to 3.5 ml. fibrinogen solution, before clotting with thrombin. It was confirmed in a set of control autographs that sufficient calcium had been added by testing the fibrin film with the potassium thiocyanate solution.

A/

A drop from each extract was placed on fibrin squares, and the autographs were exposed overnight at $4^{o}C$. as before. Every preparation showed evidence of fibrinolysis (Figs. 31, 32 and 33), but no significant difference could be detected in the degree of activity produced by any of the extracts. Activator then appears to be fairly loosely bound in the corpora amylacea, whereas activator in the tissues is relatively insoluble.

Fig. 30: Fibrinolysis by powdered corpora amylacea. X 5

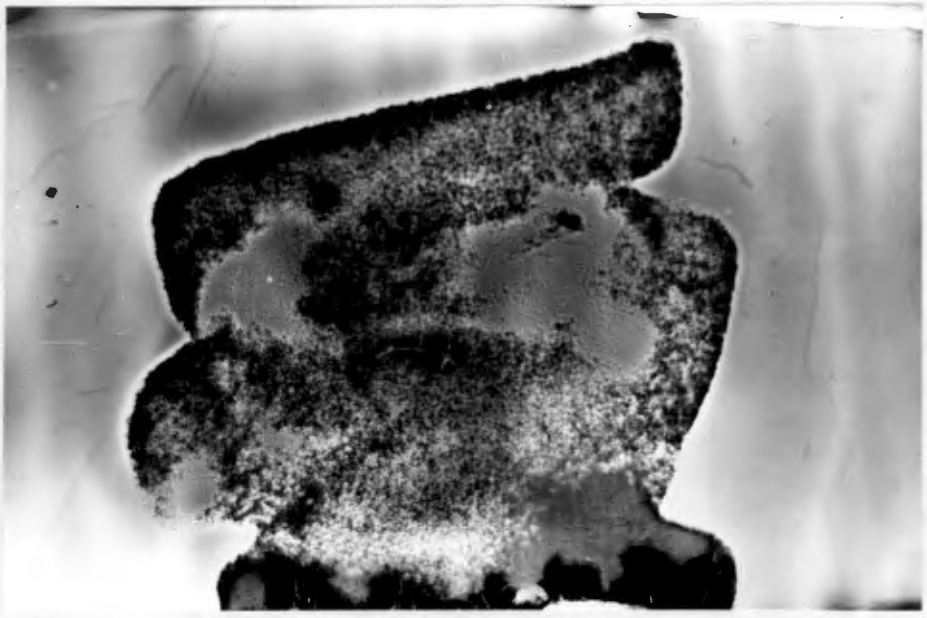


Fig. 31: Saline extract of corpora producing fibrinolysis. X 5

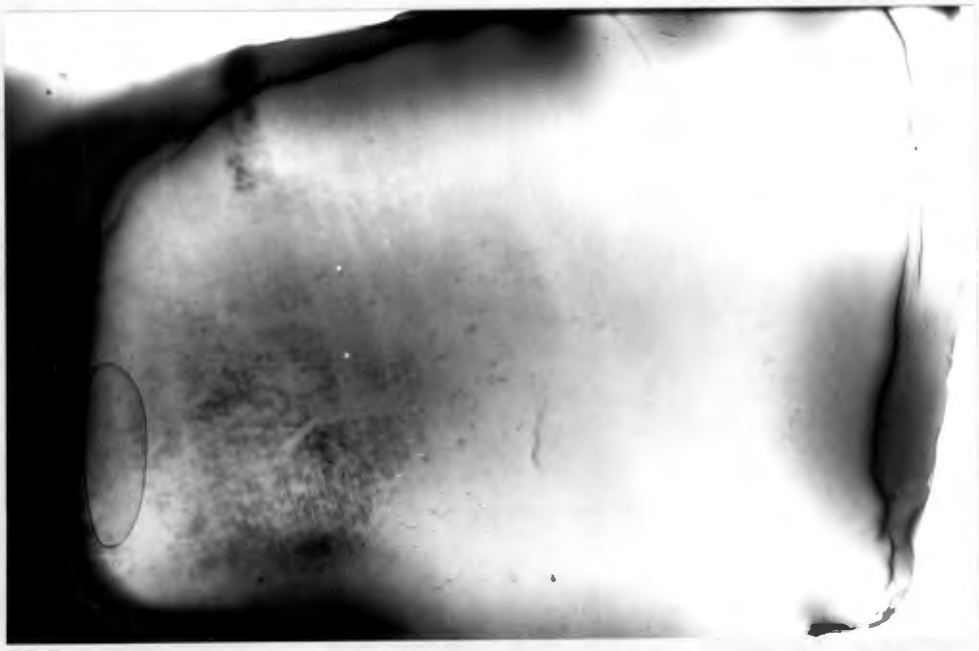


Fig. 32: Fibrin-digestion by buffer-saline extract. X 5

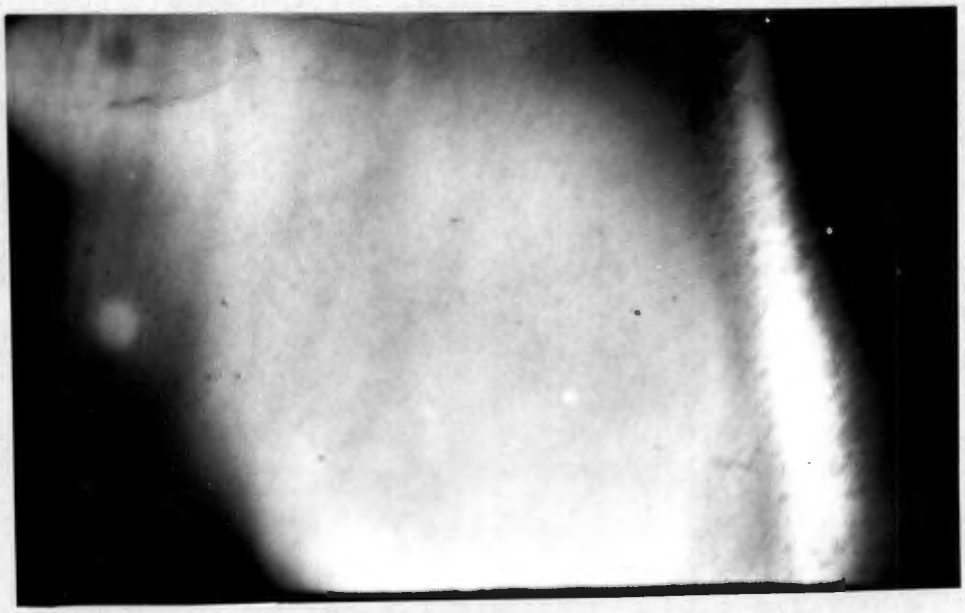


Fig. 33: Lysis by potassium thiocyanate extract. X 5

PART III

FIBRINOLYSIS BY PROSTATIC IMPRINTS

A block of freshly-obtained tissue from an enucleated prostate was pressed firmly against a glass slide. A slice of tissue was shaved off from the block's surface, and a further imprint was made. In this manner, 21 imprints were made from 3 glands, obtaining specimens of detached epithelial cells, secretions and amylaceous bodies. Fibrin-slide preparations were exposed overnight at $4^{o}C$.

Foci of fibrinolytic activity were related to these detached epithelial cells (Figs. 34, 35, 36 and 37), secretions (Fig. 36), and corpora amylacea (Figs. 38 and 39).

Figs. 34 - 37: Prominent zones of digestion related to detached epithelial cells and amylaceous bodies.

Fig. 34 x 24

Fig. 35 x 15

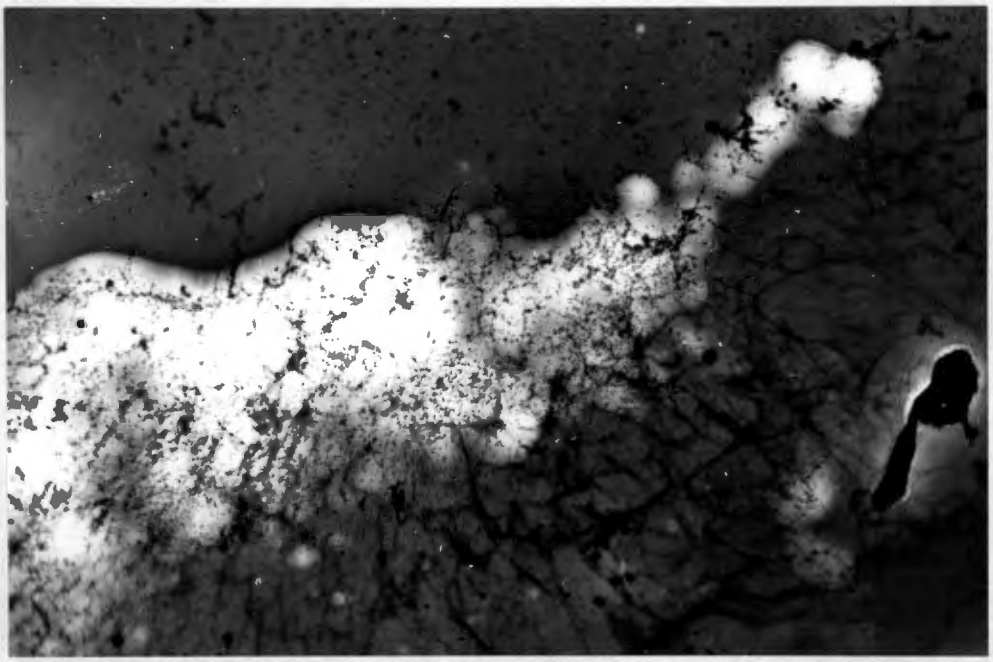


Fig. 36: Apart from epithelial activity,there is a zone of lysis related to an area of dried secretions X 15

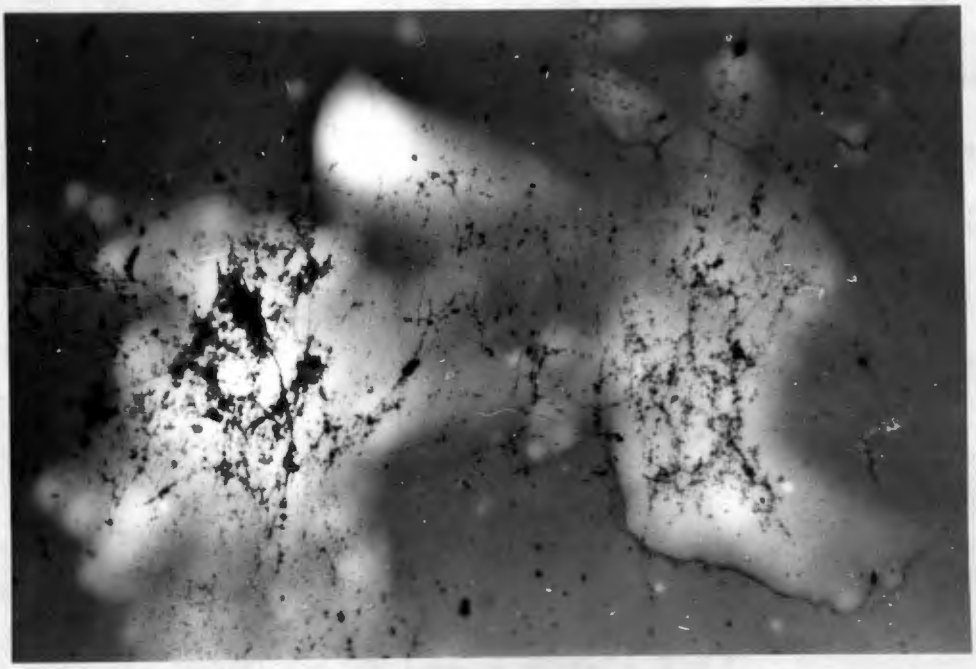
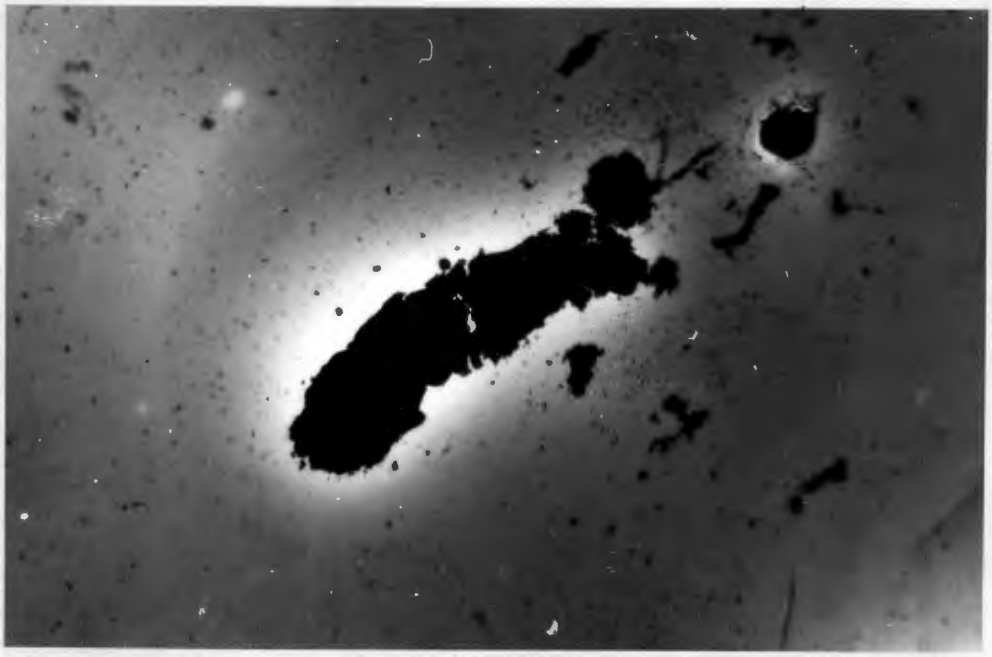
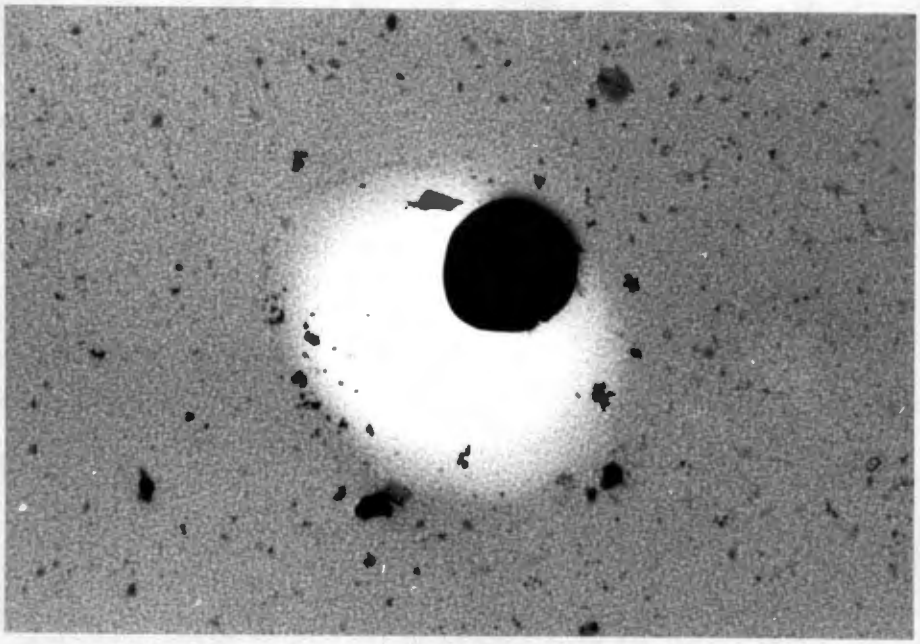


Fig. 37: X 24



Figs. 38 - 39: Broad zones of digestion surround these corpora amylacea. X 15



DISCUSSION

The "corpora amylacea" or amylaceous bodies, usually located in the larger ducts and acini of the prostate, are small, soft, concentrically laminated spheroidal bodies, pale yellow to dark brown in colour, and frequently doubly refractile. They are made up of desquamated epithelial cells and prostatic secretion (Mann, 1964), and these secretory products consist of proteins, including albumin and globulin (Wagner and Kim, 1963), mucoproteins and mucopolysaccharides (Smith, 1966).

Prostatic calculi are firm calcified bodies, usually not more than a few mm. in diameter, and of the same basic structure as that of the corpora amylacea (Moore, 1936), apart from their size, and the presence of calcium salts. The protein content of dry powdered calculi has been estimated to be about 8% (Huggins, 1947). A number of factors are responsible for the formation of prostatic calculi, viz. obstruction and dilatation of the prostatic ducts; urinary reflux and infection (Fox, 1963). There is a definite link between prostatic and urinary calculi, frequently related to hypercalciuria.

It /

It has been shown that plasmin degrades chondro-mucoprotein by attacking the protein moiety, releasing a chondro-mucopolypeptide (Lack and Rodgers, 1958). This finding was confirmed by Charlton (1966, 1967) during experiments on dogs, and he demonstrated that the protein normally found in urine is a substrate for plasmin. He showed that diminished fibrinolytic activity of dog's urine resulted in a significant rise in the non-dialyzable solids, which predisposed to the development of a mucoprotein matrix or fibrinous calculus. He presented the hypothesis that the majority of urinary calculi develop in the presence of a dminished fibrinolytic activity in urine, if at the same time, a metabolic disturbance occurs, e.g. hypercalciuria or hyperoxaluria. Indeed, if the urine has normal fibrinolytic activity, but these metabolic disturbances prevail, then a stone will not form.

It is feasible that the development of amyloid bodies is initiated by an alteration in the coagulation-fibrinolysis balance, in favour of fibrin-deposition, analagous to thrombosis occurring within the blood vessels. Indeed, it has been shown that the gland is rich in thromboplastic substances (Benzer and others, 1962; Swan and /

and others, 1965). In order to maintain the patency of the prostatic excretory system, it seems necessary that a fibrinolytic mechanism should be present to prevent fibrinous deposition around the innumerable detached epithelial cells, which themselves contain significant amounts of activator.

Supplementary to the fibrinolytic system, a further factor helps to maintain the fluid state of semen and prostatic secretion. Huggins and Neal (1942) discovered that samples of human prostatic fluid inhibited the clotting of human blood. Since coagulation of the blood proceeded normally when calcium chloride was added, they concluded that the high concentration of citrate in prostatic fluid (Huggins, Scott and Heinen, 1942) was the cause of the prolonged coagulation time.

A similar model of equilibrium to that found in the blood stream is seen in the urinary tract. Urine normally contains a thromboplastic material which contributes to haemostasis (Tocantins and Lindquist, 1947; von Kaulla, 1956). Ladehoff (1960) thought that urokinase came from the urinary conducting tissue, released by the disintegration of the highly active epithelial cells lining the conducting /

conducting sections of the urinary tract. He demonstrated that desquamated urinary epithelial cells obtained by centrifuging the bladder urine of cadavers, had a high content of activator. Indeed, there exists a concept that urokinase maintains the patency of the urinary tract by lysing fibrinous deposits (Astrup and Sterndorff, 1952b; Charlton, 1966, 1967), although it will impair haemostasis during urinary tract surgery (McNicol and others, 1961a).

In view of these previous considerations, it occurred to me that a disturbance in the coagulation-fibrinolysis equilibrium of prostatic secretion could lead to the development of corpora amylacea. The following evidence is presented in support of my hypothesis.

As with a clot in a blood vessel adsorbing fibrinolysin, it seemed necessary to determine whether the protein of corpora amylacea could adsorb activator from the surrounding medium. It has been demonstrated, too, that the mucoproteins of urine adsorb urokinase, and indeed, 5-15 ml. of urine has enough activator to dissolve 1 ml. of blood clot (von Kaulla, 1960). A collection of amylaceous /

amylaceous bodies, freshly obtained from an enucleated prostate, were washed twice with Ringer-lactate solution, and divided into 2 equal lots. One portion was placed in a sterile screw-topped jar containing 20 ml. sterile fresh urine. The second portion was placed in a similar volume Ringer's solution to act as a control. The containers were stored for 48 hours at $4^{o}C$. The corpora were next washed thrice with Ringer's solution to remove all traces of the storage solutions. Fibrinolysis autographs were made with the corpora, and were exposed at $4^{o}C$. for 24 hours, followed by incubation at $37^{o}C$. for 60 minutes. Autographs were made from drops of the stored urine and Ringer's solution.

The results (see Table 14) demonstrated that urine was rich in activator (Fig. 40) and that the Ringer's solution had extracted a significant quantity of activator from the corpora (Fig. 41). Of the corpora amylacea steeped in urine, about 50% were active (Fig. 42), while about 26% of those kept in the Ringer's solution (Fig. 43) digested fibrin. Because plasminogen activator appears to be loosely bound to corpora amylacea, extraction of activator by the Ringer's solution could contribute somewhat /

what to the lower level of activity exhibited by the controls. However, the same factor would apply to corpora soaked in urine.

It has been demonstrated that prostatic amyloid bodies can adsorb plasminogen activator, just as a mural thrombus in a blood vessel adsorbs activator from circulating blood (Fearnley, 1961). The effect of absent seminal fibrinolysins is demonstrated in rodents, where retrograde ejaculation into the bladder results in the formation of coagulated cheese-like lumps (Clayton and others, 1956; Vulpé and others, 1956).

Further indirect evidence in support of my hypothesis is the finding by Moore (1936) that corpora are most abundant in the "cephalad" portion of the posterior lobe, and in the larger ducts and adjacent acini of the lateral lobes. They are infrequent in the middle lobe, and rare in the anterior lobe. These discoveries bear relation to the findings of the present studies, that the fibrinolytic activity of the ducts in the postero-lateral regions of the prostate is less than the duct activity in the anterior lobe and the vera montanum.

It /

It would be very interesting to know whether the development of amylaceous bodies in the acini can be arrested or reversed by fibrinolysis. Vulpe and others (1956) believe that, in rodents, dissolution of the bladder masses by the action of urine, occurs as a normal physiological process. Corpora within an infected acinus are subsequently digested by the fluid of the exudate (Moore, 1936), and corpora involved in an area of caseous necrosis undergo rapid dissolution. There is evidence that the secondary softening which corpora undergo, is brought about by proteases (Moore and Hanzel, 1936).

Although characteristic of the human prostate, corpora amylacea are also found elsewhere in the animal kingdom, particularly among insectivores where they form the principal secretory product of male accessory organs (Eadie, 1948). Their presence in seminal plasma contributes substantially to the final consistency of the copulatory plug by giving "body" to the mass, and they are digested when the copulatory plug liquefies.

In order to find additional support for my hypothesis concerning the development of corpora amylacea, an investigation /

investigation is under way at present, to determine by means of electron microscopy whether the amylaceous bodies contain fibrin fibrils. Marx, Gueft and Moskal (1965) studied the structure of corpora amylacea by means of electron microscopy. The bodies consisted of branching, double-stranded fibres with banding, 200-300A in breadth. They also contained fine granules and amorphous material. These granules are thought to be secretory products of the epithelial cells. The significant feature was that the fibrils did not resemble amyloid in several respects.

TABLE 14: Fibrinolytic Activity of Corpora Amylacea steeped in Urine and Ringer's Solution

Slide No.	Corpora Steeped in Urine		Corpora Steeped in Ringer's	
	Active	Total	Active	Total
1	2	4	3	9
2	4	18	4	9
3	2	6	2	6
4	6	16	0	4
5	12	12	2	7
6	6	8	0	8
7	6	11	2	8
Total	38	75	13	51

Fig. 40: Impressive digestion by a drop of the urine in which corpora amylacea had been steeped. Exposure for 24 hours at 4°C., then 60 mins. at 37°C. X 5

Fig. 41: Lysis by a drop of the Ringer's solution in which amylaceous bodies had been steeped. X 5

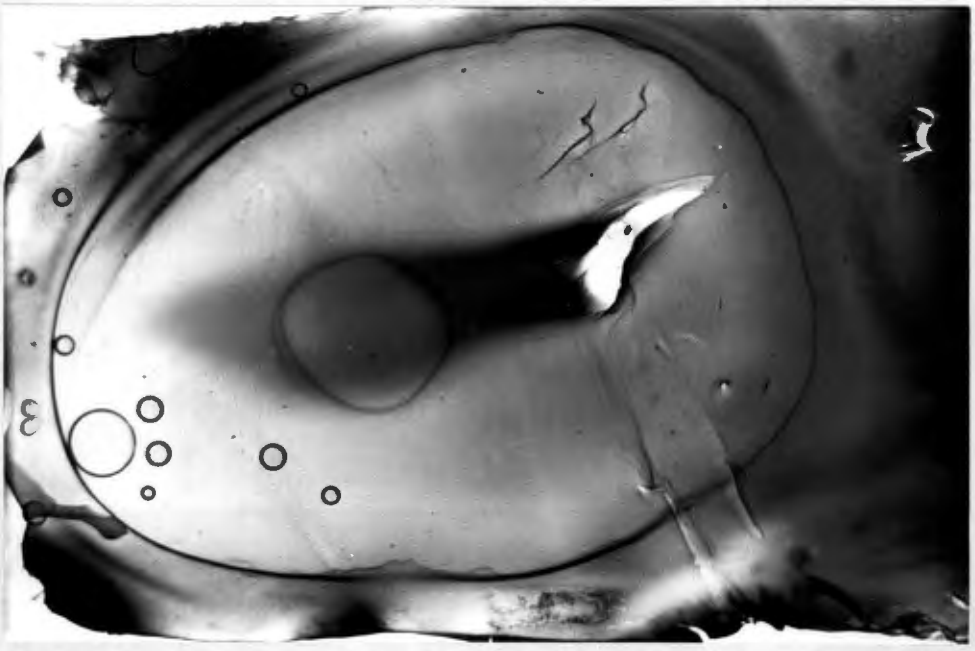
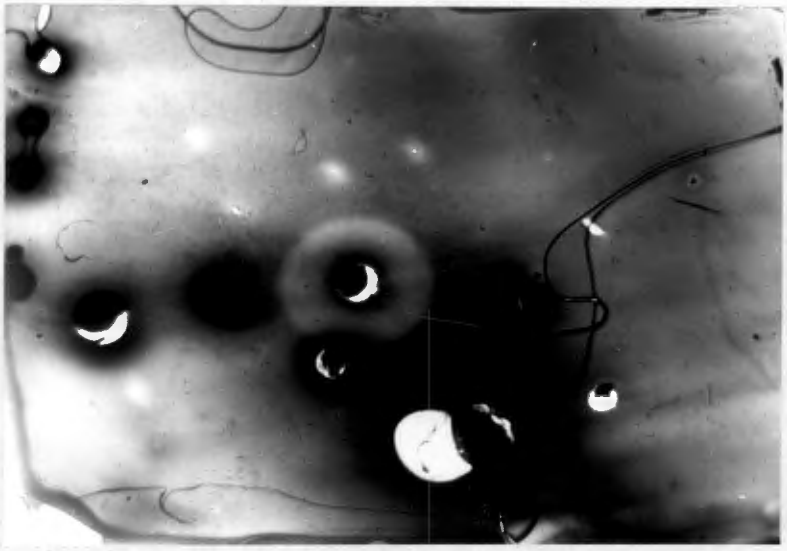


Fig. 42: Frequent areas of fibrinolysis by corpora amylacea which had been steeped in urine. X 5

Fig. 43: Less frequent zones of lysis by corpora amylacea after storage in Ringer's solution. X 5



CHAPTER VII

FACTORS INFLUENCING THE SENSITIVITY OF PLASMINOGEN ACTIVATOR IN THE PROSTATE

FACTORS INFLUENCING THE SENSITIVITY OF PLASMINOGEN ACTIVATOR IN THE PROSTATE

Using the histochemical technique, it has been confirmed that the vascular endothelium has abundant plasminogen activator. It has also been revealed that the glandular epithelium contains an inconstant amount of tissue activator. It seemed relevant, then, to determine whether the sensitivity of prostatic activator would alter by varying the conditions of storage, and by adjusting the make-up of the substrate.

(1) Effect of Storage on the Stability of Activator

METHOD

Three blocks of tissue were taken from each of 4 prostate glands, immediately after surgical removal. A block from each gland was mounted on a microtome chuck, wrapped up well in Gallenkamp's "Parafilm", and stored continuously at -30°C. in carefully closed polythene bags. At least two fibrin-slide preparations, incubated for 60 minutes at 37°C., were made from each of these samples. after specific intervals of storage, viz. 24 hours, 1 week, 1 month, 2 months, 4 months, 6 months, 9 months and one year. /

year. The degree of overall activity of the components in both slides was assessed after each interval, the scores summed, and the results of all the stages were compared to see whether any difference could be determined.

RESULTS

It can be seen from the scores in Tables 15 - 18, that the levels of activity for the components remained undiminished after one year's storage. Figs. 44, 45, 46 and 47 show the topography of fibrinolysis autographs made after 24 hours, 2 months, 6 months and one year's storage.

It has been shown that tissue activator can remain stable for long periods. Indeed, tissue activator stored as a dry powder at -20°C. has remained stable for up to 18 months (Albrechtsen, 1959). The possibility that warmer temperatures could affect the stability of activator was next examined.

In order to simulate the usual conditions of storage of a cadaver prior to a necropsy, the two remaining blocks of prostatic tissue were processed in the following manner. Immediately after prostatectomy, the two blocks were /

TABLE 15: Sample 1 - Fibrinolytic Activity in Prostatic Tissue after Storage

Duration of Storage	Overall Activity	Vascular	Glands	Ducts	Secretion
24 hours	6	6	4	6	0
1 week	6	6	4	6	0
1 month	6	6	4	6	2
2 months	6	6	4	6	4
4 months	6	6	4	4	2
6 months	6	6	4	4	0
9 months	6	6	6	4	2
12 months	6	6	6	4	4

TABLE 16: Sample 2 - Fibrinolytic Activity after Storage

Duration of Storage	Overall Activity	Vascular	Glands	Ducts	Secretion
24 hours	6	6	2	2	0
1 week	6	6	4	4	0
1 month	6	6	4	4	2
2 months	6	6	4	4	2
4 months	6	6	4	4	2
6 months	6	6	4	4	0
9 months	6	6	4	4	0
12 months	6	6	6	4	4

TABLE 17: Sample 3 - Fibrinolytic Activity after Storage

Duration of Storage	Overall Activity	Vessels	Glands	Ducts	Secretion
24 hours	6	6	4	4	0
1 week	6	6	4	4	0
1 month	6	6	4	4	0
2 months	6	6	4	2	0
4 months	6	6	4	2	0
6 months	6	6	4	4	0
9 months	6	6	4	4	0
12 months	6	6	4	4	0

TABLE 18: Sample 4

Duration of Storage	Overall Activity	Vascular	Glands	Ducts	Secretion
24 hours	6	6	4	4	0
1 week	6	6	4	4	0
1 month	6	6	0	0	0
2 months	6	6	2	2	0
4 months	6	6	4	4	0
6 months	6	6	4	4	0
9 months	6	6	2	2	0
12 months	6	6	4	4	0

Fig. 44: Fibrinolysis in prostatic tissue after its storage for 24 hours. 60 mins. X 15

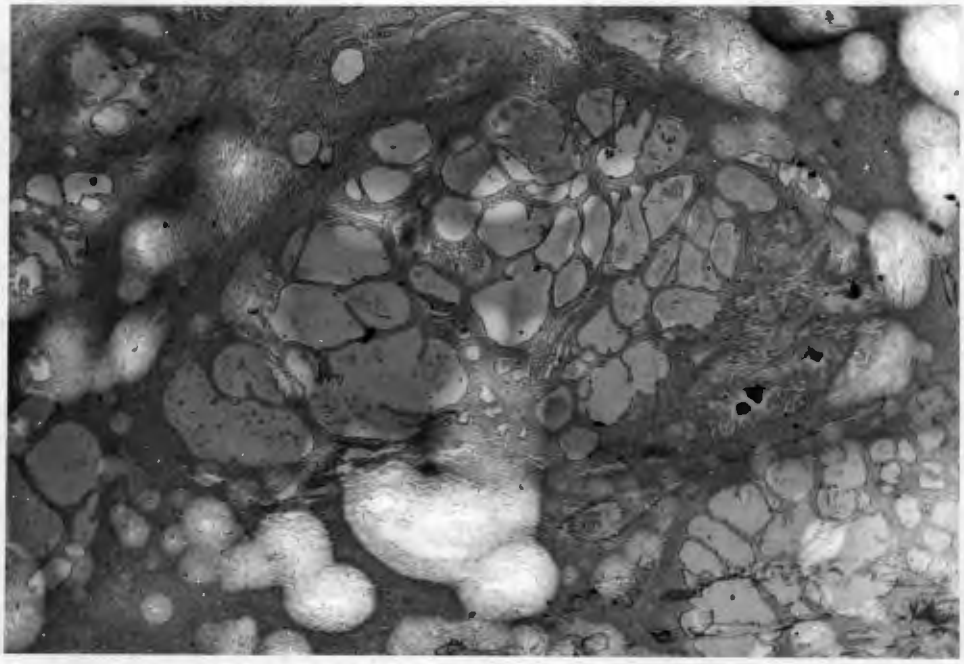


Fig. 45: Appearance of autograph from tissue stored for 2 months. 60 mins. X 19

Fig. 46: Foci of lysis in prostatic tissue stored for 6 months. 60 mins. X 19

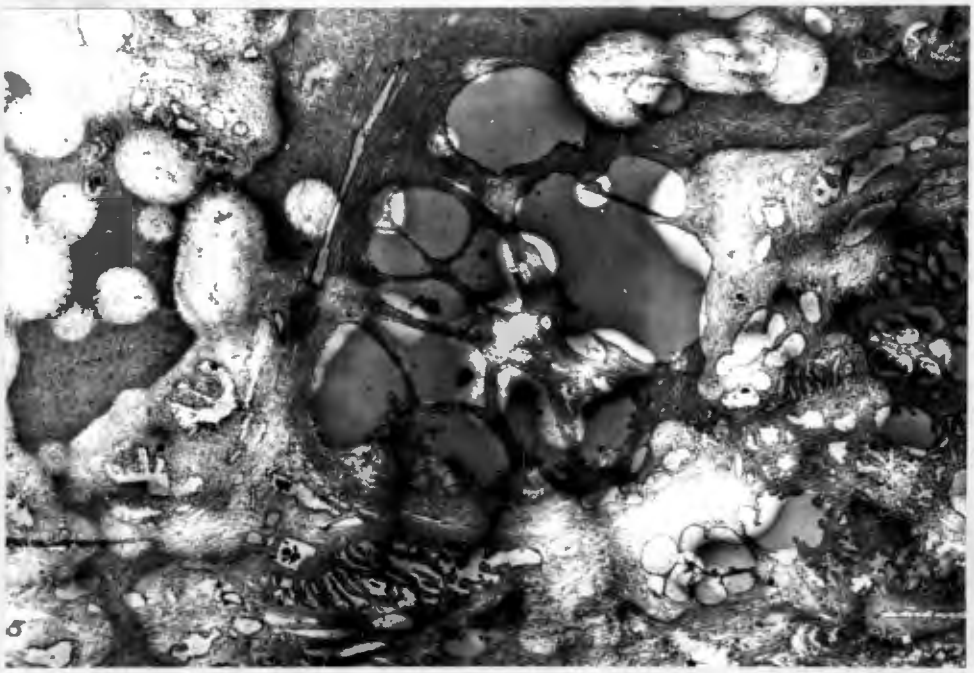
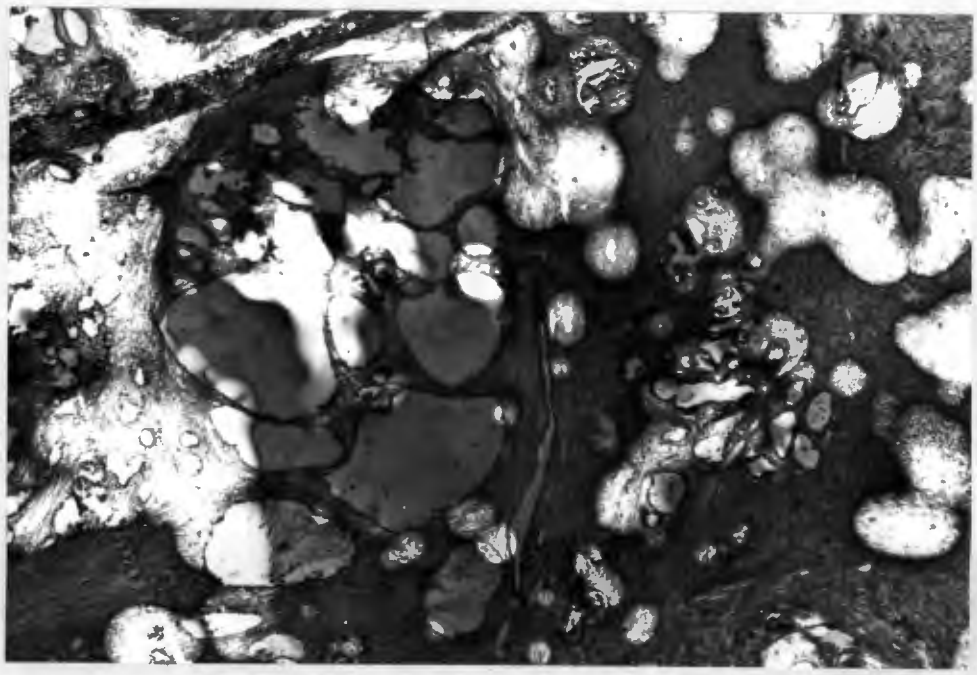


Fig. 47: Fibrin-slide made from tissue stored for 12 months. 60 mins. X 19



were immersed in buffer-saline, and one, acting as a control sample, was quick-frozen within the hour, and stored at -30°C. The second block was kept in buffer-saline at room temperature for about 6 hours, followed by storage at 4°C. for 18 hours. From the prostate of Case No. 4, a further block was taken, placed in a capped plastic container, and left unpreserved by buffer-saline at room temperature for about 24 hours. At least two fibrinolysis autographs were made from each of these tissue blocks.

That the tissue activator is extremely stable, is supported by the finding that no significant difference in the degree of activity from these samples was detected (Table 19). Figs. 48, 49 and 50 show that the distribution of fibrinolysis is comparable under the different conditions of storage, although the zones of digestion in the autolysing, unpreserved tissue tended to become confluent, and were often too diffuse to localize precisely to a particular tissue component.

TABLE 19: Comparison of Fibrinolytic Activity of "Fresh" Tissues with "Postmortem" (PM) Samples

Case No.	Method	Overall Activity	Vessels	Glands	Ducts	Secretion
1	"Fresh"	6	6	4	6	0
	"PM"	6	6	4	4	0
2	"Fresh"	6	6	4	2	0
	"PM"	6	6	6	6	0
3	"Fresh"	6	6	4	4	4
	"PM"	6	6	4	4	0
4	"Fresh"	6	6	4	4	0
	"PM"	6	6	4	4	0
	"Unpreserved"	6	6	4	4	0

Fig. 48: Fibrinolysis autograph made from "fresh" prostatic tissue. 60 mins. X 24

Fig. 49: Zones of digestion in prostate after "cadaveric" storage. 30 mins. X 24

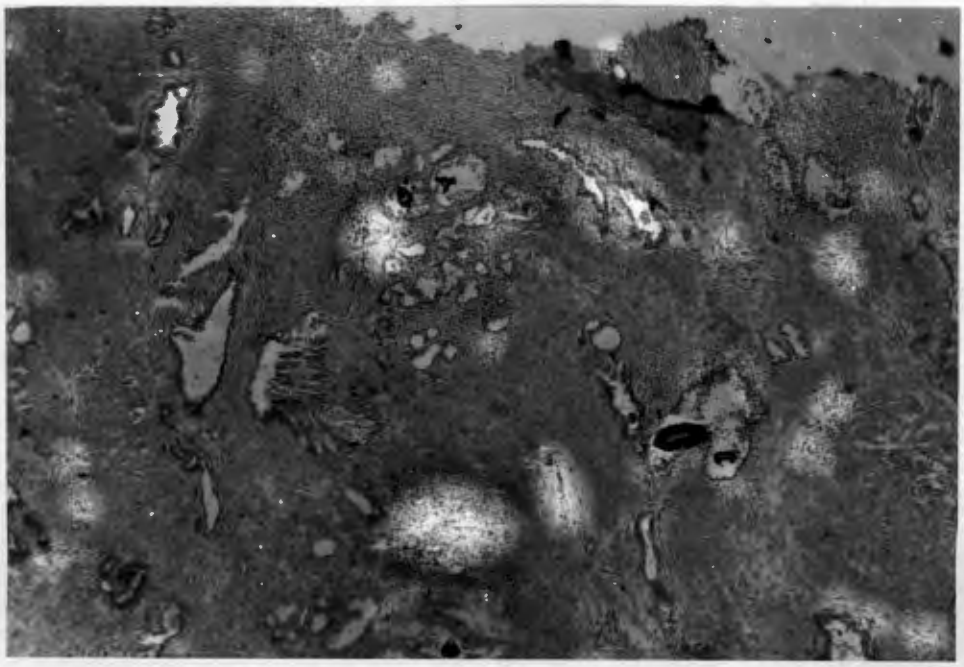


Fig. 50: Foci of activity in degenerating prostatic tissue, left unpreserved for 24 hours. 60 mins. X 24

(2) Human Fibrin as a Substrate

As prostatic epithelium shows only moderate ability to activate plasminogen, it may be that plasminogen-rich bovine fibrin is an unsuitable substrate for the semen-liquefying factor. If fibrinolysis autographs are prepared using human fibrin, it seemed likely that greater activity could be detected in the glandular epithelium. This possibility was examined using standard freeze-dried human fibrinogen*to make the fibrin film.

Tissue sections were cut from blocks taken from 5 glands removed at prostatectomy, and autographs were made using human fibrinogen, incubating the preparations for 60 minutes. A control series of autographs was made incorporating bovine fibrin. The fibrin films obtained after clotting the human fibrinogen with thrombin were stickier, less firm , and more translucent than the bovine fibrin films. The scores for the overall activity of 2 preparations from each gland were summed (Table 20).

Apart from Case No. 3, where no activity was seen in the human fibrin autographs, the overall degree of fibrinolysis from each substrate was similar. However the /

* Cohn Fraction I (cold-ethanol-precipitated)

TABLE 20: Comparison of Overall Activity from Human and Bovine Fibrin

Case No.	Human Fibrin	Bovine Fibrin
1	4	4
2	4	4
3	0	4
4	6	6
5	6	6

the zones of digestion were noticeably more numerous in the bovine fibrin preparations, (Figs. 51 and 52).

Standard freeze-dried human fibrinogen is deficient both in citrate and plasminogen, and these reasons account for the slight difference in favour of the bovine fibrin preparations.

Fig. 51: Control autograph of prostatic tissue using bovine fibrin. 60 mins. X 24

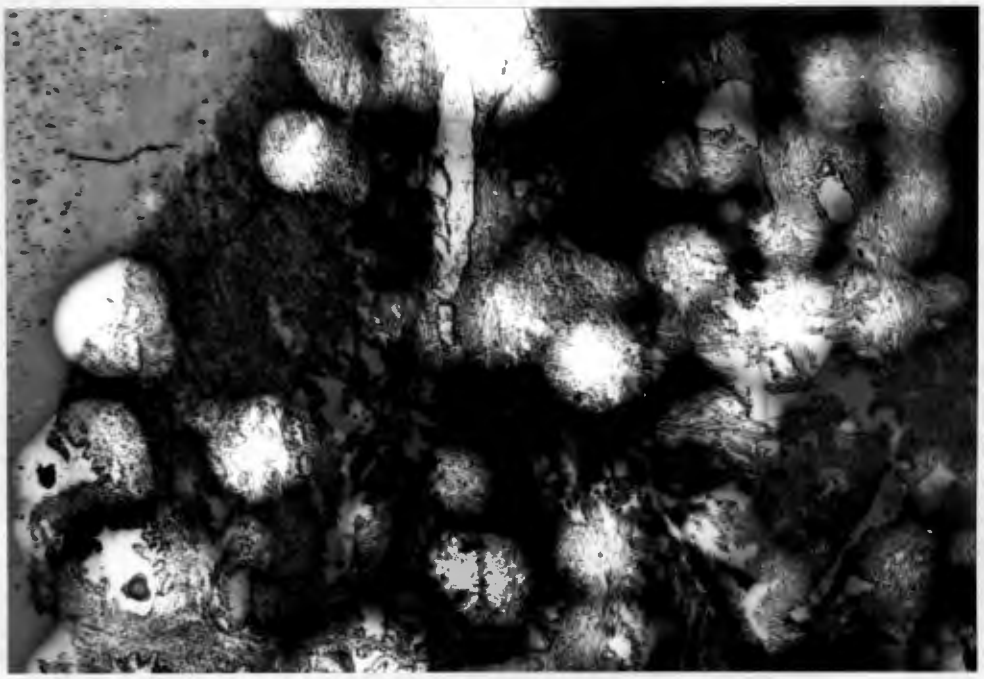


Fig. 52: Zones of fibrinolysis in fibrin-slide made with human fibrin. 60 mins. X 24

Fig. 53: No evidence of digestion when heat-denatured fibrin is incorporated in the autograph. 60 mins X 24

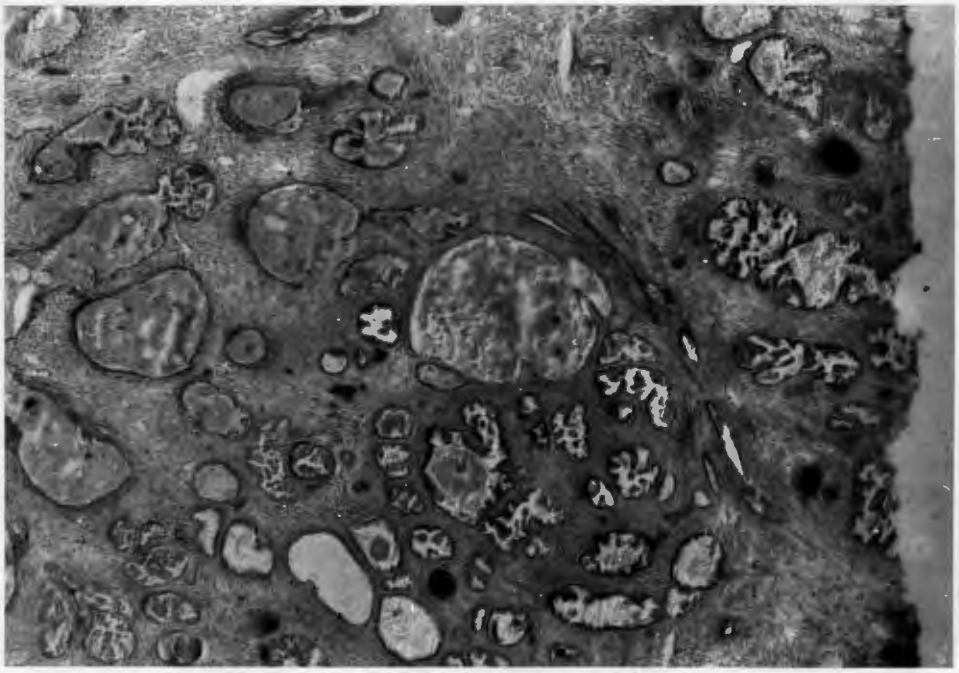


Fig. 54: Control autograph using unheated fibrin. 60 mins. X 24

TABLE 21: Fibrinolysis in Autographs made from Heated and Unheated Fibrin

Case No.	1	2	3	4	5	6	7
Heated Fibrin	0	0	0	0	0	0	0
Unheated Fibrin	6	6	6	6	6	6	6

Lassen's method of heat denaturation of plasminogen in fibrin renders the fibrin insensitive to fibrinolytic activators, while the sensitivity to proteolytic enzymes is partly retained. Even though the oven is pre-heated to 70 - 80°C. for 60 minutes, and allowing for a brief drop in temperature when the oven door is opened, traces of plasminogen can still be left in the fibrin (Brakman, 1967).

The change in character of the heated fibrin becomes apparent when it is stained with Harris's haematoxylin. The intensity of the blue stain is of a lighter shade than that obtained using unheated fibrin, and this factor can make the identification and subsequent photomicrography of pale zones of lysis a little more difficult.

Heating /

Heating also denatures the fibrin causing changes in sensitivity to some enzymes. Therefore, for these reasons, in order to distinguish between proteolytic enzymes and plasminogen activators, it would be better to use fibrinogen which is free of plasminogen (Brakman, 1967), and this preparation is now available.

(4) The Effect of Epsilon-Amino-Caproic Acid (EACA)

EACA is a potent inhibitor of plasminogen activator at a concentration of 10^{-4}M, and in greater strength, it will inhibit plasmin. It has been employed successfully in the treatment of pathological hyperfibrinolytic states. The effect of EACA on fibrinolysis by prostatic tissue was examined.

A stock solution of EACA (molecular weight of 161) of a strength 2×10^{-2}M was prepared by dissolving 0.262G powder in 100 ml. normal saline. A working solution of 2×10^{-4}M was prepared, and a sheet of bovine fibrin was soaked in a shallow bath of this solution for about 2 hours.

Fibrinolysis autographs were made with the EACA-treated fibrin, using tissue taken from 6 enucleated prostate glands. Duplicate preparations were incubated for 60 minutes, and the results were compared with the activity seen in the control slides made from standard fibrin.

In all instances, there was a reduction in the degree of fibrinolytic activity in the EACA-treated autographs, both from the blood vessels and the glands (see Table 22). The /

The number of zones of fibrinolysis was distinctly reduced in these preparations, when compared with the controls (Figs.. 55 and 56).

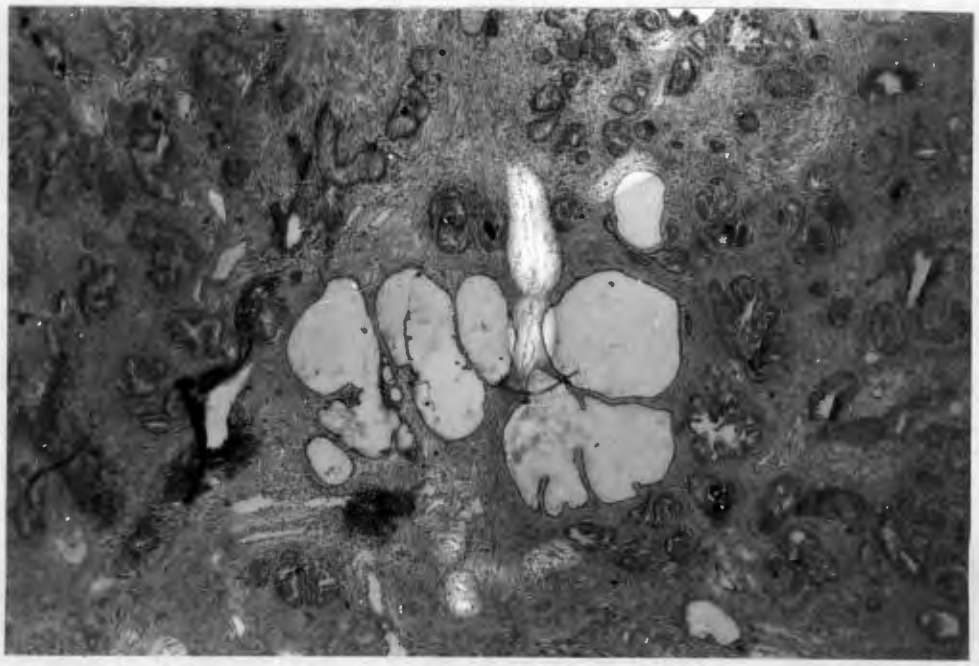
TABLE 22: Influence of EACA 2 X 10^{-4}M on Prostatic Fibrinolysis

Case No.	1	2	3	4	5	6
EACA and Fibrin	4	0	4	4	4	4
Control	6	4	6	6	6	6

As the effect of such an inhibitor is related to the concentrations of inhibitor and activator, a sheet of fibrin was soaked in EACA solution of 2 X 10^{-2}M strength. All fibrinolytic activity was completely inhibited in autographs incorporating such a fibrin film.

Fig. 55: Control autograph of prostatic tissue. 60 mins. X 24

Fig. 56: Inhibition of fibrinolysis by epsilon-amino-caproic acid. 60 mins. X 24



CHAPTER VIII

PLASMINOGEN ACTIVATOR IN TESTICULAR TISSUE

PLASMINOGEN ACTIVATOR IN TESTICULAR TISSUE

Fibrin-slide preparations were made from 13 specimens of testicular tissue, of which one sample was from a surgically excised gland. The fibrinolytic activity was assessed in arbitrary units (Table 23), and several significant features were noticed.

The overall activity was quite inconspicuous (Figs. 57 - 60). The tunica albuginea is a relatively avascular fibrous covering, and the few blood vessels seen showed only little activity after 15 - 30 minutes incubation (Fig. 57), when compared with that related to prostatic capsular vessels. The areas of digestion after 60 minutes was more striking (Figs. 58, 59). The parenchymal vessels, sparse in number, had noticeable areas of digestion, too, after the longer periods of incubation (Fig. 60).

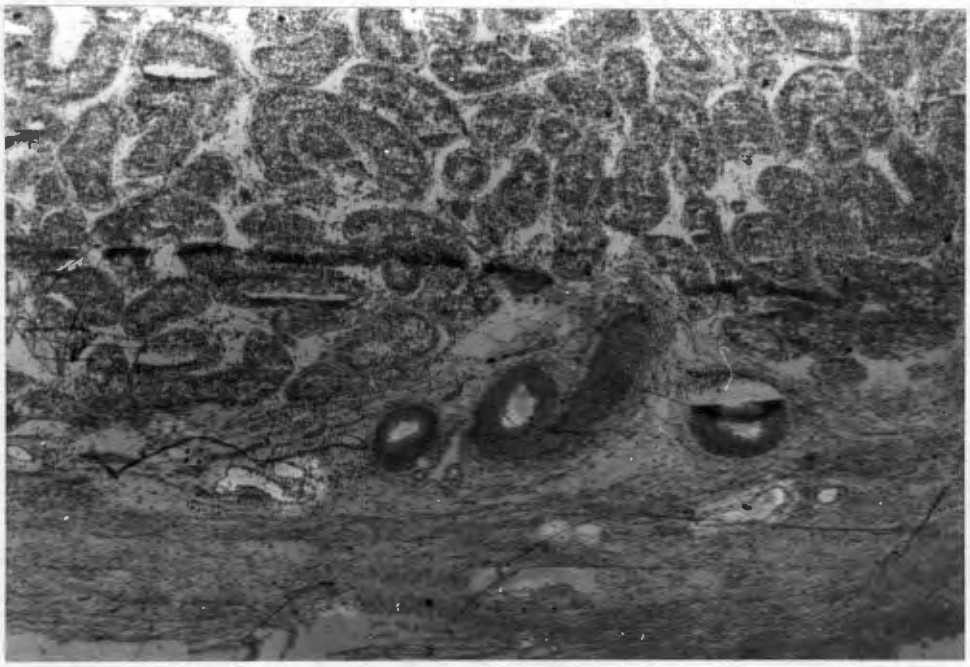
Zones of lysis related to the seminiferous epithelium (Figs. 61, 62) were most uncommon despite a careful search, and these zones were particularly related to areas of tissue disruption (Figs. 63, 64 and 65). No fibrinolytic activity whatsoever arose from the spermatozoa within the lumina of the /

of the seminiferous tubules, and none was seen related to the interstitial cells of Leydig.

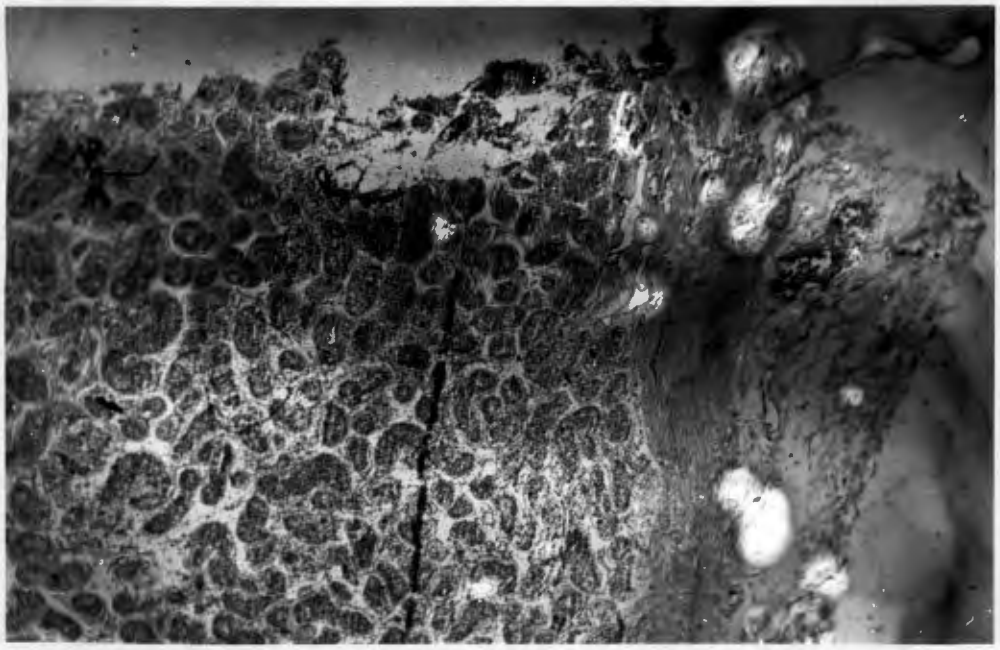
TABLE 23: Fibrinolytic Activity of Testicular Tissue Components.

Necropsy No.	Overall Activity	Vascular	Epithelium	Spermatozoa
3	10	10	0	0
4	12	12	0	0
5	12	12	0	0
6	12	12	0	0
7	12	12	0	0
8	12	12	0	0
9	16	16	0	0
10	16	16	6	0
11	14	14	3	0
12	16	16	2	0
13	14	14	0	0
14	12	12	0	0
15	10	10	1	0
Average	13	13	1	0

Fig. 57: Testis - insignificant areas of fibrinolysis are related to the blood vessels. The seminiferous tubules are quite inactive at this duration of incubation. 15 mins. X 24



60 mins. X 15



Figs. 58 and 59: Zones of digestion related to blood vessels, mainly those in the tunica. The seminiferous tubules are inactive.

60 mins. X 19

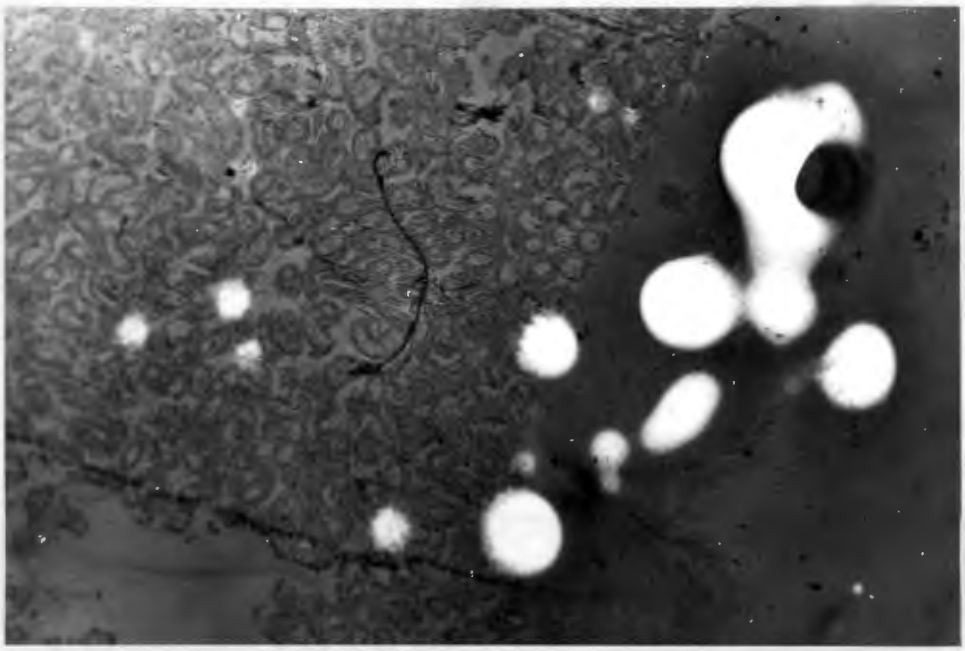


Fig. 60: Fibrinolysis arising from the parenchymal vessels. 60 mins. X 15

Figs. 61 - 65: Lysis is seen related to the seminiferous epithelium, particularly to damaged areas in Figs. 63 - 65.

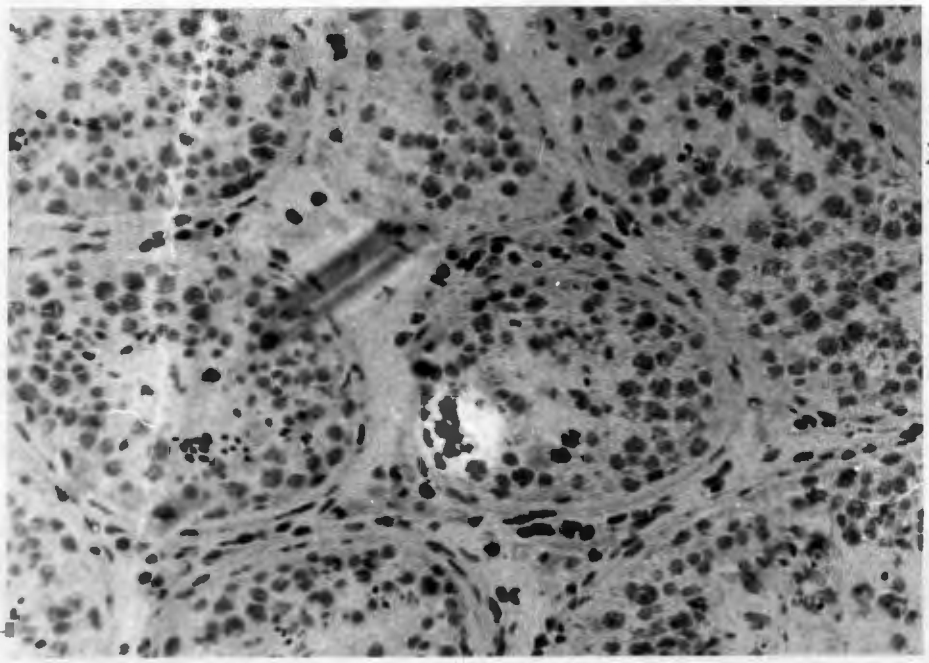


Fig. 61

60 mins

X 122

Fig. 62: 60 mins. X 95 Testis.

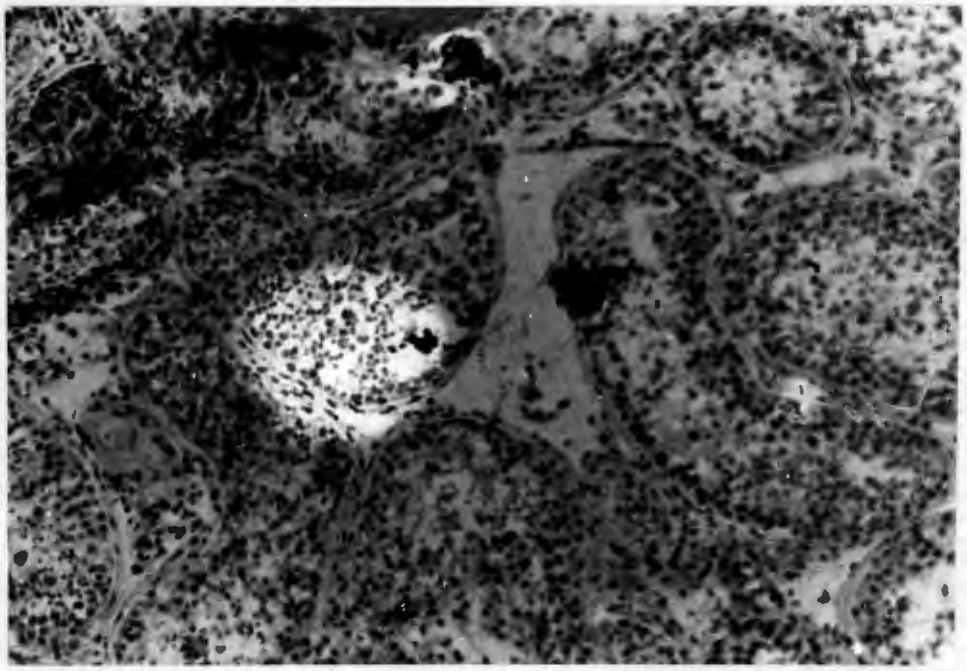


Fig. 63: 30 mins. X 122 Testis.

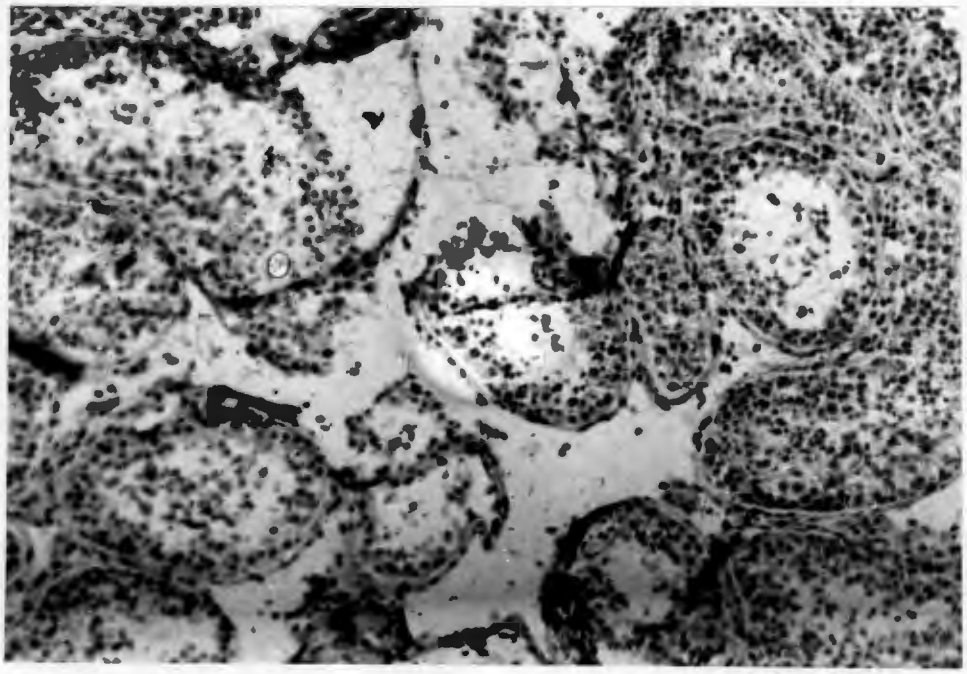


Fig. 64: 60 mins. X 152 Testis

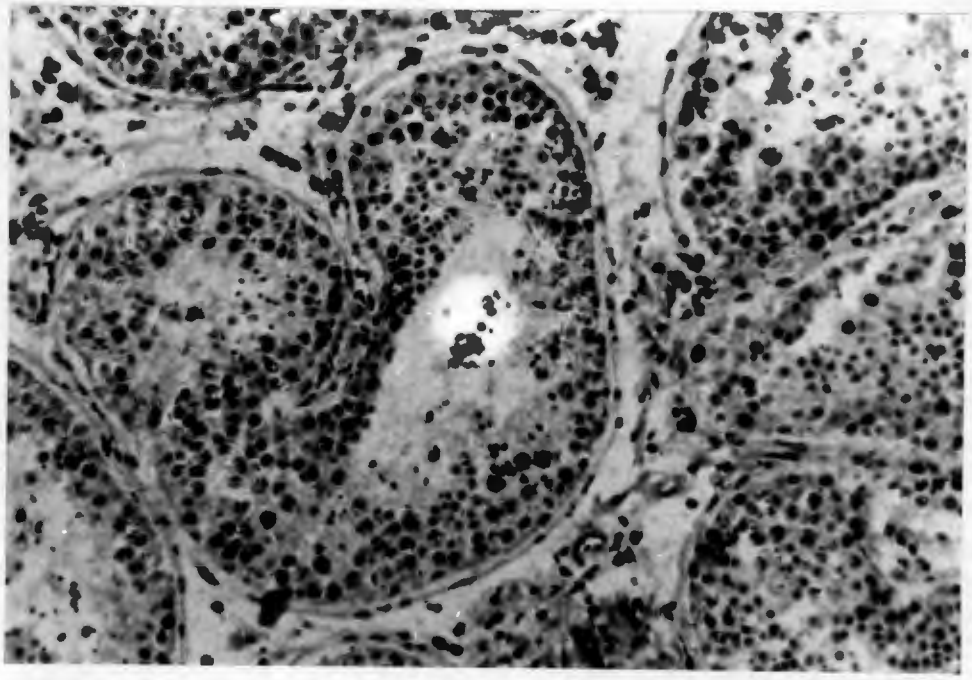
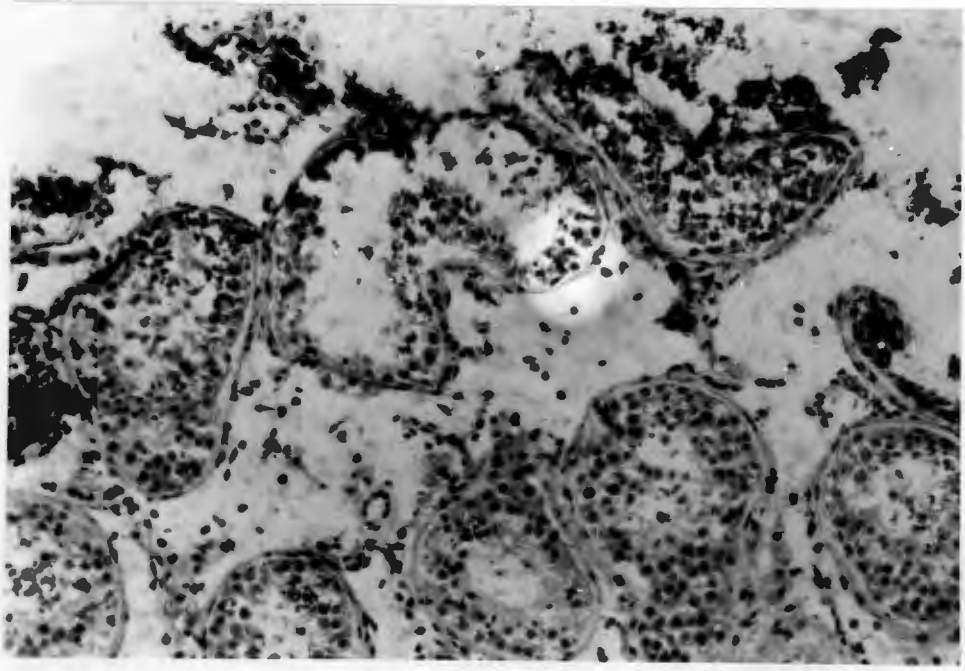


Fig. 65: 30 mins. x 95 Testis



CHAPTER IX

FIBRINOLYTIC ACTIVITY OF THE EPIDYDIMIS

FIBRINOLYTIC ACTIVITY OF THE EPIDYDIMIS

The epidydimis was dissected from each of 11 testes, and from each organ, tissue samples were taken both from the head and from the distal portion of the body. Fibrin-slide preparations were constructed, and the fibrinolytic activity arising from the head of the epidydimis was compared with that of the body of the organ, to see whether any distinction could be determined.

It can be seen that the activity of the tissue components from the proximal and distal portions of the organ appeared the same (Tables 24 and 25). Consistent and well-marked zones of fibrinolysis were related to the blood vessels throughout the sections (Figs. 66,67). Their activity was often so extensive, that the generally inactive tubules stood out prominently as blue-staining islands, encompassed by wide zones of digestion. Fig. 68 shows impressive fibrinolysis related to the endothelial lining of an arteriole, from which several of the endothelial cells are detached.

Epithelial activity was random in distribution, and only of moderate intensity (Fig. 69 and 70). The search for /

TABLE 24: Fibrinolytic Activity of Tissue Components of Head of Epidydimis

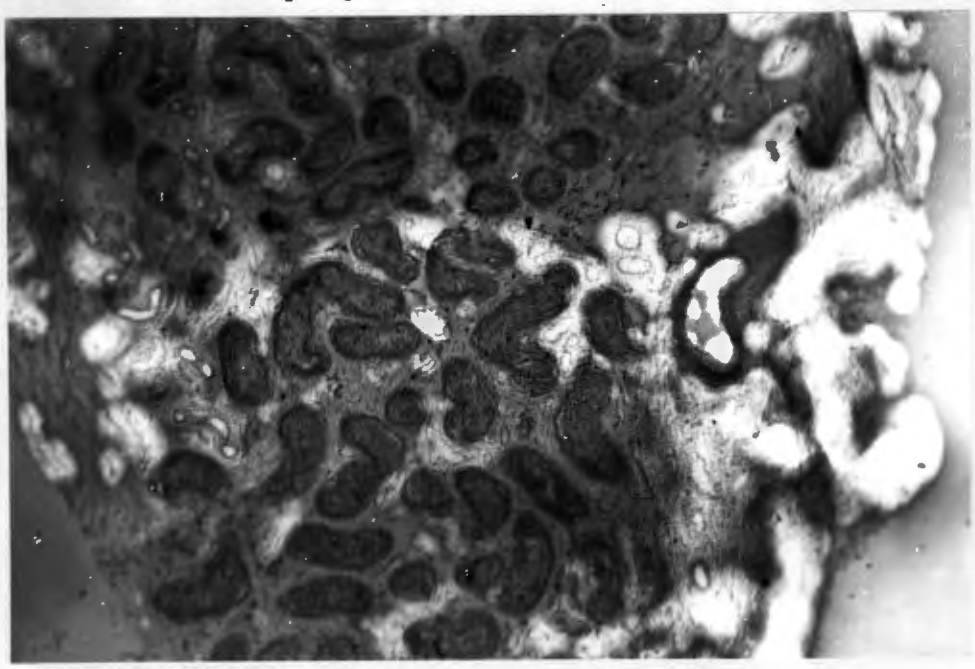
Necropsy No.	Overall Activity	Vascular	Epithelium	Secretions
5	16	16	3	0
6	16	16	3	0
7	10	10	1	0
8	16	16	2	0
9	16	16	4	0
10	16	16	10	0
11	18	18	10	0
12	16	16	10	0
13	16	16	10	0
14	16	16	7	
15	18	18	6	0
Average	16	16	6	0

TABLE 25: Fibrinolytic Activity of Tissue Components of Body of Epidydimis.

Necropsy No.	Overall Activity	Vascular	Epithelium	Secretions
5	16	16	2	0
6	16	16	6	0
7	12	12	0	0
8	16	16	2	0
9	16	16	7	0
10	16	16	9	0
11	16	16	6	0
12	16	16	00	0
13	18	18	14	0
14	16	16	0	0
15	18	18	4	0
Average	16	16	5	0

for isolated areas of epithelial activity was impeded by prominent overlap of the tubules by vascular fibrinolysis. The spermatozoa and secretions exhibited no evidence of fibrinolytic power (Fig. 71).

Fig. 66: Head of Epidydimis. 60 mins. X 15



Prominent zones of fibrinolysis localized to the blood vessels.

Fig. 67: Body of Epidydimis. 30 mins. X 15

Fig. 68: Arteriole in the head of the epidydimis, with areas of lysis related to the endothelial cells, especially where these have been detached. 60 mins. X 95

Fig. 69: Body of epidydimis, with activity related to the tubular epithelium. 60 mins. X 122

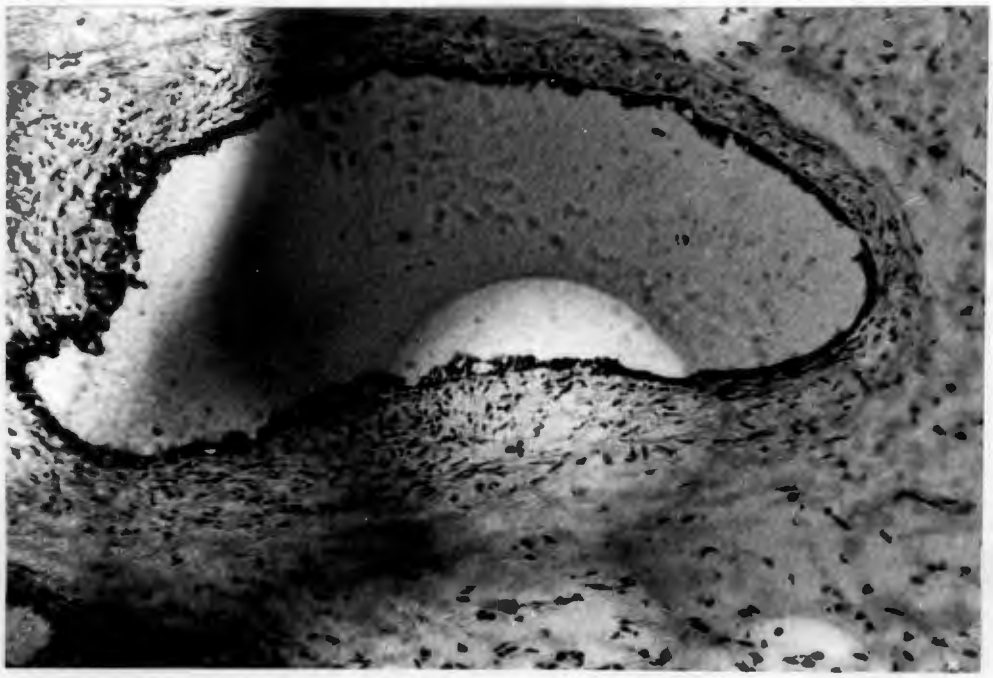


Fig. 70: Head of epidydimis showing fibrinolysis localized to a group of tubules. 60 mins. X 95

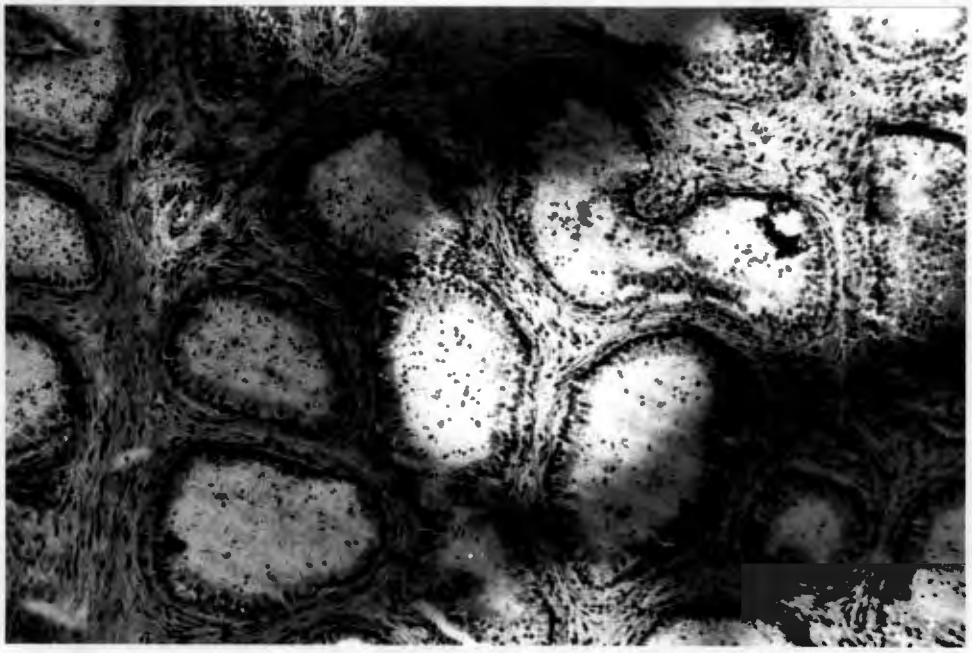
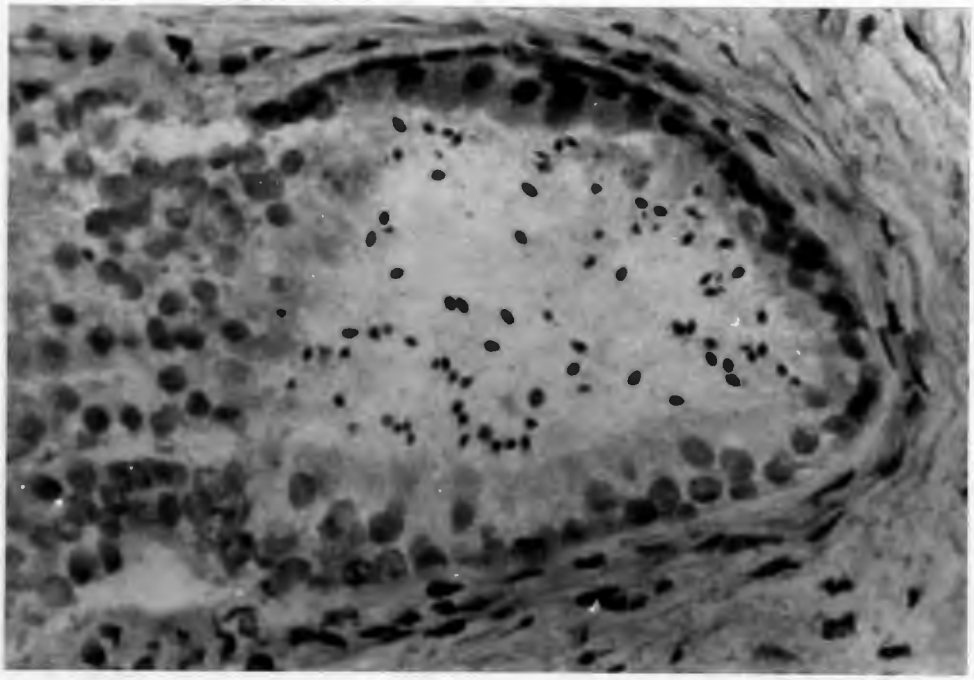


Fig. 71: Head of epidydimis - no activity related to spermatozoa or the secretion. 60 mins. X 475



CHAPTER X

LOCALIZATION OF ACTIVATOR IN THE VAS DEFERENS

LOCALIZATION OF ACTIVATOR IN THE VAS DEFERENS

In 14 cases, fibrinolysis autographs were made from samples of the main trunk of the vas deferens. Fibrin-slide preparations were made, in 9 cases, from the duct's dilated termination - the ampulla of the vas deferens. The fibrinolytic activity of these two regions of the vas was assessed in arbitrary units (Tables 26 and 27), and the results obtained for both regions were compared.

In both regions, the blood vessels exhibited uniform and impressive activity (Figs. 72 and 73), particularly those that were sinusoidal. Fig. 74 shows considerable activity related to a thin-walled vein, while the adjacent artery is inactive. Epithelial activity was random in distribution and moderate in intensity (Figs. 75, 76), but such fibrinolytic activity was especially enhanced where the cells had been torn off from their attachments (Figs. 77, 78).

Using the semiquantitative method of evaluation, it is shown that the concentration of plasminogen activator in the epithelium remains constant in the proximal and distal/

TABLE 26: Fibrinolytic Activity of Vas Deferens Tissue Components

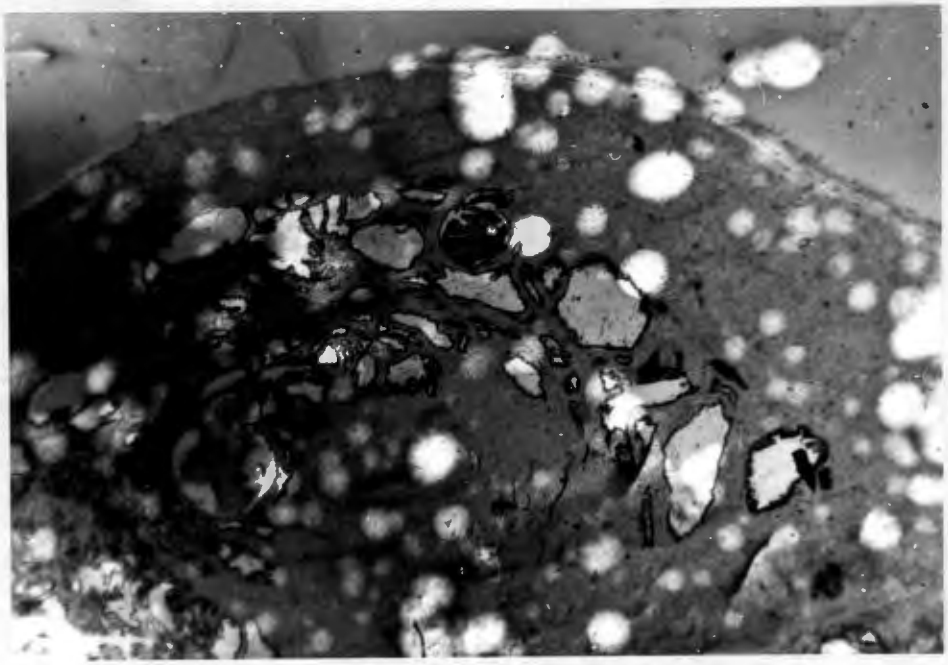
Necropsy No.	Overall Activity	Vascular	Epithelium	Secretions
1	8	8	0	0
2	16	16	12	0
3	16	16	6	0
5	16	16	0	0
6	10	10	4	0
7	16	16	6	0
8	16	16	12	0
9	12	12	6	0
10	16	16	6	0
11	16	16	6	0
12	12	12	6	0
13	18	18	10	0
14	16	16	12	0
15	16	16	6	0
Average	15	15	7	0

TABLE 27: Fibrinolytic Activity of Tissue Components of the Ampulla of Vas Deferens

Necropsy No.	Overall Activity	Vascular	Epithelium	Secretions
5	12	12	6	0
6	12	12	4	0
7	12	12	0	0
8	16	16	6	0
11	18	18	14	0
12	10	10	6	0
13	16	16	10	0
14	16	16	10	0
15	16	16	10	0
Average	14	14	7	0

distal portions of the vas deferens. Fibrinolysis was also noticeable at the edges of the sections where damage was likely. Scattered foci of digestion were related to the smooth muscle fibres in the wall of the vas (Fig. 79). No activity arose from the secretions.

Fig. 72: Vas deferens. 30 mins. x 15



Fibrinolysis related mainly to small blood vessels, but the epithelium also shows haphazard activity; Figs. 72, 73

Fig. 73: Ampulla of vas deferens. 30 mins. x 15

Fig. 74: Prominent lysis by a thin-walled vein. Adjacent artery is inactive. 30 mins. X 95

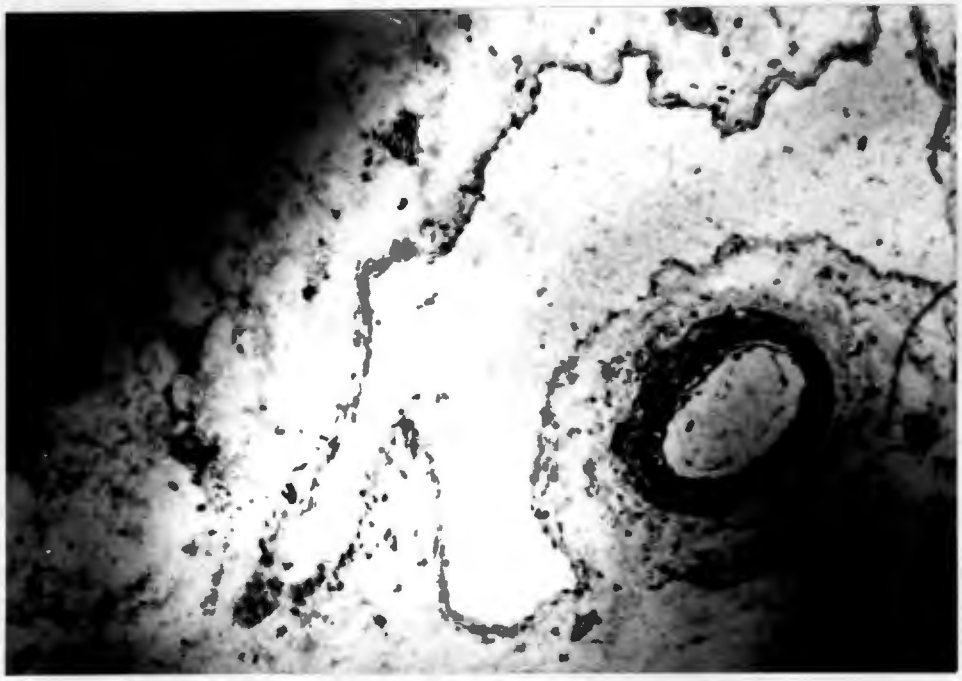


Fig. 75: Ampulla of vas deferens. Fibrin-digestion by the epithelium and subepithelial vessels. 60 mins. X 95

Fig. 76: Vas deferens. Epithelial activity. 30 mins. X 95

Fig. 77: Vas deferens. Fibrinolysis arising from disrupted epithelial cells. 60 mins. X 95

Fig. 78: Activity from a detached clump of epithelial cells, and the adjacent vas epithelium. 60 mins. X 95

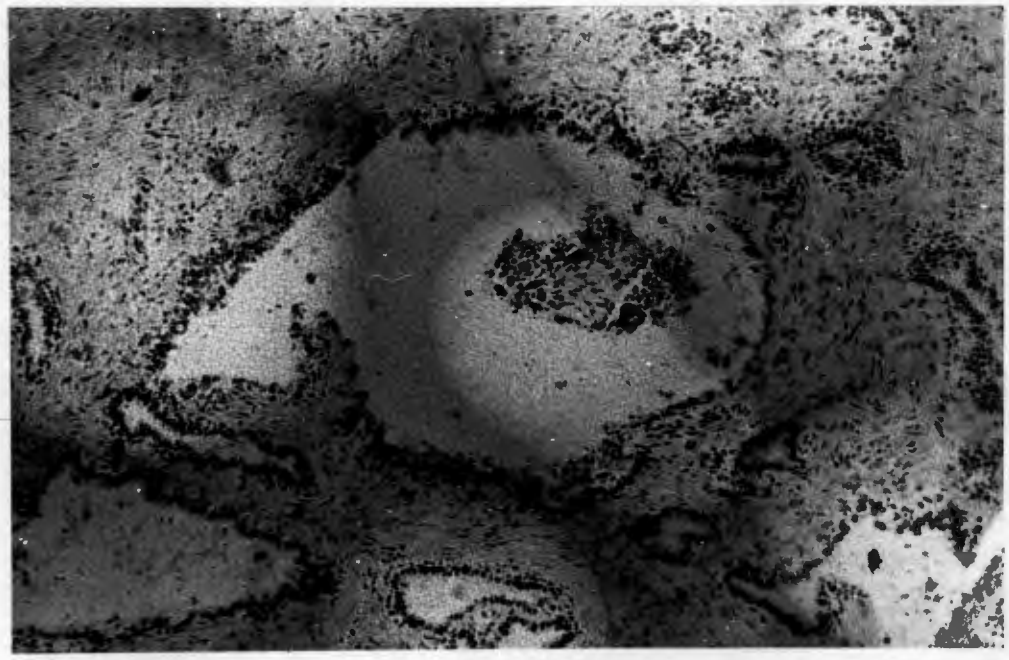
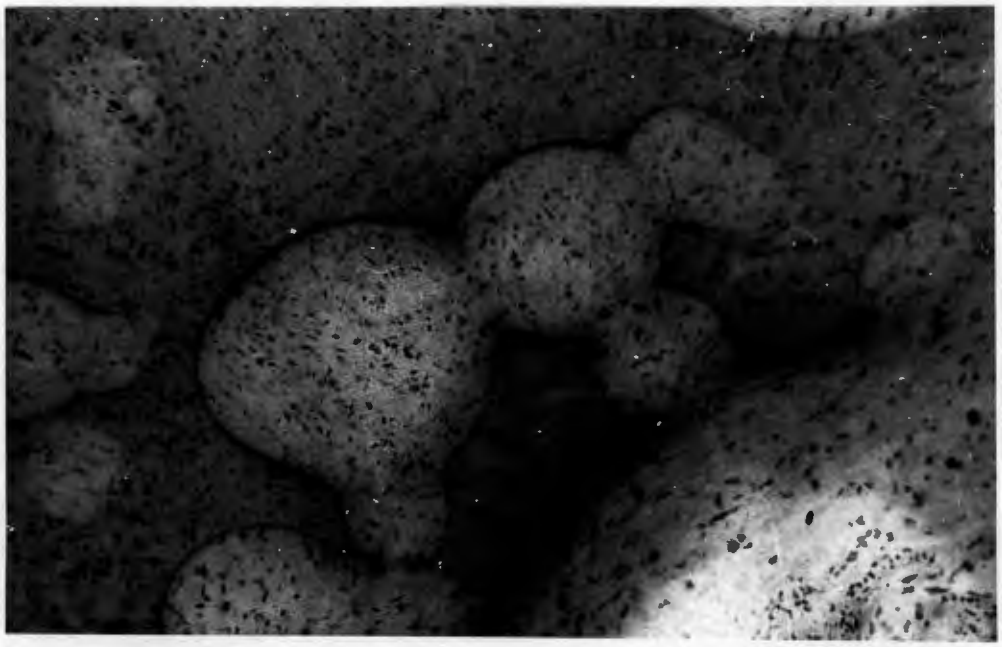


Fig. 79: Zones of digestion localized to smooth muscle in the wall of the vas. 60 mins. X 95



CHAPTER XI

PLASMINOGEN ACTIVATOR IN THE SEMINAL VESICLE

PLASMINOGEN ACTIVATOR IN THE SEMINAL VESICLE

Fibrin-slide preparations were made from the seminal vesicles taken from 14 cases. As before, the fibrinolytic activity of the tissue components in 6 autographs from each case was assessed in arbitrary units, and the results are shown in Table 28.

The blood vessels were consistently active, and the diverticula were encircled by wide bands of fibrinolysis in the fibromuscular stroma (Figs. 80, 81 and 82). However the most interesting feature was the impressive epithelial activity seen in the sections. The epithelium of the diverticula, arranged in numerous tall ridges, recesses and anastomosing folds to form an alveolar surface, often exhibited striking fibrinolysis. Foci of activity arose from this honey-combed surface, punctuating the epithelial lining of the diverticula (Figs. 80, 82).

Scattered within the lumen of each diverticulum, were many disrupted cells and fragments of cellular debris, and areas of fibrinolysis were often related to these (Figs. 83, 84 and 85). Because of the dispersed cellular debris, it was difficult to find zones of digestion related only to the/

TABLE 28: Fibrinolytic Activity of Tissue Components of the Seminal Vesicles

Necropsy No.	Overall Activity	Vascular	Epithelium	Secretions
1	12	12	12	0
2	16	16	8	6
3	16	16	8	8
5	16	16	10	2
6	16	16	10	2
7	16	16	10	0
8	16	16	16	8
9	16	16	6	0
10	16	16	10	4
11	18	18	16	6
12	16	16	16	8
13	18	18	16	10
14	16	16	16	2
15	16	16	12	0
Average	16	16	12	4

the secretions, but at infrequent intervals, such foci were noticed (Fig. 80). Careful examination of the spermatozoa within the vesicular secretion revealed that these remained inactive (Fig. 86).

Specimens of vesicular fluid were obtained at 6 necropsies, and without overmuch delay, thick smears of the fluid from each case were made on 6 coverslips. Next, fibrinolysis autographs were made, incubating half of the preparations for 60 minutes at 37°C.; the remaining number were exposed at 4°C. for 24 hours, followed by incubation at 37°C. for 30 minutes. In every sample, well-marked digestion of fibrin was produced by the vesicular fluid (Fig. 87). However, no fibrinolysis was localized to spermatozoa in the specimens (Fig. 88).

It is possible that substances in the secretory material of the vesicle may inhibit the potency of plasminogen activator in the epithelial cells. To test this idea, seminal vesicles from 3 cases were slit open and their contents were thoroughly washed out in running tap water. Fibrinolysis autographs were constructed, and the fibrinolytic activity of the tissue components was assessed (Table 29).

It/

TABLE 29: Fibrinolytic Activity of Tissue Components of Washed Seminal Vesicles

Necropsy No.	Overall Activity	Vascular	Epithelium	Secretions
1	10	10	2	0
2	12	12	12	0
3	16	16	12	0
Average	13	13	9	0

It is seen that the overall activity was only mildly diminished, and that the general pattern remained identical to the activity in unwashed vesicles. Epithelial activity still remained as striking as before (Fig. 89), confirming that plasminogen activator is firmly bound to the intact cells, and showing that removal of the vesicular fluid, containing possible inhibitory factors, had not affected fibrinolysis by the epithelium.

Fig. 80: Diverticulum of the seminal vesicle, with zones of digestion related to blood vessels, glandular epithelium and secretion. 30 mins. X 24

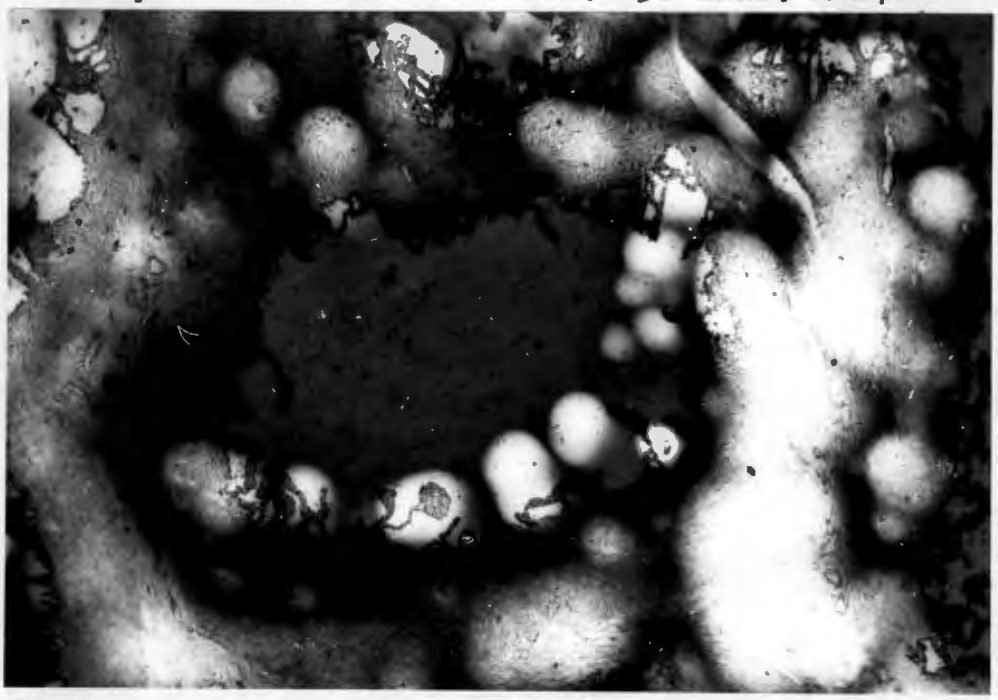


Fig. 81: Fibrinolysis occurring at the epithelial alveolar surface. 30 mins. X 95

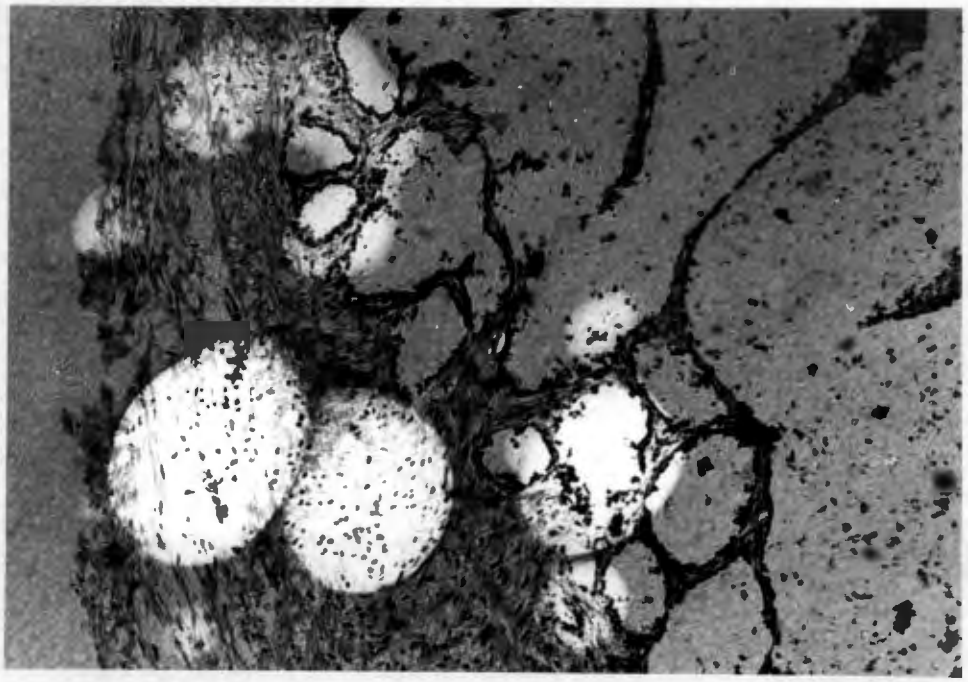


Fig. 82: Lysis produced by stromal vessels, glandular epithelium, and desquamated epithelial cells. 30 mins. X 24

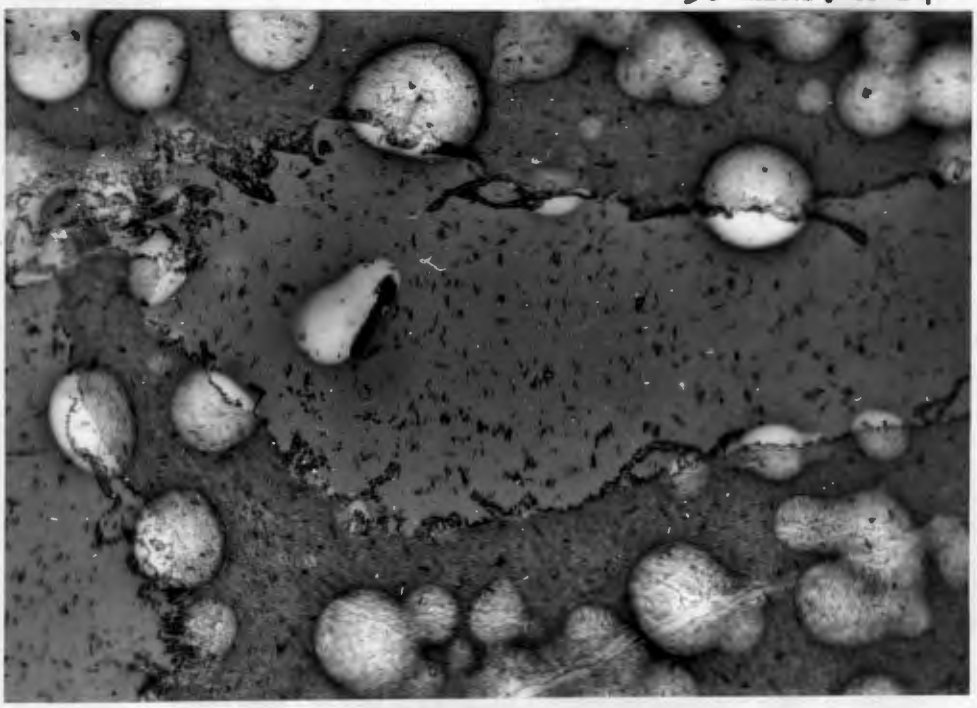


Fig. 83: Zones of digestion related to disintegrating cells. 60 mins. X 152

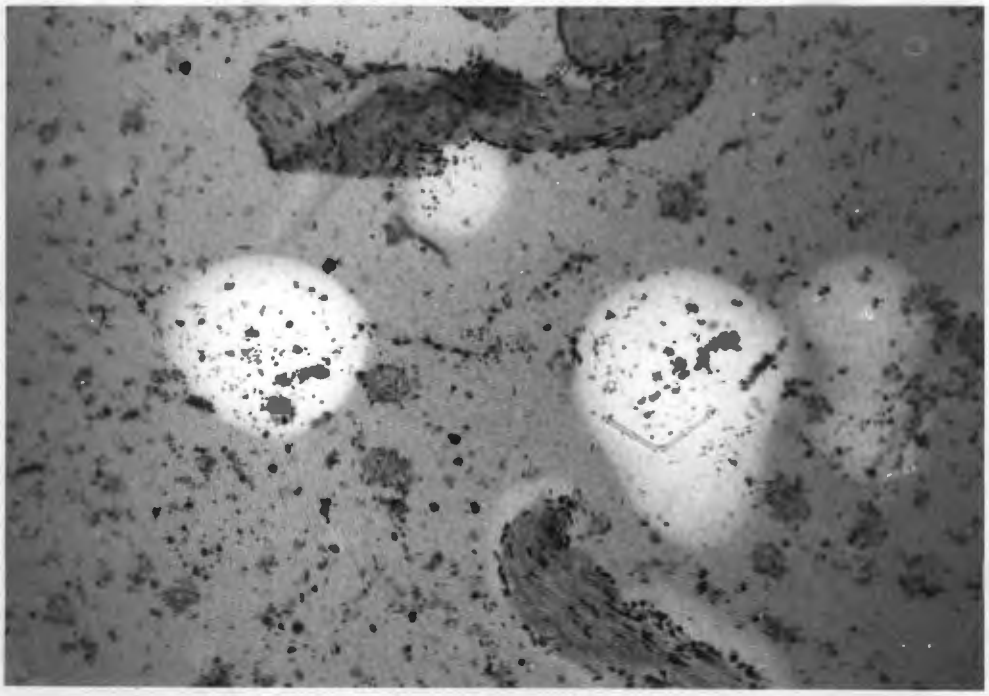
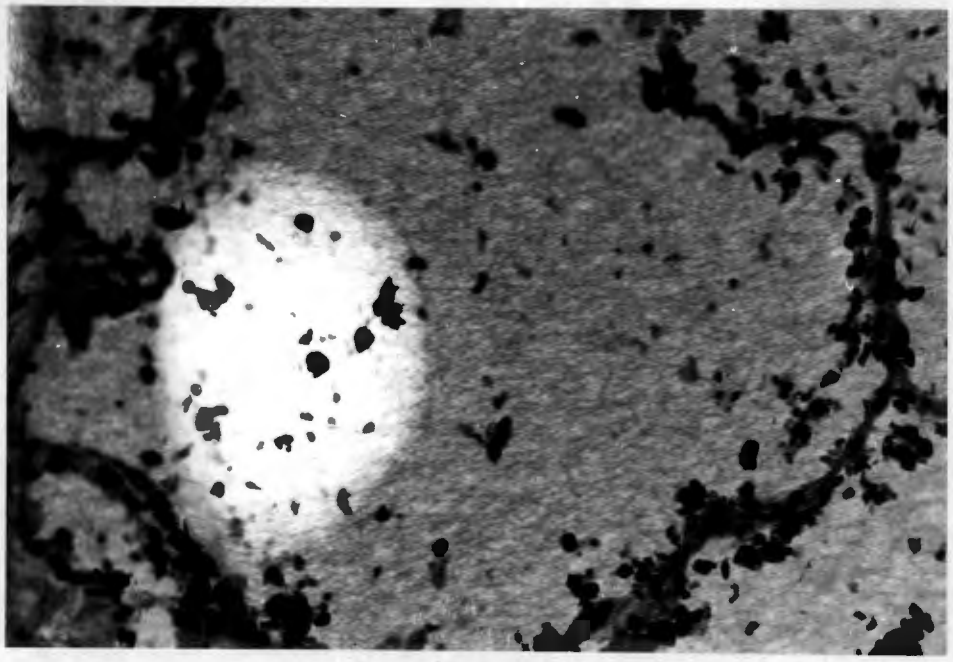


Fig. 84: 30 mins. X 608 Seminal vesicle



Wide areas of fibrinolysis related to cellular debris.

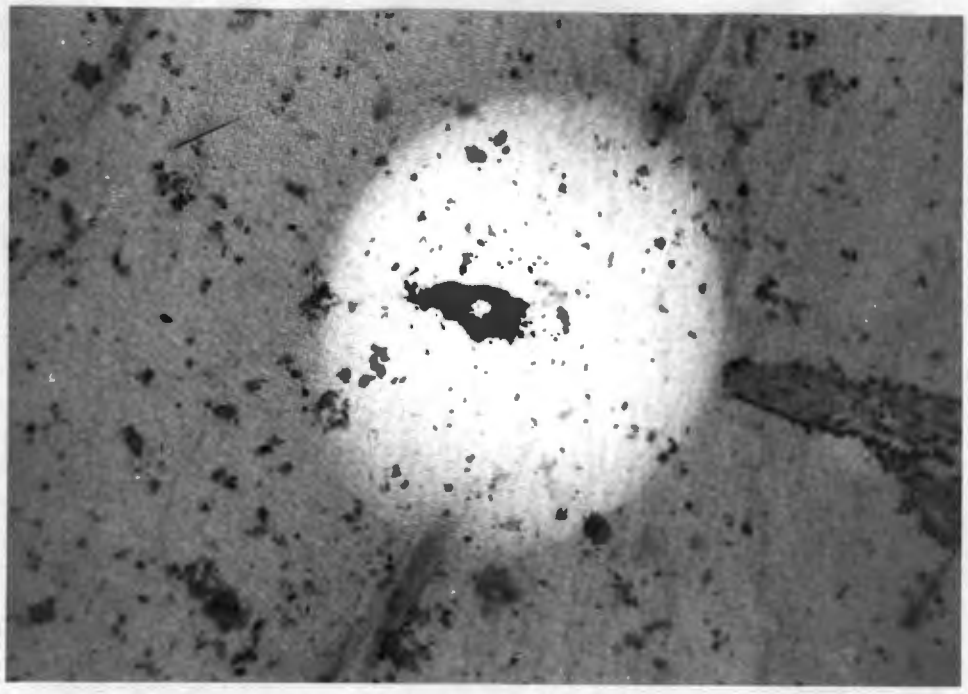


Fig. 85: 60 mins. X 152 Seminal vesicle

Fig. 86: Inactive spermatozoa in the secretion within a diverticulum. 60 mins. X 950

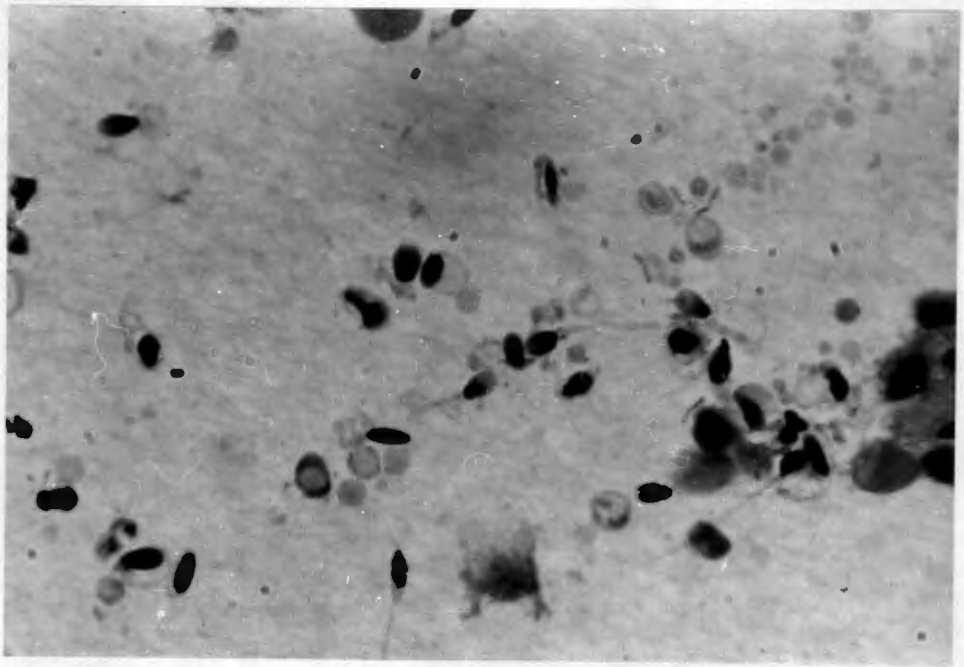


Fig. 87: Fibrin-digestion by vesicular fluid. 60mins. X 24

Fig. 88: Inactive spermatozoa within the vesicular fluid. Overnight at 4°C. X 760

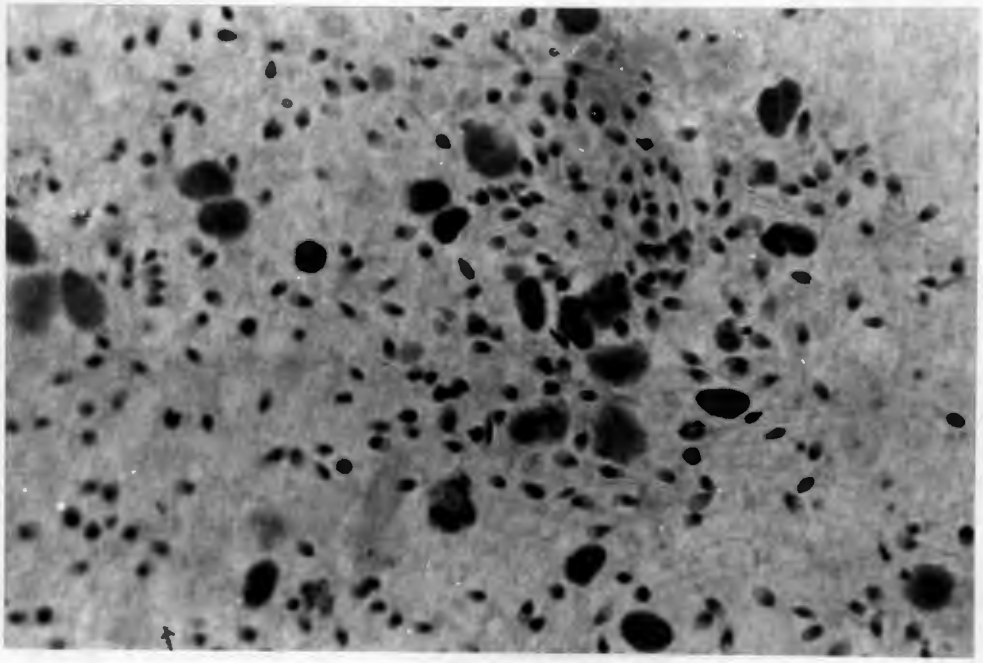


Fig. 89: Fibrinolytic activity arising from the epithelium and blood vessels of a washed seminal vesicle. 60 mins. X 95

CHAPTER XII

TOPOGRAPHY OF FIBRINOLYTIC ACTIVITY IN THE BULBO-URETHRAL GLAND

TOPOGRAPHY OF FIBRINOLYTIC ACTIVITY IN THE BULBO-URETHRAL GLAND

The secretion of the bulbo-urethral (Cowper's) glands is an additional constituent of seminal fluid. More is known about the biochemistry of their secretions in animals, where the glands can be quite large, than in man. For instance, in the boar, their thick white secretion is rich in sialo-protein which contributes to the content of mucoprotein in the seminal plasma (Mann, 1964). An "egg-yolk-coagulating" enzyme, secreted by Cowper's glands, has been found in goat semen (Roy, 1957), but this ferment is more likely to be a lipase, rather than a proteolytic enzyme. In man, the glands are small, are of the compound tubulo-acinar (alveolar) type, and it is thought that the part they play is probably very subsidiary.

Eight bulbo-urethral glands were carefully dissected out, quick-frozen, and subjected to fibrinolysis autography. The fibrinolytic activity of the tissue components was evaluated in 6 autographs from each case, and the results are given in Table 30.

In comparison with the scores achieved by tissue components/

TABLE 30: Fibrinolytic Activity of Tissue Components in the Bulbo-urethral Glands

Necropsy No.	Overall Activity	Vascular	Glands	Ducts	Secretion
5	18	18	16	16	16
6	18	18	16	16	0
7	18	18	16	16	14
8	18	18	16	16	10
11	18	18	14	6	0
12	18	18	14	6	0
13	18	18	12	12	7
14	18	18	10	0	0
Average	18	18	14	11	6

components of the male accessory glands examined previously, the blood vessels, glandular and ductal epithelium, and secretions each respectively recorded the highest levels of fibrinolytic activity. Indeed, the early 15 minute preparations (see Fig. 90) developed activity almost as maximum as that in the autographs incubated for 60 minutes.

Although the blood vessels showed the most striking and/

and consistent activity (Fig. 90), the fibrinolysis exhibited by the flattened epithelial cells lining the glandular acini was remarkable (Figs. 91 and 92). The ducts were equally active, especially the larger ducts (Figs. 93, 94 and 95). The long ducts communicating with the bulbar urethra, too, exhibited prominent fibrinolytic activity (Fig. 96).

Haphazard zones of digestion arose within the secretions (Fig. 95), but infrequently, the score of activity attained was of considerable magnitude. Foci of fibrinolysis were often related to striated muscle fibres (Fig. 97), especially where damaged (Figs. 98, 99), and occasionally to the numerous nerve fibres seen in the sections (Fig. 99).

Fig. 90: Autograph of bulbo-urethral gland showing prominent zones of digestion after 15 mins. incubation. X 24

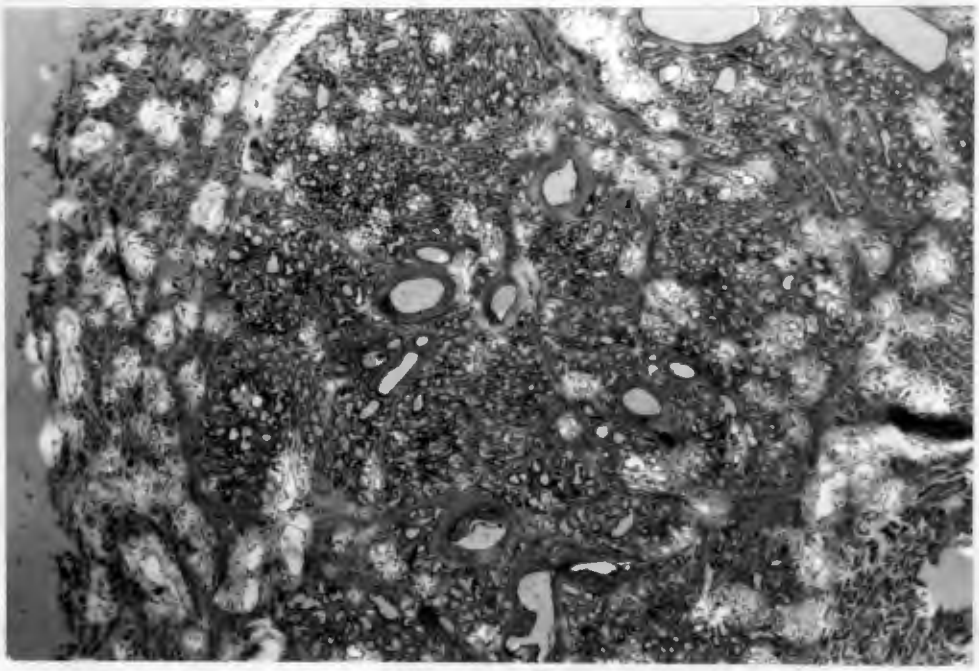


Fig. 91: Cowper's gland - lysis related to several acini. 60 mins. X 95

Fig. 92: Acini in the bulbo-urethral gland exhibiting fibrinolytic activity. 30 mins. X 95

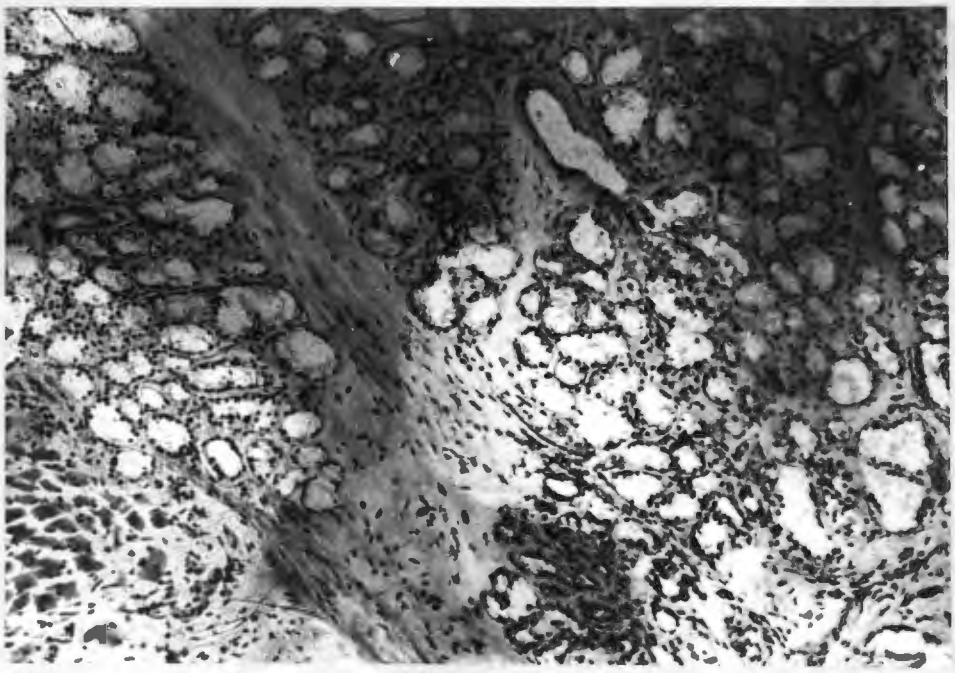


Fig. 93: Epithelial activity in a large duct, particularly where the cells are detached. 60 mins. X 24

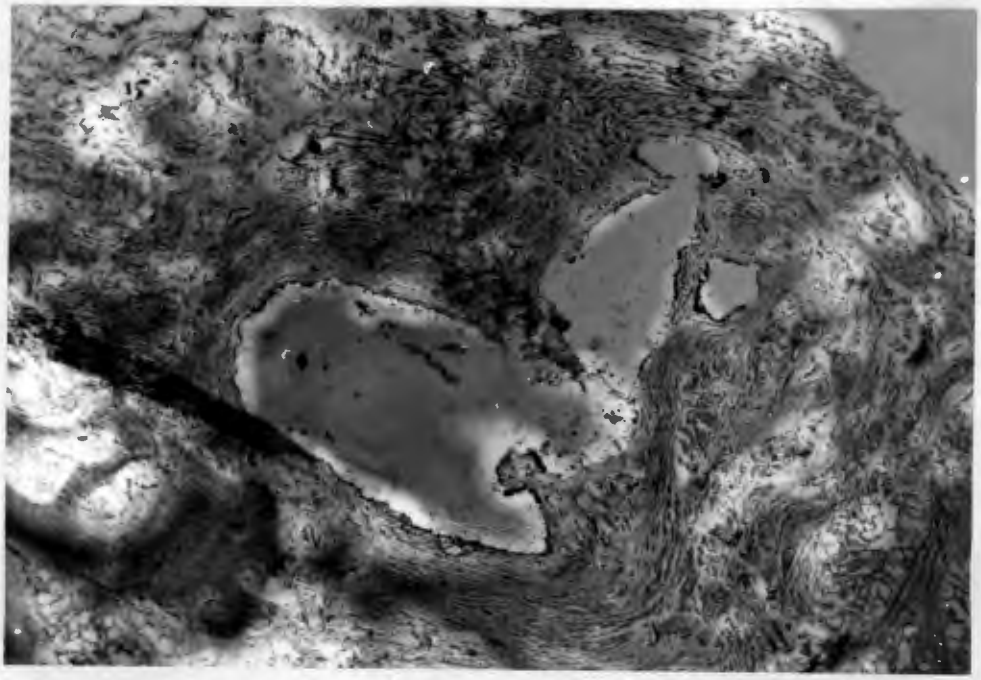


Fig. 94: Lysis related to the epithelial lining of a large duct in Cowper's gland. 30 mins. X 24

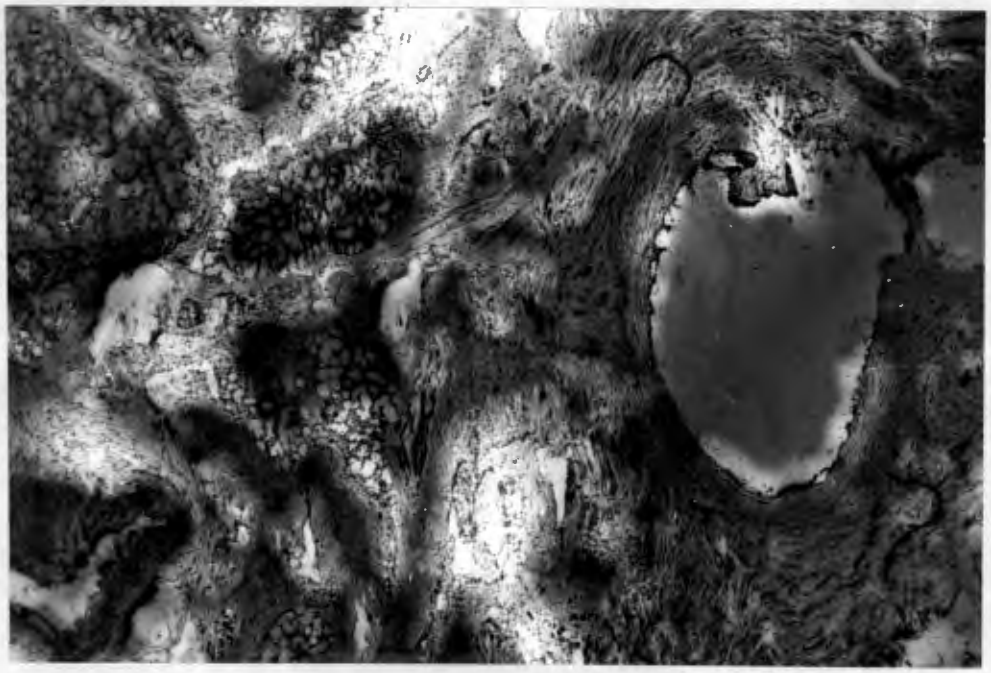


Fig. 95: Activity from the epithelium and secretions of large ducts. 30 mins. x 24

Fig. 96: Bulbo-urethral gland - striking activity from the epithelium of a long excretory duct. 30 mins. X 95

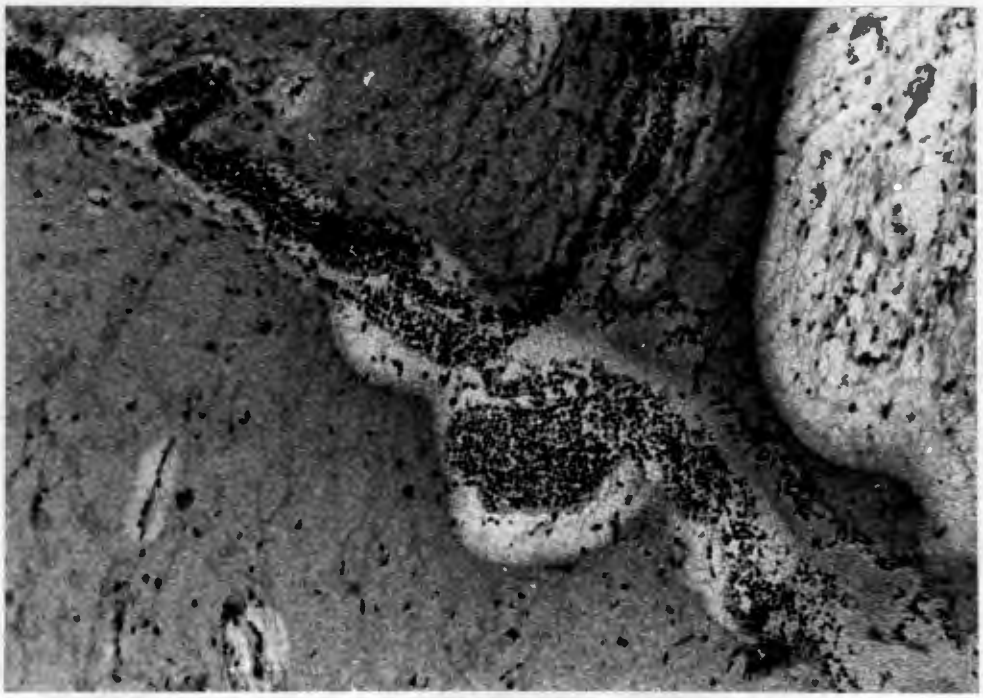


Fig. 97: Fibrinolysis related to striated muscle fibres, particularly where damaged. 15 mins. x 95

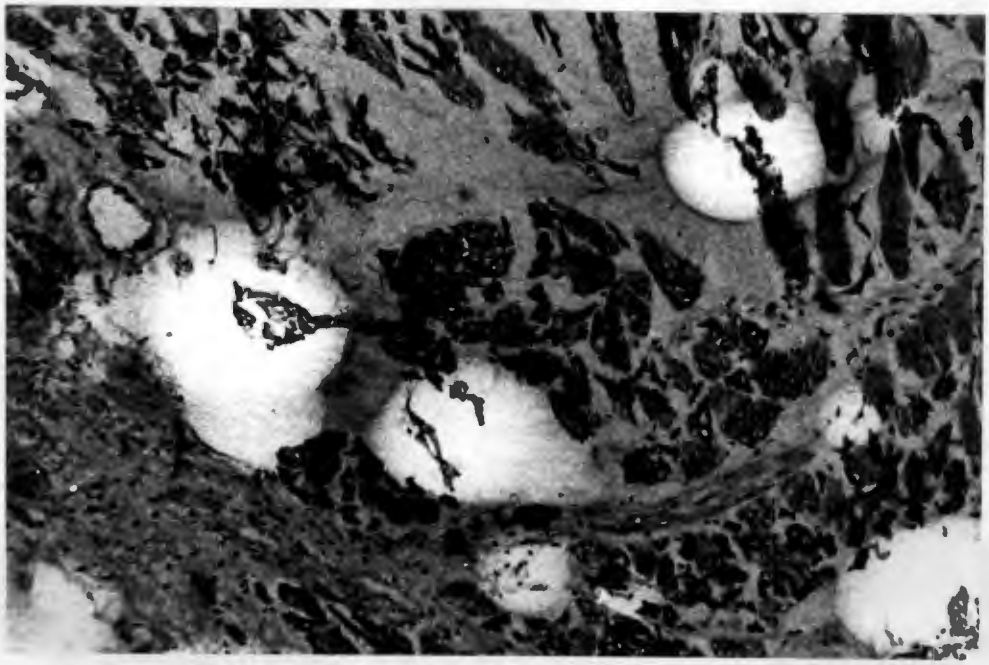


Fig. 98: Area of lysis related to a small blood vessel situated in centre of photograph. Fibrinolysis by striated muscle fibres located on the right side of the autograph. 30 mins. X 95

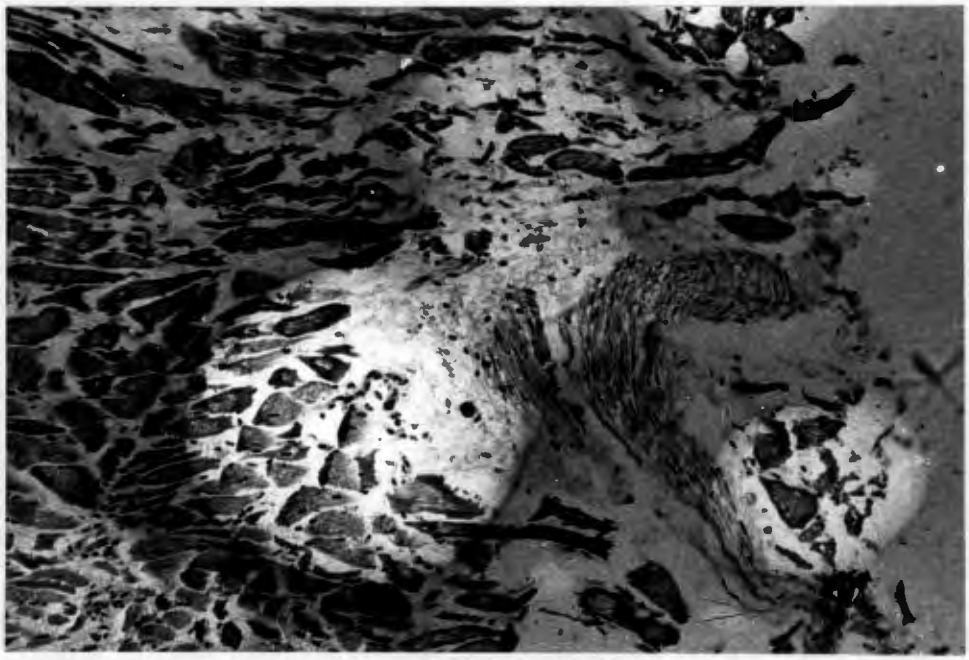


Fig. 99: Lysis arising from nerve fibres situated on the left side of the autograph. Other foci from muscle fibres. 60 mins. X 95

CHAPTER XIII

FIBRINOLYSIS BY ERECTILE TISSUE

FIBRINOLYSIS BY ERECTILE TISSUE

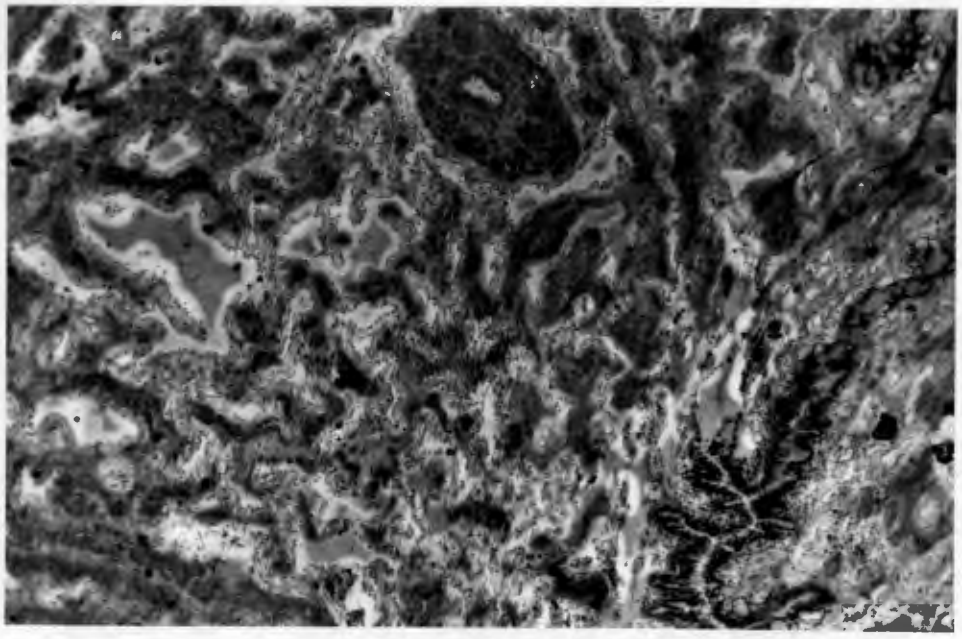
Blocks of tissue were taken from the bulb of the penis and corpus spongiosum in 12 cases, in order to obtain specimens of the penile or "spongy" urethra, and a series of fibrinolysis autographs from each case was constructed as before. Blocks of corpus cavernosum, too, were taken, so that further samples of erectile tissue could be studied.

The endothelial lining of the large venous spaces or lacunae exhibited the most outstanding fibrinolysis yet seen in these studies, and excellent examples were often observed after only 15 minutes incubation (Figs. 100, 101 and 102). Digestion was so extensive after 60 minutes of incubation, that the autographs were mostly unsuitable for the identification of microscopic detail.

Remarkable zones of lysis were constantly related to detached endothelial cells (Figs. 103, 104). Although foci of activity were often related to the cells of the urethral mucosa, it was difficult to distinguish suitable areas for photography due to overlap by gross vascular activity. Impressive areas of digestion were frequently related /

related to acini of the short and long urethral glands of Littré (Fig. 105).

Fig. 100: Corpus spongiosum. 15 mins. X 24



Fibrinolysis arising from the endothelial-lined lacunae.

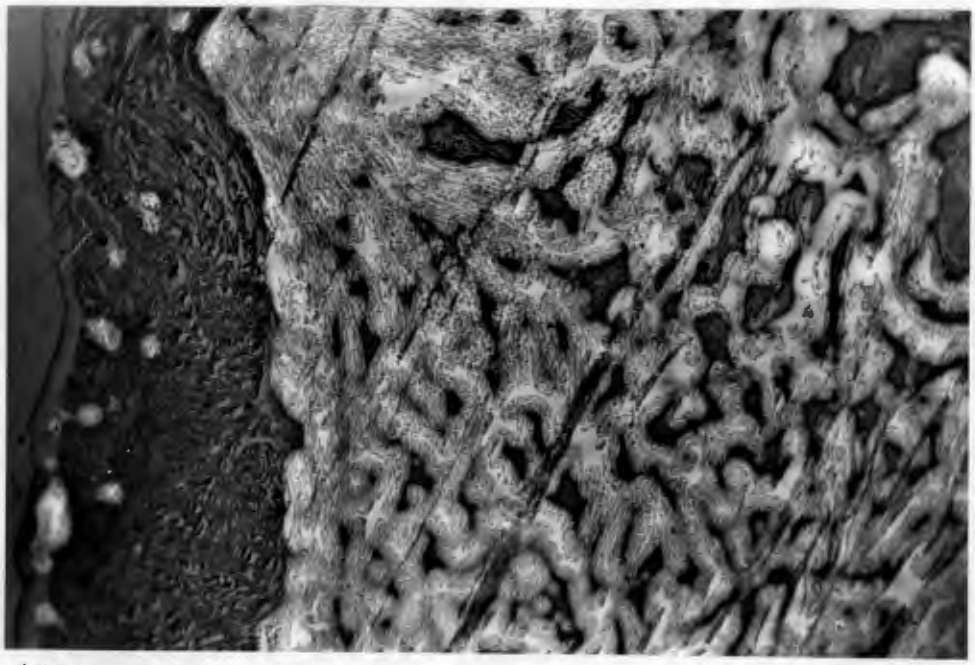


Fig. 101: Corpus cavernosum. 30 mins. X 15

Fig. 102: Bulb of penis - consistent endothelial activity. 15 mins. X 24

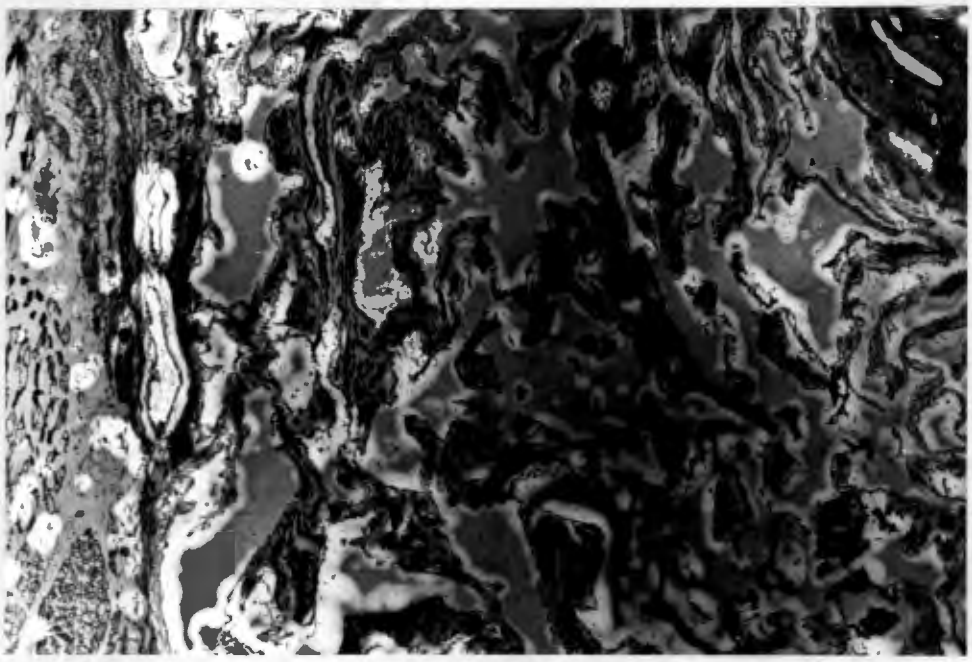


Fig. 103: Bulb of penis - striking digestion by detached endothelial cells. 15 mins. X 95

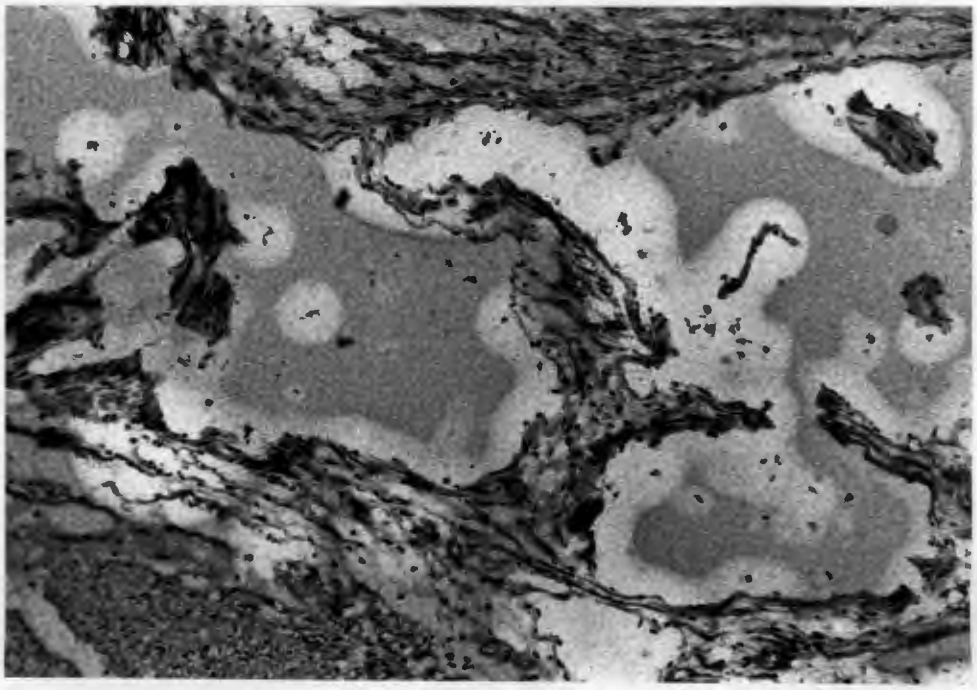


Fig. 104: Corpus cavernosum - lysis arising from endothelium, particularly where disrupted. 15 mins X 95

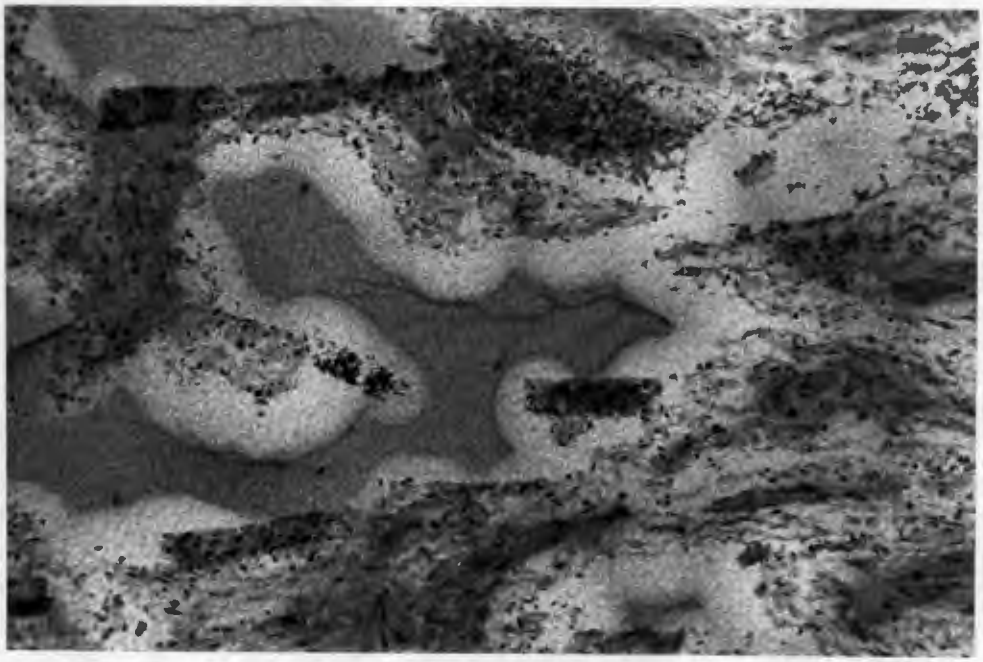
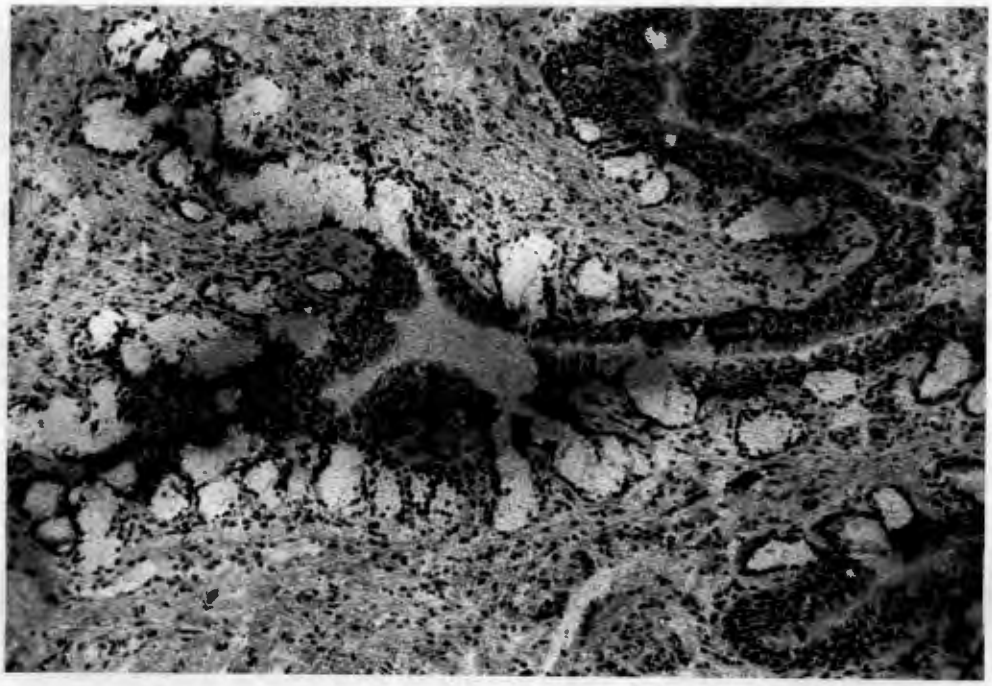


Fig. 105: Corpus spongiosum - fibrinolysis related to acini of the urethral glands of Littré. 30 mins. X 95



CHAPTER XIV

FIBRINOLYTIC ACTIVITY IN THE MALE REPRODUCTIVE ORGANS

FIBRINOLYTIC ACTIVITY IN THE MALE REPRODUCTIVE ORGANS

Human seminal plasma is comprised of the secretions produced by the male accessory organs, viz. epidydimides, vasa deferentia, ampullae of the vas, seminal vesicles, prostate, bulbo-urethral (Cowper's) glands, urethral (Littré's) glands and the preputial gland. The development of the male accessory organs, their secretory capacity, and the highly specialized nature of their glandular secretions, is directly influenced by male sex hormone.

After examining fluid obtained by prostatic massage, Huggins and his associates (1942, 1943) presumed that the source of seminal fibrinolysin was the prostate. A direct relationship was established between the number of leucocytes in prostatic secretion from patients with chronic prostatitis and the fluid's activity (Huggins and McDonald, 1944). Attempts have been made to correlate the size of the prostate (estimated by rectal examination) with the fibrinolytic activity of seminal fluid. Karhausen and Tagnon (1955) attributed the fibrinolytic activity of the prostate to a trypsin-like protease, and not /

not to the presence of plasminogen activator.

These previous findings were proved to be fallacious. Harvey (1949) showed that the fibrinolytic activity of seminal plasma was independent of the volume of prostatic fluid. Ying and others (1956) believed that only half of the fibrinolytic activity of semen could be ascribed to prostatic secretion. They suspected, too, that fluid obtained by prostatic massage would be contaminated by vesicular or urethral fluid. Furthermore, it is now known that the prostate is rich in plasminogen activator (Albrechtsen, 1957; Rasmussen, Albrechtsen and Astrup, 1958; Rasmussen and Albrechtsen, 1960a).

It has been demonstrated clearly and abundantly in these studies that the glandular epithelium of the accessory organs contains plasminogen activator in varying amounts, and it will be confirmed later that activator is responsible for the fibrinolytic activity of semen. Albrechtsen (1957) has shown the plasminogen activator content of testis to be low, viz. 25 tissue-activator units; that of striated muscle was a little higher, viz. 110 units; whilst the prostate, with 334 units, was one of /

of the richest tissues in activator concentration. It was interesting to note the presence of active striated muscle fibres within the prostate. Although not recorded in standard histological descriptions of the prostate, striated muscle fibres have often been observed within the prostatic capsule (Kost and Evans, 1964).

By means of a semi-quantitative method of fibrinolysis autography, it is shown in these studies that although vascular activity remains fairly constant (testicular vessels apart) in each of the male reproductive organs, the degree of epithelial fibrinolysis increases progressively down the genital tract (see Table 31). The concentration of plasminogen activator both in epithelium and secretion appears to be greatest in the bulbo-urethral gland, followed by the seminal vesicle, and next, the prostate. The observation that human testicular vessels were only moderately active, is similar to the findings by Tympanidis and Astrup (1968) that lysis was rarely related to the vessels of rabbit testis and epidydimis. On the other hand, the blood vessels of the rat testis and epidydimis were quite active.

TABLE 31: /

TABLE 31: Distribution of Fibrinolytic Activity in the Male Genital Tract

Tissue		Overall Activity	Vascular	Epithelium	Secretion
Testis		13	13	1	0
Epidydimis	Head	16	16	6	0
	Body	16	16	5	0
Vas Deferens	Duct	15	15	7	0
	Ampulla	14	14	7	0
Seminal Vesicle		16	16	12	4
Prostate	Anterior	17	17	11	3
	Posterior	16	16	9	2
	Vera Montanum	16	16	11	2
Bulbo-urethral gland		18	18	14	6

The seminiferous tubules were almost totally inactive, and the foci seen were usually related to areas of trauma. The seminiferous epithelium is made up by the germinal spermatogenic cells and the supporting cells of Sertoli. Throughout the studies, no evidence of fibrinolytic activity was seen related to spermatozoa. Activator has been located to the microsome portion of cells (Tagnon and Petermann, 1949a), and as the cells of Sertoli contain inclusion granules and mitochondria, and part of their function is to nourish the spermatogenic cells, it is likely that these supporting cells are responsible for the zones of fibrinolysis.

Slight amounts of activator were detected in the epithelium of the epidydimis and vas deferens. Observation of the epidydimal autographs reveal that there is often a sharp demarcation between overlapping activity from the interstitial stromal vessels and the inactive secretions within the epidydimal tubules. These observations raise a question about the concentration of fibrinolytic inhibitors in the epidydimis, and for that matter, about the presence of inhibitors in the testis and the remaining accessory glands. Indeed, an organ-specific and /

and sex-specific proteinase inhibitor has been discovered in large quantities in the vesicles and semen of many mammals, including man (Vogel, Trautschold and Werle, 1968).

It has been stated before that 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 reveals not only the secretion from the intact cells, but also the contents of disintegrating cells. These results are amply confirmed by the results of the histochemical technique. Although foci of fibrinolysis occurred at random and inconstant intervals along the epithelial surface, yet wherever cells were detached or damaged, their fibrinolytic activity was enhanced. Numerous examples have been shown where disrupted or detached epithelial cells in prostatic imprints; prostatic excretory ducts; the ejaculatory ducts in the vera montanum; seminal vesical fluid and the bulbo-urethral ducts have produced well-marked zones of fibrinolysis. Areas of digestion frequently arose from intact or detached cells of the urethral mucosa.

Only /

Only the secretions of the bulbo-urethral gland, seminal vesicle and prostate displayed fibrinolytic activity, the greatest source being the bulbo-urethral gland, with the prostate evincing the least activity. It is obvious that fluid obtained by prostatic massage will contain quantities of prostatic, vesicular, bulbo-urethral and urethral gland secretions, and such fluid will have striking fibrinolytic activity, influenced by the trauma of massage.

Studying the localisation of activator in the cornea and conjunctiva by means of fibrinolysis autography, Pandolfi and Astrup (1967) reported that injury to the epithelial cells caused the release of plasminogen activator, and the more damaged the cells were, the greater was the fibrinolytic response. The present studies confirm these previous observations that the degree of lysis is proportionate to the severity of the injury.

As in the blood stream, where there exists a fine balance between coagulation and fibrinolysis, so in the urinary tract, where identical systems exist, the fibrinolytic mechanism maintains the patency of the tube system by /

by digesting fibrinous deposits (Astrup and Sterndorff, 1952a; Ladehoff, 1960; Charlton, 1966). A further example of fibrin-deposition obstructing a narrow duct is the case described by Kussmaul (1879) in which Stensen's duct of the parotid was obstructed.

In the male genital tract, thromboplastic and fibrinolytic activators are released into the fluid from intact secreting cells and disintegrating cells. The potential build-up of fibrinous deposits is prevented by the phenomenon of fibrinolysis, and the tube system remains patent. Where the fibrinolytic mechanism fails, I suggest that fibrinous deposits will collect, and in the case of the prostate, lead to the formation of corpora amylacea (see Chapter VI).

No similar bodies have been observed in the other accessory glands, particularly the seminal vesicle, which should be liable to the formation of fibrinous bodies, when considering that it secretes a fibrinogen-like protein. Indeed, vesicular fluid is rich in protein, is viscous, and stasis is favoured by the intricately interlaced epithelial folds. However, it has a higher level of fibrinolytic activity than the prostate, which prevents coagulation. Formation /

Formation of cheese-like lumps has been observed in the vesicles of guinea-pigs and rats (Hunter, 1786). The impressive activity of the bulbo-urethral glands, together with their relatively simple gland and duct system, would preclude the formation of fibrinous plugs.

By studying autographs made with unheated and heated bovine fibrin, the finding by Ying and others (1956) and Rasmussen and Albrechtsen (1960a), that no plasmin is present in the prostate has been confirmed. The latter workers also detected pro-activator in saline extracts of prostatic tissue, which they thought came from the blood in the prostate, and not the parenchyma. They found two types of activator, of which one was stable tissue activator, and the other was thermolabile at acid reaction and identical to activator found in the blood. No lysokinase or trypsin-inhibitors were discovered.

In order to complete the story of the fibrinolytic mechanism in the secretory epithelium, further studies would be required to search for the presence of pro-activators and lysokinases, and to evaluate the influence of fibrinolytic inhibitors.

CHAPTER XV

LOCALIZATION OF ACTIVATOR IN SEMINAL FLUID

LOCALIZATION OF ACTIVATOR IN SEMINAL FLUID

It has been shown in the previous studies, that the source of activator in human seminal fluid is the epithelium of the male genital tract, particularly that of the bulbo-urethral glands, seminal vesicle and prostate, in that order of importance. It seemed relevant to the previous studies to confirm the presence of activator in seminal fluid and to determine whether spermatozoa contained fibrinolysin.

Six specimens of seminal fluid were stored at $4^{o}C.$ until they could be examined within 12 hours after ejaculation. Microscopic examination of the fluid determined the sperm density and ascertained that most of the spermatozoa became motile when the fluid had been gently warmed. A sample of fluid was taken from each specimen, centrifuged to separate the cells from the plasma, and both the deposit and the supernatant fluid were reserved. Microscopic examination confirmed that the samples of seminal plasma were cell-free. The spermatozoa were re-suspended in 5.0 ml. Ringer-lactate solution, and washed three times with the same volume of fluid.

Small drops of semen were placed on films of unheated and heat-treated bovine fibrin, and incubated at 37°C. for 60 minutes. The fibrinolytic activity in duplicate autographs was assessed in arbitrary units, and the results are given in Table 32. Impressive zones of digestion were seen in the unheated fibrin (Fig. 106) and in the heated fibrin (Fig. 107), demonstrating that seminal fluid contains plasminogen activator and plasmin in large amounts. In the small number of cases examined, it is confirmed that the sperm density bears no relationship to the fibrinolytic activity of seminal fluid. It has already been demonstrated that the fibrinolytic activity (Harvey, 1949; von Kaulla and Shettles, 1953) and the viscosity (Tjioe and Oentoeng, 1968) of semen is not related to the sperm count.

Next, autographs were made using drops of seminal plasma, and the areas of fibrinolysis obtained were as large (Fig. 108) as those made by semen. Specimens of seminal fluid were obtained from two vasectomy cases. Microscopic examination of the fluid, consisting chiefly of prostatic, vesicular and bulbo-urethral gland secretions, confirmed that these specimens were sperm-free. Autographs /

Autographs made with such fluid revealed striking zones of lysis (Fig. 109). Huggins and Johnson (1933) have determined that the chemistry of semen was not appreciably altered by vasotomy, which eliminates testicular and epidydimal secretions.

Wet smears of washed spermatozoa were made on coverslips and allowed to dry. Fibrinolysis autographs were constructed from these, exposed at 4°C. for 24 hours, and then incubated at 37°C. for 30 minutes. No fibrinolytic activity was seen related to the spermatozoa (Figs. 110 and 111), and one small clear zone in the fibrin was thought to be an artefact (Fig. 110). Flat epithelial cells occasionally exhibited fibrinolysis (Fig. 111).

Specimens of cervical mucus were obtained from patiants undergoing gynaecological procedures. Washed spermatozoa from two cases were resuspended in 5.0 ml. Ringer's solution, and each suspension was thoroughly mixed with a sample of cervical mucus. The mixtures were incubated at 37°C. for 30 minutes, and from these, a series of autographs were made, exposing the preparations at 4°C. for 20 hours, followed by incubation at 37°C. for 45 minutes. Fibrin-

olysis /

Fibrinolysis autographs were prepared from a sample of vaginal fluid obtained from a patient attending for post-coital examination.

In both the spermatozoa treated with cervical mucus (Fig. 112) and those in postcoital fluid (Fig. 113), no evidence of fibrinolytic activity was seen. Figs. 114 and 115 show striking zones of fibrinolysis related to epithelial cells in the female genital fluids. Indeed, vaginal epithelial cells have been found to be rich in plasminogen activator (Astrup, Henrichsen, Tympanidis and King, 1967).

Clermont, Glegg and Leblond (1955), and Hathaway and Hartree (1963) were able to damage or dissolve the acrosomes by treating spermatozoa with a 0.0125 - 0.25N solution of sodium hydroxide, so releasing complex biochemical compounds and enzymes. The acrosomes could also be damaged by sodium carbonate solutions, CTAB, or by shaking up spermatozoa with glass beads. When the sperms had been stained with Giemsa or P.A.S., such disruption was visible at microscopic examination. It may be that damage to the cell membrane or acrosome can release a lytic factor, the so-called "zona lysin". If this factor is a tissue activator /

activator or a plasmin-like enzyme, its presence will be revealed by fibrinolysis autography. The following experiment was therefore conducted to test this idea.

Washed spermatozoa were re-suspended in 10 ml. Ringer's solution. Several autographs were made from wet smears of the suspension, to act as controls, and these were exposed at 4°C. for 18 hours, followed by incubation at 37°C. for 45 minutes. The suspension was now divided into two halves. On the assumption that ice crystals would damage the cell membrane, one portion, in a plastic test-tube was subjected to repeated freezing and thawing by plunging the test-tube into dry ice-acetone mixture, and alternately, into warm water. Autographs incorporating the spermatozoa were exposed overnight at 4°C., followed by incubation at 37°C. The second portion was mixed with an equal volume of 0.125N sodium hydroxide solution, and incubated for 45 minutes at 37°C. A series of wet smears, taken from the suspension, was allowed to dry. Half of these were stained with Harris's haematoxylin, and the other half were stained with Giemsa, so that the effect of alkali on the spermatozoa could be observed. A set of fibrinolysis autographs were made as before.

Fig. 117 /

Fig. 117 shows damaged spermatozoa following treatment with sodium hydroxide, and then stained with Giemsa. Spermatozoa treated by freezing and thawing (Fig. 116), or with sodium hydroxide (Fig. 118) showed no evidence of fibrinolysis.

TABLE 32: Fibrinolytic Activity of Seminal Fluid

Case No.	Sperm Density millions/ml.	Activity Unheated Fibrin	Activity Heated Fibrin
1	52	6	6
2	143	6	6
3	136	6	6
4	75	6	6
5	14	6	6
6	105	6	6

Fig. 106: Fibrinolysis of unheated bovine fibrin by semen. 60 mins. X 5

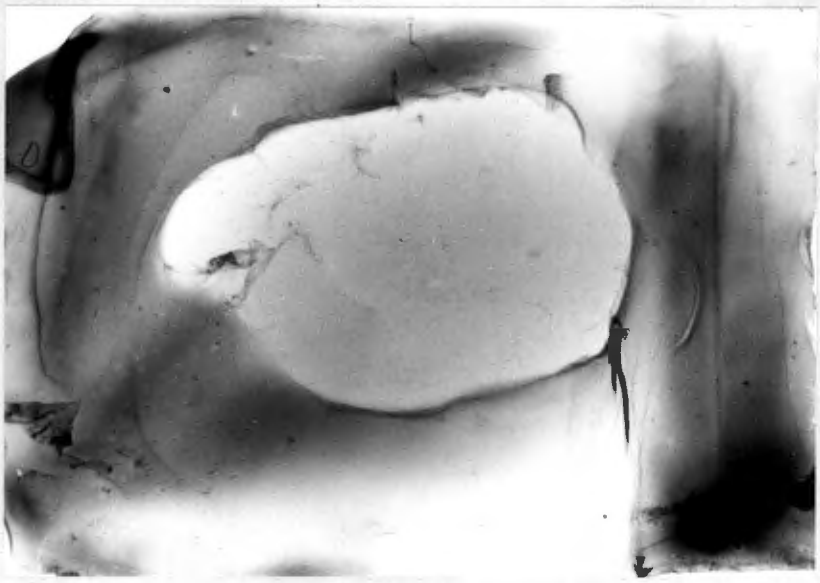


Fig. 107: Digestion of heated bovine fibrin by semen. 60 mins. X 5

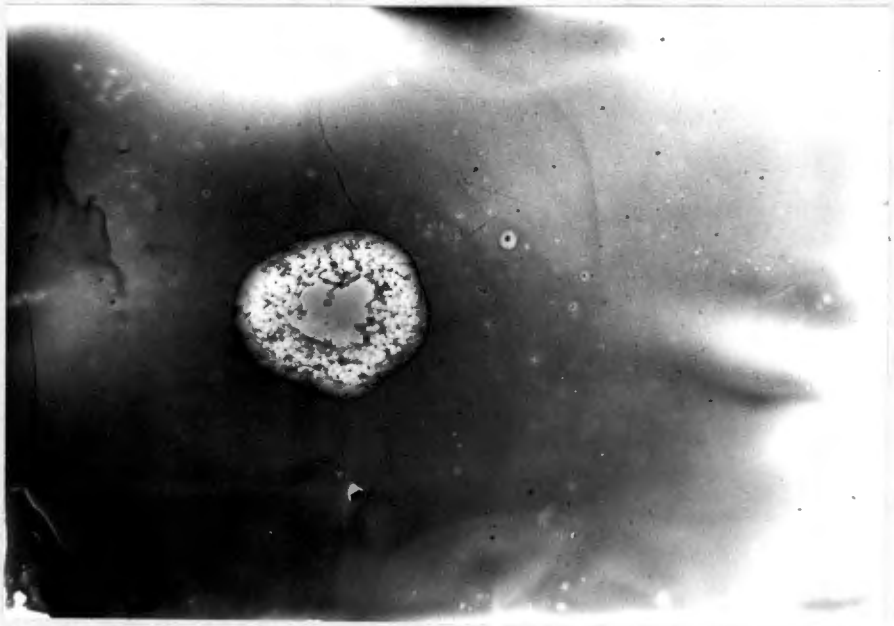


Fig. 108: Digestion of fibrin by seminal plasma. 60 mins. X 5

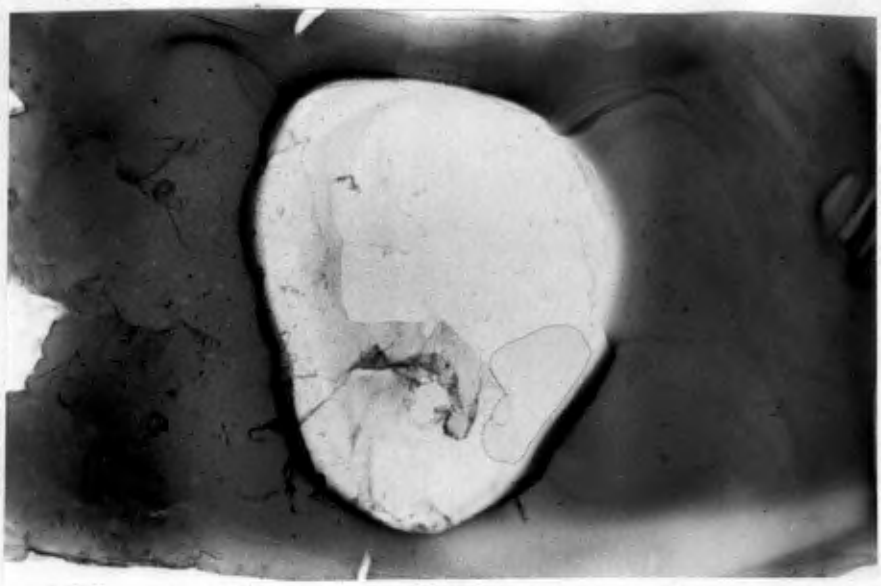


Fig. 109: Fibrinolysis by seminal fluid from a vasectomy case. 60 mins. X 5

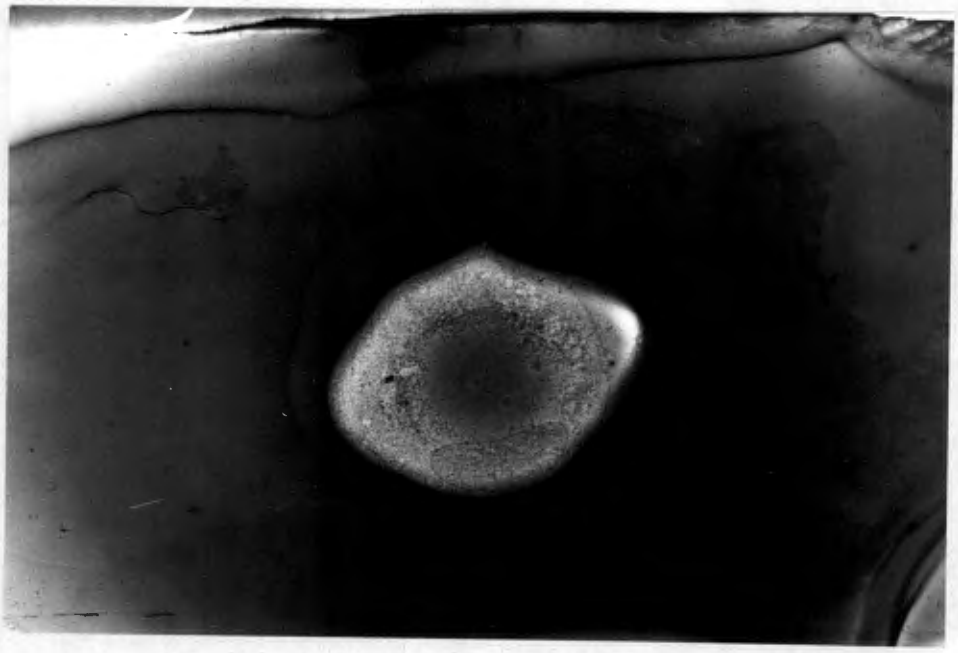


Fig. 110: Inactive washed spermatozoa. Artefact near centre of autograph. Overnight exposure at 4°C. for 24 hours, then incubation at 37°C. for 30 mins. X 608

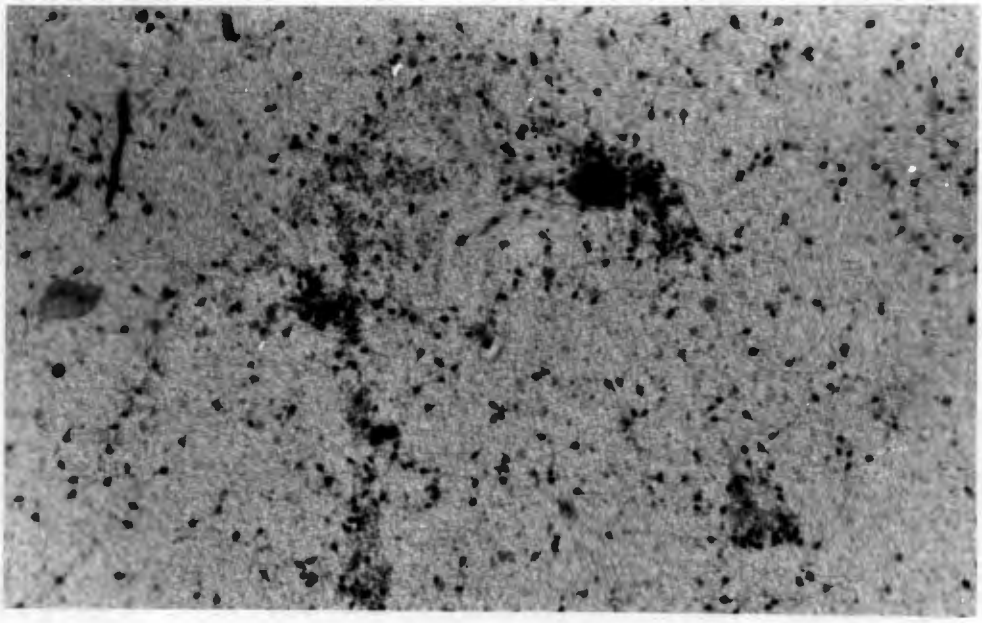


Fig. 111: Inactive washed spermatozoa. Digestion of fibrin by epithelial debris. X 608

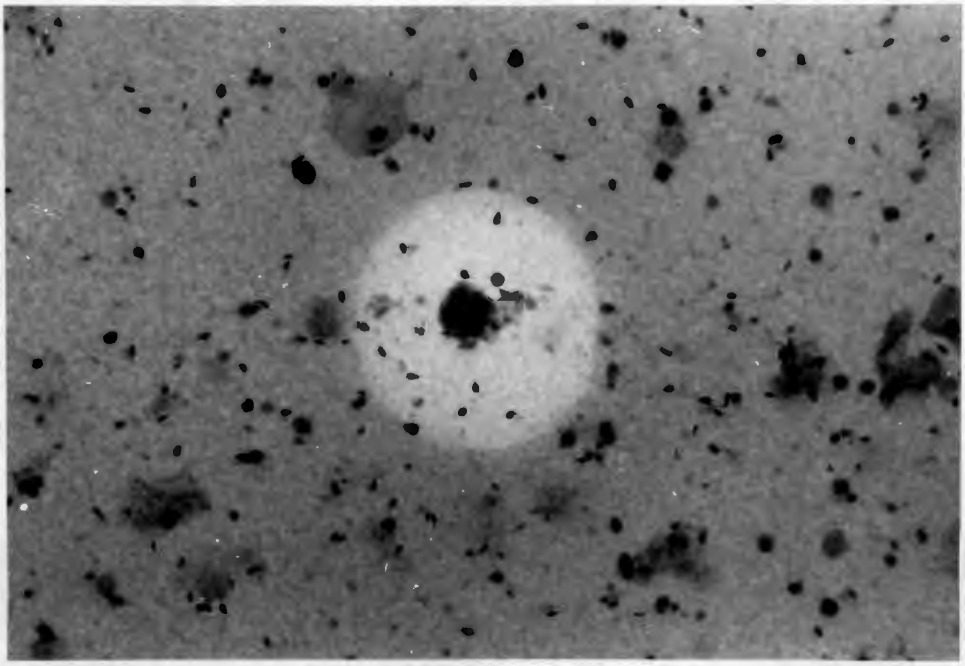


Fig. 112: Spermatozoa remain inactive after treatment with cervical mucus. Overnight exposure X 950

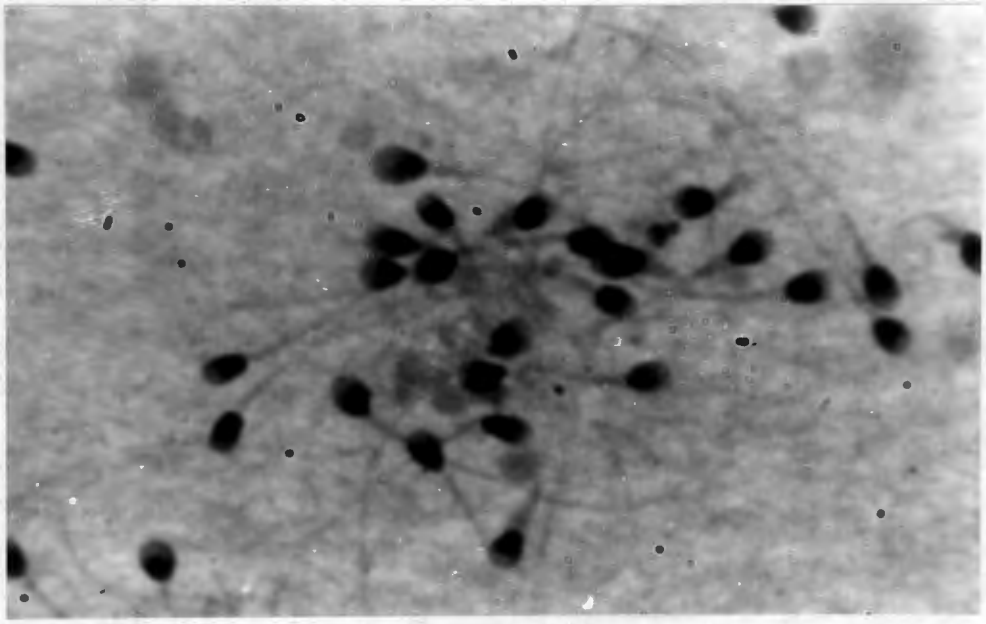


Fig. 113: Inactive spermatozoa in vaginal fluid. X 950

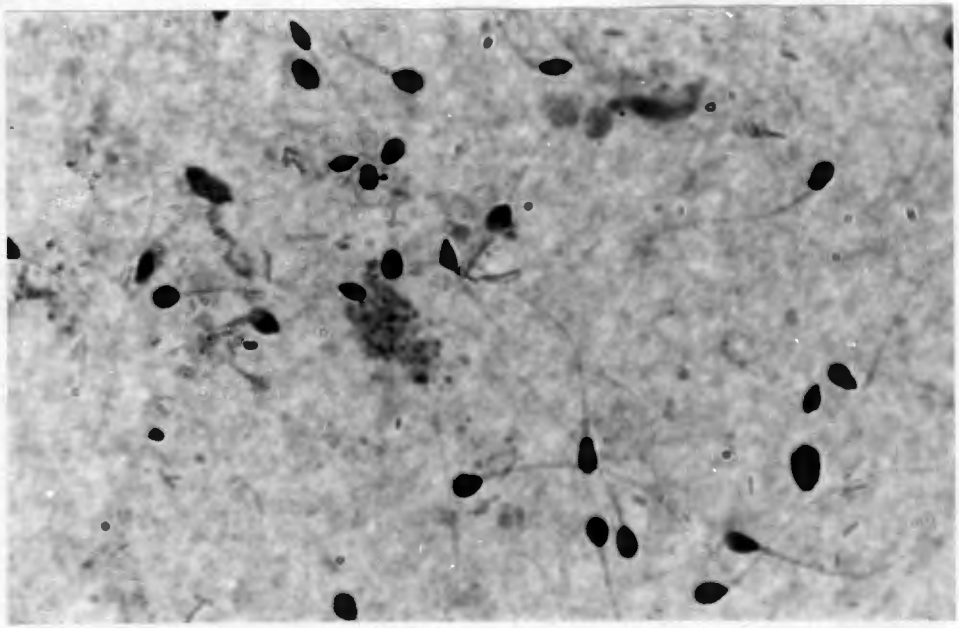


Fig. 114: Active epithelial cells in cervical mucus. X 608

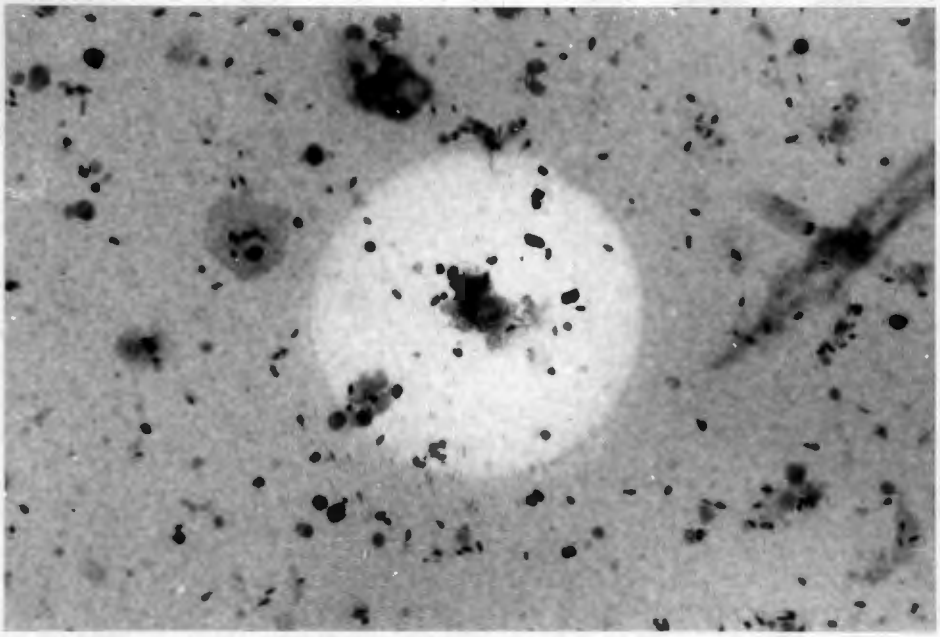


Fig. 115: Fibrinolysis by vaginal epithelial cells. X 380

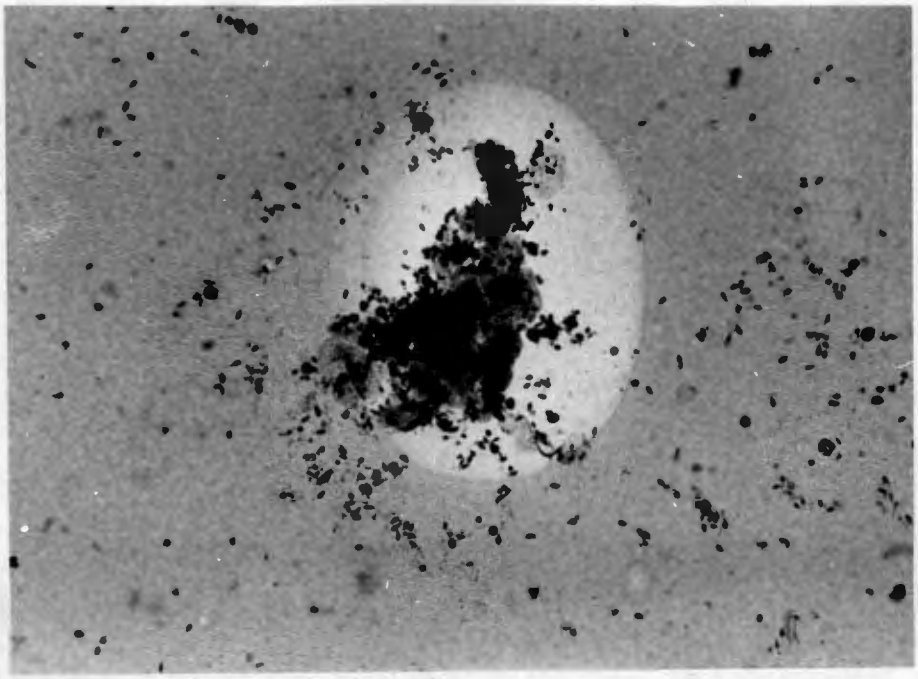


Fig. 116: Spermatozoa treated by freezing and thawing remain inactive. Overnight exposure at 4°C. X 760

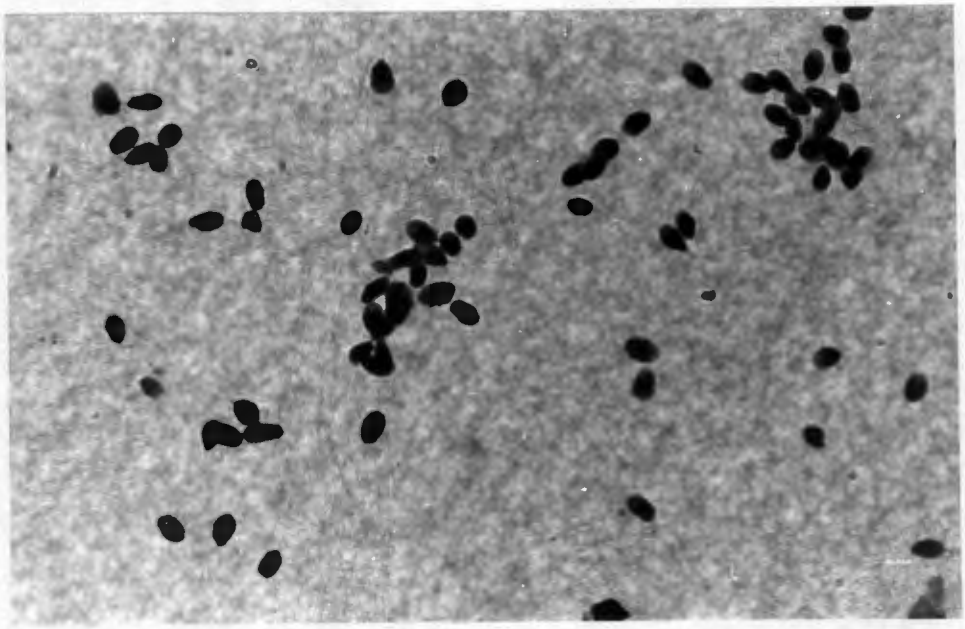


Fig. 117: Spermatozoa treated with sodium hydroxide, then stained with Giemsa. X 760

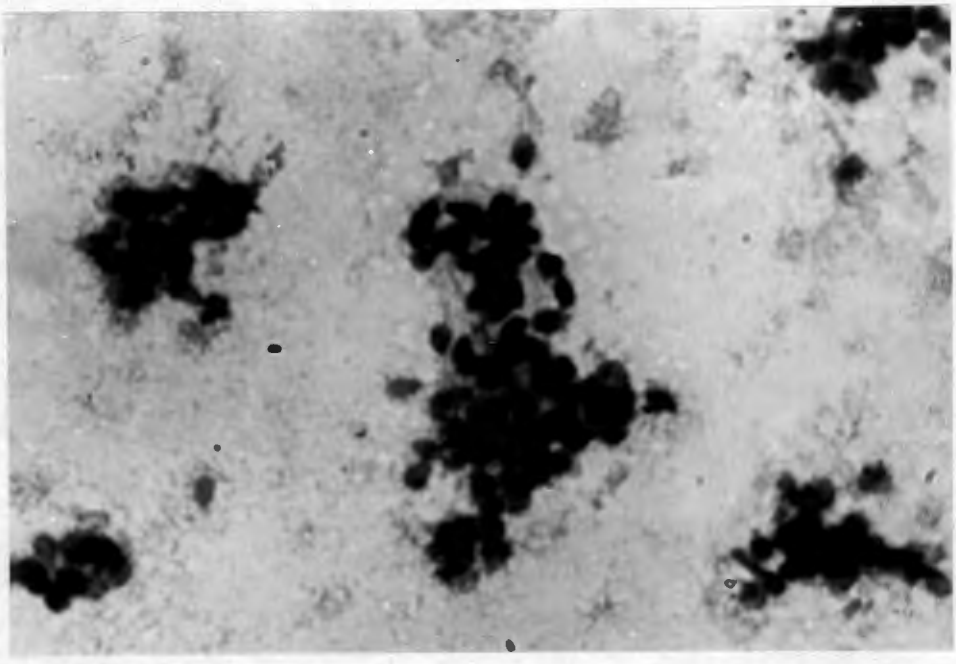
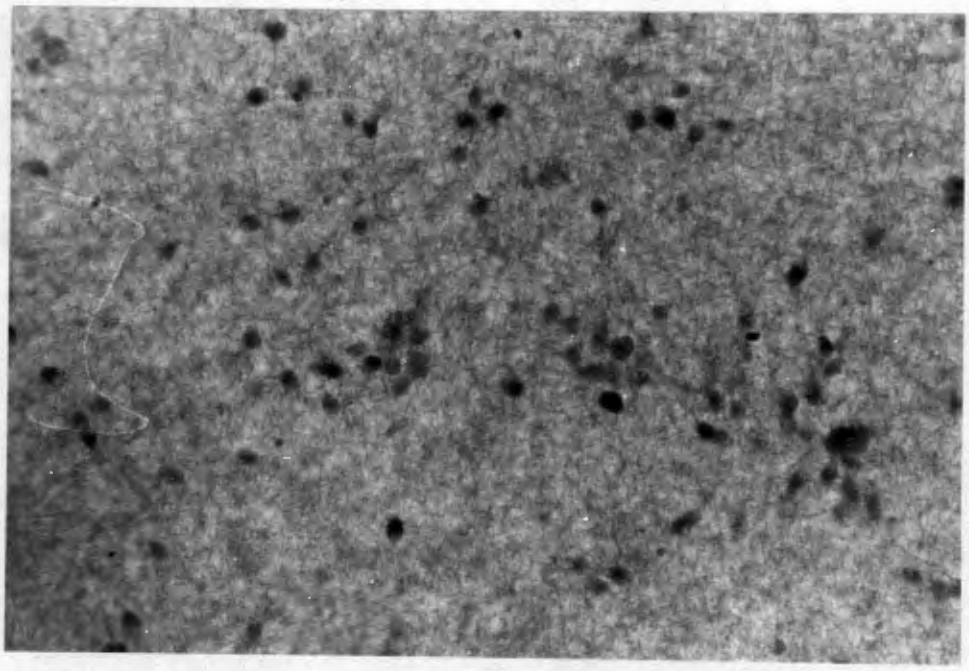


Fig. 118: Fibrinolysis autograph of inactive spermatozoa, damaged after treatment with sodium hydroxide. Overnight exposure at 4°C. X 760



DISCUSSION

It has been shown by von Kaulla and Shettles (1953, 1954) that human semen contains powerful fibrinolytic proteases. By testing heated and unheated bovine fibrin plates with samples of seminal fluid, they concluded that the fluid contained plasminogen activator and plasmin, and they also detected the presence of pro-activator and fibrinolytic inhibitors. Dilution enhanced the fibrinolytic activity of seminal fluid, due to the dilution of enzyme inhibitors. Their findings were corroborated by Lundquist, Thorsteinsson, and Buus (1955) and Rasmussen, Albrechtsen and Astrup (1958).

That fibrinolysin is secreted by epithelial cells lining the male reproductive tract has been shown by these studies, but there is as yet no unequivocal proof that the normal liquefying activity of human semen is in fact directly due to the concentration of fibrinolysin. Although a rough test of fibrinolytic capacity is that a given sample of semen should liquefy within 20 minutes or so after ejaculation, delay in liquefaction is by no means a cause of male infertility. Other enzymes, too, may be important /

important, since liquefaction of abnormal non-liquefying samples of human semen can be induced by adding alpha-amylase to the clotted specimen (Bunge and Sherman, 1954). The content of protein soon after ejaculation undergoes rapid changes of a proteolytic nature, as manifested by a progressive decrease in the concentration of nondialyzable protein nitrogen, and a simultaneous increase of non-protein nitrogen, free amino acids, and later, free ammonia (Mann, 1963). Pathological conditions of the male accessory glands especially in the prostate, can produce definite changes in the pattern and rate of proteolysis.

Huggins and Neal (1942) have localized the fibrinolytic activity of semen to the seminal plasma, and not to the spermatozoa, since the fibrinolytic activity is not influenced by passing the fluid through a Seitz filter. Thorough washing is required to remove any coating of seminal plasma from spermatozoa. Centrifugation, dilution and washing may inflict an injury upon the sperm cell not apparent to ordinary microscopic examination, and result in the loss or leakage of proteins and enzymes into the surrounding medium (Mann, 1964). Such factors need to be taken into account when appraising the lack of fibrin-olysis /

fibrinolysis by spermatozoa in these experiments.

In complete contrast to the findings of the present studies, plasminogen activator has been found present in spermatozoa, by Tympanidis and Astrup (1968). Washed rat sperms were more active than sperms from testicular and epidydimal tissue. Although untreated rabbit spermatozoa became fibrinolytic only after prolonged incubation, washed cells were quite active. They believed that washing with saline removed inhibitors which were only loosely bound to plasminogen activator.

However, the finding which captured the most profound attention was that vaginal smears from human volunteers, and also mated rats contained spermatozoa which exhibited significant fibrinolysis. It was suggested that the activity of vaginal spermatozoa may be related to capacitation, a process which spermatozoa undergo in the female genital tract prior to fertilisation. Nevertheless, the finding of plasminogen activator in human spermatozoa must be accepted with reserve, since no attempt was made in their experiments to exclude the possibility of contamination of the spermatozoa by seminal plasma activator.

No fibrinolytic mechanism has been demonstrated in spermatozoa in these studies, despite attempts to trigger off such a process in isolated spermatozoa by treating them with female genital fluids, or by damaging their cell membranes. If components of the fibrinolytic system are present in spermatozoa, other methods may be necessary to expose such factors. Plasminogen activator might be demonstrable in sperms if the cells are initially treated with activator-releasing agents, such as "Triton X 100", saponin, citric acid or endotoxin (Lack and Ali, 1964). Alternatively, the fibrinolytic component may be in pro-activator form, and the sperm would need to be treated with a lysokinase, e.g. streptokinase, before fibrinolysis is evident.

Spermatozoa change in nature during their passage through the genital tract. For instance, they acquire antigens from the accessory glands, mainly the prostate and seminal vesicles (Weil and Rodenburg, 1960). There is evidence that the process of sperm maturation is not halted at ejaculation, but continues in the female reproductive tract, where the sperms undergo a definite change called "capacitation" Before becoming capable of penetrating the egg /

egg surface (Austin, 1951; Chang, 1951).

Capacitation of the spermatozoa occurs during their traverse of the uterus and Fallopian tubes, and this process involves certain structural changes in the sperm-head. The acrosome becomes elevated and detached from the sperm before it reaches the zona pellucida (Austin and Bishop, 1958), reminiscent of the "acrosome reaction" of the sperms of marine invertebrates. The modified acrosome is then said to release hyaluronidase which burrows a tunnel through the cumulus oöphorus in advance of the penetrating sperm.

Removal of the acrosome exposes the perforatorium, which is thought to carry zona lysin, probably a mucolytic enzyme. The zona lysin alters the zona pellucida, so that the sperm can get into the perivitelline space. Finally the sperm has to penetrate a final barrier, the surface of the vitellus. The sperm possibly carries an inhibitor which must be removed before the mucolytic enzyme can act on the zona. The inhibitor has been termed "decapacitating factor". Recent work at Cambridge has revealed that it can be removed by immersion of the sperm in /

in follicular fluid (Edwards, 1969). The enigma concerning penetration of the egg may be solved by capacitating sperms either by retaining them temporarily in the uterine cavity or Fallopian tube, or by treating them with follicular fluid, and then examining them by means of fibrinolysis autography.

All genital fluids contain components of the fibrinolytic system, but their biological significance is not yet clear. The finding by Goldblatt (1935) that seminal fluid was rich in thromboplastic substances, has been confirmed by von Kaulla and Shettles (1956), who detected their presence, too, in cervical mucus, amniotic and follicular fluid. A globulin has been found in cervical mucus which inhibits trypsin (Schumacher and Pearl, 1968), and von Kaulla (1952) has observed that cervical mucus is free of antiplasmin at midcycle. The secretion of these inhibitors is influenced by sex hormones, and their function may be connected with sperm migration.

The physiological significance of the fibrinolytic enzyme system in seminal plasma is not yet clearly understood. Its main purpose may be to maintain the semen in a /

a fluid state during its storage and passage through the genital tract (Mann, 1964). By dissolving the copulatory plug, it expedites sperm migration from the site of deposition until they make contact with the ovum. Motile spermatozoa may carry a coating of seminal fluid with them, which may influence the process of capacitation. The fibrinolytic activity of seminal fluid may facilitate both the entry of the sperm into the uterine cavity by dissolving cervical mucus, and their penetration of the zona pellucida.

CHAPTER XVI

SUMMARY OF THE THESIS

SUMMARY OF THE THESIS

The cycle of coagulation and liquefaction of seminal fluid resembles the clotting and fibrinolysis of blood. Indeed, human semen contains a substance which can activate the blood fibrinolytic system; it has been assumed that this "activator" is of prostatic origin, and is an enzyme identical with the semen-liquefying protease.

The histochemical technique of "fibrinolysis autography" reveals the topography of plasminogen activator in tissues. Using a semi-quantitative method of the technique, tissues of the male genital tract were studied. Samples of tissues were taken from enucleated prostate glands at operation, and at necropsy from testis, epidydimis, vas deferens, seminal vesicle, prostate, bulbo-urethral gland, corpus spongiosum and corpus cavernosum. The regional distribution of fibrinolytic activity in the prostate was surveyed, and the effect of various factors on the sensitivity of plasminogen activator was studied. Spermatozoa and samples of seminal fluid were likewise tested.

Preparations from such samples confirmed that the greatest fibrinolytic activity arose from the blood vessels, except /

except in the testis, where their activity was less striking. Randomly distributed activity was localized to the epithelium in all parts of the male genital tract, except the testicular tubules, which were almost inactive.

Epithelial activity was more impressive in the distal parts of the male genital tract, viz. bulbo-urethral gland, seminal vesicle and prostate, than in the proximal tissues. Fibrinolysis by epithelial cells was enhanced by desquamation and disruption.

The development of prostatic corpora amylacea may be related to a disturbance in the equilibrium of coagulation-fibrinolysis. Seminal plasma is rich in plasminogen activator. No fibrinolytic activity seems to be related to spermatozoa.

The presence of activator maintains semen in a fluid state during its storage and passage through the genital tract. By liquefying the clot formed in ejaculated semen, activator facilitates sperm migration from the site of deposition, and it may play a role in the process of fertilisation.

REFERENCES

Ablondi, F.B., Hagan, J.J., Philips, M., de Renzo, E.C., 1959 — Inhibition of Plasmin, Trypsin and the Streptokinase - Activated Fibrinolytic System by E-Aminocaproic Acid. Arch. Biochem., 82, 153

Aboulker, P., Soulier, J.P., Larrieu, M.J., Wartelle, O., 1955 — Syndrome hémorragique avec fibrinolyse associé au cancer de la prostate. Presse Med., 63, 353

Aggeler, P.M., Seale, R.A., 1951 — Quoted in: Seale, R.A., Jampolis, R.W., Bargen, J.A.: Clotting defect in the presence of metastatic carcinoma of prostate; a case report. Surg. Clin. N.Amer., 31, 1111

Albrechtsen, O.K., 1957 — The Fibrinolytic Activity of Human Tissues. Brit. J. Haemat., 3, 284

Albrechtsen, O.K., 1959 — Fibrinolytic Activity in the Organism. p.26. Universitets forlaget I: Aarhus

Albrechtsen, O.K., Storm, O., Claassen, M., 1958 — Fibrinolytic activity in some human body fluids. Scand. J. Clin. Lab. Invest., 10, 310

Albrechtsen, O.K., Thaysen, J.H., 1955 — Fibrinolytic Activity in Human Saliva. Acta Physiol. Scand., 35, 138

Albrechtsen /

Albrechtsen, O.K., Trolle, D., 1955 — A Fibrinolytic System in Human Amniotic Fluid. Acta Haemat., 14, 376

Alkjaersig, N., Fletcher, A.P., Sherry, S., 1959 — E-Aminocaproic Acid: an Inhibitor of Plasminogen Activation. J. Biol. Chem., 234, 832

Allen, J.G., 1951 — Homeostatic hemostasis. The Dynamics of Blood Coagulation. Arch. Surg., 63, 279

Ambrus, C.M., Weintraub, D.H., Dunphy, D., Dowd, J.E., Pickren, J.W., Niswander, K.R. Ambrus, J.L., 1963 — Studies on hyaline membrane disease. I. The Fibrinolysin System in Pathogenesis and Therapy. Pediatrics, 32, 10

Andersen, M.N., Mendelow, M., 1963 — Fibrinolysis During and After Extracorporeal Circulation. Arch. Surg., 86, 649

Andersson, L., 1963a — Fibrinolysis in Patients with Prostatic Cancer. Acta Chir. Scand., 126, 172

Andersson, L., 1963b — Fibrinolytic States in Prostatic Disease and Their Treatment with Epsilon-Amino-Caproic Acid. Acta Chir. Scand., 126, 251

Andersson, L., Nilsson, I.M., 1961 — Effect of E-amino-n-caproic acid (E-ACA) on Fibrinolysis and Bleeding Conditions in Prostatic Disease. Acta Chir. Scand., 121, 291

Anglesio, E., Pelocchino, A.M., Badà, C., 1959 — La fibrinolisi nei portatori di neoplasie maligne. Arch. Sci. Med., 107, 51

Arthus, M., 1893 — Sur la fibrine Arch. de Physiol., 5, 392

Astrup, T., 1951 — Fibrinokinase. Acta Physiol. Scand., 24, 267

Astrup, T., 1952 — The Activation of a Proteolytic Enzyme in Blood by Animal Tissue. Biochem. J., 50, 5

Astrup, T., 1955a — Recent Developments in Fibrinolysis. In: Thrombosis and Embolism, Proceedings of the First International Conference, P.95. Basel: Benno Schwabe

Astrup, T., 1955b — Fibrinolysis and the formation of connective tissue. In: Contemporary Rheumatology, Proceedings of the Third European Rheumatology Congress, P.124. Amsterdam: Elsevier Publishing Co., 1956

Astrup, T., 1956a — Fibrinolysis in the Organism Blood, 11, 781

Astrup, T., 1956b — The biological significance of fibrinolysis, Lancet, 2, 565

Astrup,/

Astrup, T., 1958
Neuere Aspekte in der Blutgerinnung und der Fibrinolyse und ihren Beziehungen zur Koronarthrombose und Koronarsklerose.
Wein. Z. Inn. Med., 39, 373

Astrup, T., Claassen, M., 1958
The Fibrinolytic and Thromboplastic Activities of the Arterial Wall.
In: Trans. Sixth Congress Europ. Soc. Haemat., 2, 455. Basel: Karger

Astrup, T., Henrichsen,J., Tympanidis, K., King, A.E., 1967
Fibrinolytically Active Vaginal Epithelial Cells.
Nature, 214, 297

Astrup, T., Permin, P.M., 1947
Fibrinolysis in the Animal Organism.
Nature, 159, 681

Astrup, T., Permin, P.M., 1948
Fibrokinase and Fibrinolytic Enzymes.
Nature, 161, 689

Astrup, T., Stage, S., 1952
Isolation of a Soluble Fibrinolytic Activator from Animal Tissue.
Nature, 170, 929

Astrup, T., Sterndorff,I., 1952a
Fibrinolytic Activity of Tissue Extracts and of Trypsin.
Nature, 170, 981

Astrup, T., Sterndorff,I., 1952b
An Activator of Plasminogen in Normal Urine.
Proc. Soc. Exp. Biol. Med., 81, 675

Astrup, T., Sterndorff, I., 1953 — A Fibrinolytic System in Human Milk. Proc. Soc. Exp. Biol. Med., 84, 605

Astrup, T., Sterndorff, I., 1956a — The Plasminogen Activator in Animal Tissue. Acta Physiol. Scand., 36, 250

Astrup, T., Sterndorff, I., 1956b — Fibrinolysokinase Activity in Animal and Human Tissue Acta Physiol. Scand., 37, 40

Astrup, T., Sterndorff, I., 1960 — The Effects of Sodium Citrate and an Excess of Plasminogen on Fibrinolytic Agents. Thromb. Diath. Haemorrh., 4, 462

Austin, C.R., 1951 — Observations on the penetration of the sperm into the mammalian egg. Aust. J. Sci. Res., B, 4, 581

Austin, C.R., 1959 — In: Reproduction in Domestic Animals, P.411. Edited by Cole, H.H., Cupps, P.T. New York: Academic Press.

Austin, C.R., Bishop, M.W.H., 1958 — Role of the rodent acrosome and perforatorium in fertilisation. Proc. Roy. Soc. Lond., B, 149, 241

Balfour, J., 1959 — Circulating fibrinolysin in carcinoma of the prostate. Canad. J. Surg., 2, 138

Barker, /

Barker, B.I., 1908 — The enzymes of fibrin. J. Exper. Med., 10, 343

Baurys, W., Jacobson, H.H.H., 1959 — Proteolytic enzymes and prostatectomy. J. Urol., 81, 676

Beck, E., Schmutzler, R., Duckert, F., 1963 — Inhibition of Fibrinolysis and Fibrinogenolysis in Man: Comparison of E-Aminocaproic Acid and Kallikrein Inhibitor. Thrombo. Diath. Haemorrh., 10, 106

Bennike, T., Mülleritz, S., 1952 — Haemorrhagic Diathesis Associated with Fibrinogenopenia and Fibrinolysis. (A Report of two cases). Acta Haemat. (Basel), 8, 147

Benzer, H., Blümel, G., Piza, F., 1962 — Experimentelle Untersuchungen zur Thrombose und Blutung bei Prostatekomien mit gleichzeitigem Beitrag zur Pathogenese der "Thromboembolie". Wein. Klin. Wschr., 74, 601

Bergen, S., Schilling, F.J., 1958 — Circulating fibrinolysin in a case of prostatic carcinoma with bony metastases. Ann. Intern. Med., 48, 389

Bergmann, C., Leuckart, R., 1852 — In: Anatomisch - physiologische Uebersicht des Thierreichs, P. 567. Stuttgart: Müller.

Bierstedt, /

Bierstedt, P., 1955

Das Gewebe als Quelle der fibrinolytischen Aktivität der Körperflüssigkeiten. In: Proc. First Int. Conf. Thrombosis and Embolism, p.99. Basel: Benno Schwabe.

Biggs, R., Macfarlane, R.G., 1963

Human Blood Coagulation and its Disorders. 3rd Edition, p.370. Oxford: Blackwell.

Bischoff, T.L.W., 1852

Entwicklungsgeschichte des Meerschweinchens, p.12 Giessen: Ricker.

Boomgaard, D.J., Moers, A.M.J., 1963

Hemorragische diatheses gepaard gaande met versterkte fibrinolyse bij een patiënte met gemetastataseerd maagcarcinoom. Nederl. T. Geneesk., 107,903

Boros, M., Tarnoky, K., Vereczkey, C., Rāk, K., 1962

Kryofibrinogenämie und pathologisch gesteigerte Fibrinolyse bei einem Kranken mit Prostata carcinom. Zeit. Klin. Med., 157, 68

Bose, A., Brose, S.S., Hartley, G., 1969

Experiences with intra-arterial streptokinase perfusion in the treatment of acute arterial thrombosis. J. Clin. Path., 22, 374

Brakman, P., 1967

Fibrinolysis. A Standardised Fibrin Plate Method and a Fibrinolytic Assay of Plasminogen. M.D. Thesis, Univ. Amsterdam: Scheltema and Holkema N.V.

Brakman /

Brakman, P., Mohler, E.R., Astrup, T., 1966
A Group of Patients with Impaired Plasma Fibrinolytic System and Selective Inhibition of Tissue Activator-Induced Fibrinolysis. Scand, J. Haemat., 3, 389

Braun, P., Horányi, M., 1951
Hamorrhagische Diathese verursacht durch Fibrinopenie bei Knochenmark-karzinose. Folia Haemat., 71, 164

Bredt, J., 1962
Hyperfibrinolyse bei Prostatakarzinom. Blut. 8, 22

Brown, R.C., Campbell, D.C., Thompson, J.H., 1962
Increased Fibrinolysin with Malignant Disease. Report of Two Cases. Arch. Intern. Med., 109, 201

Bunge, R.G., Sherman, J. K., 1954
Liquefaction of Human Semen by Alpha-amylase. Fertil. Steril., 5, 353

Burghele, T., Papadopol, S., Hozoc, D., Burchi, D., 1961
Le syndrome de protéolyse prostatique. La fibrinolyse en urologie. J. Urol. Nephrol. (Paris), 67, 795

Caffier, P., 1930
Die Rolle des menschlichen Uterus als mesodermales Verdauungsorgan. München. Med. Wschr., 77, 389

Camus, /

Camus, L., Gley, E., 1896 — Action coagulante du liquide prostatique sur le contenu des vésicules séminales. C.R. Acad. Sci. Paris, 123, 194

Camus, L., Gley, E., 1922 — Action coagulante du liquide prostatique de la Viscache sur le contenu des vésicules séminales. C.R. Soc. Biol. (Paris), 87, 207

Carrell, A., Burrows, M.T., 1911 — Cultivation in vitro of malignant tumors. J. Exper. Med., 13, 571

Chakrabarti, R., Fearnley, G.R., Hocking, E.D., 1964 — Effect of Corticosteroid Therapy on Fibrinolysis Patients With Inflammatory and Non-Inflammatory Conditions. Brit. Med. J., 1, 534

Chakrabarti, R., Fearnley, G.R., Hocking, E.D., Delitheos, A., Clarke, G.M., 1966 — Fibrinolytic activity related to age in survivors of myocardial infarction. Lancet, 1, 573

Chalnot, P., Michon, P., Lochard, Mme., 1952 — Afibrinémie mortelle a l'occasion d'une intervention endothoracique. Problémes pathogéniques et thérapeutiques. Rev. Hémat., 7, 25

Chang, M.C., 1951 — Fertilizing Capacity of Spermatozoa deposited into the Fallopian Tubes. Nature, 168, 697

Charlton, /

Charlton, C.A.C., 1966 — Pathogenesis of urolithiasis: Relation of urinary fibrinolytic activity to nondialyzable urinary solids. Surg. Forum, 17, 503

Charlton, C.A.C., 1967 — Fibrinolysis and urolithiasis. Lancet, 1, 1199

Christensen, L.R., 1945 — Streptococcal fibrinolysis: A proteolytic reaction due to a serum enzyme activated by streptococcal fibrinolysin. J. Gen. Physiol., 28, 363

Christensen, L.R., MacLeod, C.M., 1945 — A proteolytic enzyme of serum: Characterization, activation, and reaction with inhibitors. J. Gen. Physiol., 28, 559

Cibert, J., Favre-Gilly, J., Traeger, J., Durand, L., 1952 — Purpuras thrombocytopéniques au cours de cancers de la prostate, traités par les oestrogènes de synthèse. Le Sang, 23, 541

Clayton, B.E., Hammant, J. E., Hawkins, J.B., 1956 — Occurrence of "lumps" of coagulated material in the bladder of adrenalectomized guinea pigs. Nature, 178, 1296

Clermont, Y., Glegg, R.E., Leblond, C.P., 1955 — Presence of carbohydrates in the acrosome of the guinea pig spermatozoon. Exp. Cell Res., 8, 453

Cliffton, /

Cliffton, E.E., 1960 — Enzymes in Health and Disease, p.212. Thomas: Springfield, Ill.

Cohen, S.N., Kupfer, H.G., 1958 — Fibrinolysis. Report of a Case and Clinical Review. New Engl. J. Med., 259, 1103

Colwin, L.H., Colwin, A.L., 1960 — Formation of Sperm Entry Holes in the Vitelline Membrane of Hydroides hexagonus (Annelida) and Evidence of their Lytic Origin. J. Biophys. Biochem. Cytol., 7, 315

Colwin, L.H., Colwin, A.L., 1961 — Changes in the spermatozoon during fertilisation in Hydroides hexagonus (Annelida). I. Passage of the Acrosomal Region through the Vitelline Membrane. J. Biophys. Biochem. Cytol., 10, 231

Copley, A.L., 1954 — Effet capillorragique de la fibrinolysine et de l'anti-fibrinolysine sur la membrane nictitante du lapin normal et exposé aux rayons X. Arch. Int. Pharmacodyn., 99, 426

Copley, A.L., 1957 — Fibrinolysis and atherosclerosis. Lancet, 1, 102

Cosgriff, /

Cosgriff, S.W., Leifer, E., 1952 — Factor 5 deficiency in hæmorrhagic diathesis (Parahemophilia) J.A.M.A., 148, 462

Cottier, P., Leupold, R., Scheitlin, W., 1955 — Die Hämorrhagische Diathese bei Prostatakarzinom und ihre Behandlung. Schweiz. Med. Wschr., 85, 781

Crane, J.J., Ware, A.G., Hamilton, J., 1955 — Spontaneous Afibrinogenemia in Cancer of Prostate: Report of Two Cases. J. Urol., 73, 379

Crehan, J.P., 1957 — Abnormal Bleeding in the Surgical Patient. Surg. Clin. N.Amer., Lahey Clinic, 37.

Croizat, P., Revol, L., Favre-Gilly, J., Thouverez, J.-P., 1955 — Diathèse hémorragique complexe avec fibrinolyse au cours d'un cancer de la prostate. Lyon Méd., 87, 397

Dale, H.H., Walpole, G.S., 1916 — Some experiments on factors concerned in the formation of thrombin. Biochem. J., 10, 331

Dan, J.C., 1952 — Studies on the Acrosome. I. Reaction to egg-water and other stimuli. Biol. Bull. Woods Hole, 103, 54

Dan, J.C., 1956 — The Acrosome Reaction. Int. Rev. Cytol., 5, 365

Das, /

Das, P.C., Allan, A.G.E., Woodfield, D.G., Cash, J.D., 1967 — Fibrin Degradation Products in Sera of Normal Subjects. Brit. Med. J., 4, 718

Dastre, A., 1893 — Fibrinolyse dans le sang. Arch. Physiol. Norm. Path., 5th series, 5, 661

Dastre, A., 1894a — Digestion sans ferments digestifs. Arch. Physiol. Norm. Path., 5th series, 6, 464

Dastre, A., 1894b — La digestion saline de la fibrine. Arch. Physiol. Norm. Path., 5th series, 6, 919

Delezenne, C., Pozerski, E., 1903 — Action du sérum sanguin sur la gélatine en présence du chloroforme. C.R. Soc. Biol. (Paris), 55, 327

Demuth, F., von Riesen, I., 1928 — N-Stoffwechsel in vitro gezüchteter Gewebe. Arch. Exper. Zellforsch., 6, 146

Denis, P.S., 1838 — Essai sur l'application de la chimie á l'étude physiologique du sang de l'homme, et à l'étude physiologico-pathologique, hygiénique et thérapeutique des maladies de cette humeur. Thesis, Paris: Béchet Jeune.

Denys, /

Denys, J., Marbaix, H. de 1889 — Sur les peptonisations provoquées par le chloroforme et quelques autres substances. La Cellule, 5, 197

Dillard, G.H.L., Chanutin, A., 1949 — The Protease and Antiprotease of Plasmas of Patients with Cancer and Other Diseases. Cancer Research, 9, 665

Dolaz, E.J.,Dolehide, R.A., Friedman, I.A., 1955 — Fibrinolysis in Patients with Metastatic Carcinoma of the Prostate Gland. Illinois Med. J., 107, 142

Dossogne, M., 1959 — Le syndrome de fibrinolyse prostatique. Acta Urol. Belg., 27, 145

Duckert, F., Jung, E., Shmerling, D.H., 1961 — A Hitherto Undescribed Congenital Haemorrhagic Diathesis Probably Due To Fibrin Stabilizing Factor Deficiency. Thrombo. Diath, Haemorrh., 5, 179

Duguid, J.B., 1946 — Thrombosis as a factor in the pathogenesis of coronary atherosclerosis. J. Path. Bact., 58, 207

Eadie, W.R., 1948 — The male accessory reproductive glands of Condylura with notes on a unique prostatic secretion Anat. Rec., 101, 59

Edwards, /

Edwards, R.G., 1969 — In: Snakes-and-ladders in the tunnel of love. Pulse International, 18, No. 15, April 26, 1969, p.12-13

Ende, N., Auditore, J.V., 1963 — Circulating Fibrinolytic Activator and Hemorrhagic Diathesis. Ann. Surg., 158, 117

Fearnley, G.R., 1953 — Fibrinolysis by Adsorption. Nature, 172, 544

Fearnley, G.R., 1961 — A concept of natural Fibrinolysis. Lancet, 1, 992

Fearnley, G.R., 1965 — Fibrinolysis. 1st Edition, p.131. Edward Arnold: London

Fearnley, G.R., Chakrabarti, R., 1962 — Increase of blood fibrinolytic activity by Testosterone. Lancet, 2, 128

Fearnley, G.R., Chakrabarti, R., 1964 — Pharmacological enhancement of fibrinolytic activity of blood. J. Clin. Path., 17, 328

Fearnley, G.R., Chakrabarti, R., 1969 — Pharmacological enhancement of Fibrinolysis. J. Clin. Path., 22 368,

Fearnley, G.R., Chakrabarti, R., Avis, P.R.D., 1963 — Blood fibrinolytic activity in Diabetes mellitus and its bearing on ischaemic heart disease and obesity. Brit. Med. J., 1, 921

Fearnley, G.R., Chakrabarti, R., Vincent, C.T., 1960 — Effect of the sulphonylureas on fibrinolysis. Lancet, 2, 622

Fearnley, G.R., Ferguson, J., 1958 — Presence of fibrinolytic activity in whole blood, masked by the clotting process. Clin. Sci., 17, 555

Fearnley, G.R., Tweed, J.M., 1953 — Evidence of an active fibrinolytic enzyme in the plasma of normal people with observations on inhibition associated with the presence of calcium. Clin. Sci., 12, 81

Fermi, C., 1891 — Die Auflösung des Fibrins durch Salze und verdünnte Säuren. Zeit. Biol., 28, 229

Fidon, L., Gautier, C., Martin, E., 1908 — Recherches physiologiques sur le sang des noyés. C.R. Soc. Biol. (Paris), 65, 474

Fiehrer, A., Bordas, P., Migault, P., Renard, R., 1957 — Fibrinolyse latente dans un cas de cancer de la prostate. Taux normal du facteur A et de la pro-accélérine. Rev. Path. Gen. Physiol. Clin., 57, 287

Fischer, A., 1946 — Mechanism of the Proteolytic Activity of Malignant Tissue Cells. Nature, 157, 442

Fleischhacker, /

Fleischhacker, H., 1940 — Über die Herkunft der Plasmaeiweiskörper. Wein. Arch. Inn. Med., 34, 96

Fleisher, M.S.,Loeb, L., 1915 — On tissue fibrinolysins. J. Biol. Chem., 21, 477

Fletcher, A.P., 1960 — Pathophysiology of thrombolysis. Proceedings of The Conference on Thrombolytic Agents, Hematology Study Section P.H.S., p.148. Chicago, April 8-9, 1960

Fletcher, A.P., Alkjaersig, N., Sherry, S., 1962 — Pathogenesis of the coagulation defect developing during pathological plasma proteolytic ("fibrinolytic") states. I. The significance of fibrinogen proteolysis and circulating fibrinogen breakdown products. J. Clin. Invest., 41, 896

Foret, J., Bounameaux, Y., Lecarte, J., 1959 — Sur une observation de fibrinolyse dans un cas de cancer prostatique sans métastase. J. Urol. Nephrol. (Paris) 65, 54

Fox, M., 1963 — The natural history and significance of stone formation in the prostate gland. J. Urol., 89, 716

Frankl, O., Aschner, B., 1911 — Zur quantitativen Bestimmung des tryptischen Ferments in der Uterusmukosa. Gynaek. Rundschau, 5, 647

Freeman, /

Freeman, T., 1964 — The Study of ^{131}I-fibrinogen. J. Clin. Path., 17, 347

Frick, P.G., 1956 — Acute hemorrhagic syndrome with hypofibrinogenemia in metastatic cancer. Acta Haemat. (Basel), 16,11

Fürstenberg, H., 1962 — Fibrinolyse in der Urologie. J. Int. Coll. Surg., 38, 268

Galeone, A., Pelocchino, A.M., 1956 — La sindrome emorragica da carcinoma della prostata; contributo clinico. Minerva Med., 2, 875

Geiger, W.B., 1952 — Involvement of a complement-like factor in the activation of blood protease. J. Immunol., 69, 597

Goldblatt, M.W., 1935 — Constituents of human seminal plasma. Biochem. J., 29, 1346

Goldhaber, P., Cornman, I., Ormsbee, R.A., 1947 — Experimental Alteration of the Ability of Tumour Cells to Lyse Plasma Clots in vitro. Proc. Soc. Exper. Biol. Med., 66, 590

Goodpasture, E.W., 1914 — Fibrinolysis in chronic hepatic insufficiency. John Hopkins Hosp. Bull., 25, 330

Gordon, /

Gordon, A.M., McNicol, G. P., Dubber, A.H.C., McDonald, G.A., Douglas, A.S., 1965 — Clinical Trial of Epsilon-aminocaproic Acid in Severe Haemophilia. Brit. Med. J., 1, 1632

Gratia, A., 1921 — Action fibrinolytique du staphylocoque. Arch. Int. Physiol., 18, 355

Green, J.R., 1887 — Note on the action of sodium chloride in dissolving fibrin. J. Physiol., 8, 372

Hadorn, W., 1953 — Personal communication to Cottier, P., Leupold, R., Scheitlin, W., quoted in Schweiz. Med. Wschr., 85, 781, 1955

Hahn, M., 1897 — Zur Kenntniss der Wirkungen des extravasculären Blutes. Berl. Klin. Wschr., 34, 499

Halban, J., Frankl, O., 1910 — Zur Biochemie der Uterusmukosa. Gynaek. Rundschau, 4, 471

Hardaway, R.M., 1966 — Syndromes of disseminated intravascular coagulation. Charles C. Thomas: Springfield, Illinois.

Hardisty, R.M., Ingram, G.I.C., 1965 — Bleeding Disorders. Investigation and Management. Blackwell Scientific Publications: Oxford.

Harris, /

Harris, H.F., 1900 — On the rapid conversion of haematoxylin into haematein in staining reactions. J. App. Micro., 3, 777

Harvey, C., 1949 — Fibrinolysin in human semen. A method of assay, and some preliminary observations. Proc. Soc. Study of Fertility, 1, 11. Edinburgh

Hathaway, R.R., Hartree, E.F., 1963 — Observations on the mammalian acrosome: experimental removal of acrosomes from ram and bull spermatozoa. J. Reprod. Fertil., 5, 225

Hayashi, I., Yano, H., Sakurai, T., Murakami, F., Kadota, H., Kusakari, K., 1962 — Fibrinolysis and Hypofibrinogenemia associated with cancer of the prostate: Report of a case. Acta Urol. Jap., 8, 369

(Japanese authors) 1961 — Acta Urol. Jap., 7, 481. Quoted by Hayashi and others, 1962.

Hedin, S.G., 1904 — On the presence of a proteolytic enzyme in the normal serum of the ox. J. Physiol., 30, 195

Herbst, W.P., 1942 — Thoughts and observations on the relation of altered hormonal balance to prostatic malignancy. Urol. Cutan. Rev., 46, 691

Hildebrandt, /

Hildebrandt, H., 1893 — Weiteres über hydrolytische Fermente, deren Schicksal und Wirkungen, sowie über Ferment festig keit und Hemmung der Fermentationen im Organismus. Virchow Arch. Path. Anat., 131, 5

Howell, W.L., Manion, W.C., 1960 — The Low Incidence of Myocardial Infarction in Patients With Portal Cirrhosis of the Liver: A Review of 639 Cases of Cirrhosis of the Liver from 17,731 Autopsies. Amer. Heart J., 60, 341

Huggins, C., 1947 — The prostatic secretion. Harvey Lect., 42, 148

Huggins, C.B., Johnson, A.A., 1933 — Chemical observations on fluids of the seminal tract. 1. Inorganic phosphorus, calcium, non-protein nitrogen and glucose content of semen, and of seminal vesicle, prostate and spermatocele fluids in man. Amer. J. Physiol., 103, 574

Huggins, C., McDonald, D.F., 1944 — Proteolytic enzymes and acid phosphatase in the prostatic fluid in chronic prostatitis. J. Urol., 52, 472

Huggins, C., Neal, W., 1942 — Coagulation and liquefaction of Semen. Proteolytic enzymes and citrate in prostatic fluid. J. Exp. Med., 76, 527

Huggins, /

Huggins, C., Scott, W.W., Heinen, J.H., 1942 — Chemical composition of human semen and of the secretions of the prostate and seminal vesicles. Amer. J. Physiol., 136, 467

Huggins, C.,Vail, V.C., 1943 — Plasma coagulation and fibrinogenolysis by prostatic fluid and trypsin. Amer. J. Physiol., 139, 129

Hunter, J., 1786 — Observations on certain parts of the animal oeconomy. "Sold at No. 13, Castle-street, Leicester-square," London.

Hunter, J., 1793 — A treatise on the blood, inflammation, and gun-shot wounds. Vol. 1. Written at Leicester-square, May 20, 1793. Printed for E. Cox, Bookseller, etc., by T.Bensley, London, 1812

Innerfield, I., 1960 — Clinical Syndromes Associated with Impaired Fibrinolytic Activity. Amer. Practit., 11, 189

Jensen, H., 1956 — Dynamic Concept of Fibrin Formation and Lysis in Relation to Hemorrhage (Capillary Permeability) and to Thrombosis. Exp. Med. Surg., 14, 189

Jürgens, R., Trautwein, H., 1930 — Über Fibrinopenie (Fibrinogenopenie) beim Erwachsenen, nebst Bemerkungen über die Herkunft des Fibrinogens. Deut.Arch.Klin.Med., 169, 28

Kakkar, V.V., Flanc, C., O'Shea, M.J., Flute, P.T., Howe, C.T., Clarke, M.B., 1969
Treatment of deep-vein thrombosis with streptokinase.
Brit. J. Surg., 56, 178

Källén, B., Röhl, L., 1960
The fibrinolytic activity of human hyperplastic prostatae studied in tissue culture.
Acta. Chir. Scand., 118, 240

Kaplan, M.H., 1944
Nature and Role of the Lytic Factor in Hemolytic Streptococcal Fibrinolysis.
Proc. Soc. Exp. Biol. Med., 57, 40

Karhausen, L., Tagnon, H., 1955
Le syndrome de fibrinolyse prostatique. Nature de l'activité protéolytique de la prostate.
Acta Clin. Belg., 10, 471

Kellock, I.A., Gallagher, N., 1958
Metastatic carcinoma of the prostate and bleeding.
Brit. Med. J., 2, 487

Kester, R.C., 1969
Plasminogen activator in the human prostate.
J. Clin. Path., 22, 442

Kjeldgaard, N.O., Ploug, J., 1957
Urokinase. An activator of plasminogen from human urine. II. Mechanism of plasminogen activation.
Biochim. Biophys. Acta, 24, 283

Kline, /.

Kline, D.L., 1960 — "Comments on the Conference" in: Proceedings of the Conference on Thrombolytic Agents, Hematology Study Section, P.H.S., Chicago, April, 1960, p.253

Koller, F., 1963 — Über die Therapie der post-operativen Blutung und Thrombose bei Prostatektomie unter besonderer Berücksichtigung der E-amino-capronsäure. Helv. Chir. Acta 30, 514

Kost, L.V., Evans, G.W., 1964 — Occurrence and significance of striated muscle within the prostate. J. Urol., 92, 703

Kussmaul, A., 1879 — Anfallsweise auftretende speichelgeschwulst in Folge von chronischer eitrig-fibrinöser Entzündung des Stenonschen Ganges. Berl. Klin. Wschr., 16, 209

Kwaan, H.C., Astrup, T., 1964a — Fibrinolytic activity of reparative connective tissue. J. Path. Bact., 87, 409

Kwaan, H.C., Astrup, T., 1964b — Localisation of Fibrinolytic Activity in Myocardial Infarcts in Rats. Thrombo. Diath. Haemorrh., 12, 407

Kwaan, H.C., Lo, R., McFadzean, A.J.S., 1958 — On the lysis of thrombi experimentally produced within the veins. Brit. J. Haemat., 4, 51

Kwaan, /

Kwaan, H.C., McFadzean, A.J.S., 1956 — On plasma fibrinolytic activity induced by ischaemia. Clin. Sci., 15, 245

Kwaan, H.C., McFadzean, A.J.S., Cook, J., 1957 — On plasma fibrinolytic activity in cryptogenic splenomegaly. Scot. Med. J., 2, 137

Lack, C.H., Ali, S.Y., 1964 — Tissue Activator of Plasminogen.Nature, 201, 1030

Lack, C.H., Rodgers, H.J., 1958 — Action of Plasmin on Cartilage. Nature, 182, 948

Ladehoff, A.A., 1960 — The content of plasminogen activator in the human urinary tract. Scand. J. Clin. Lab. Invest., 12, 136

Lambert, R.A., Hanes, F.M., 1911 — Characteristics of growth of sarcoma and carcinoma cultivated in vitro. J. Exp. Med., 13, 495

Landsteiner, K., 1900 — Zur Kenntnis der antifermentativen, lytischen und agglutinierenden Wirkungen des Blutserums und der Lymphe. Cent. f. Bakt., 27, Abt.1, 357

Lassen, /

Lassen, M., 1952 — Heat denaturation of Plasminogen in the Fibrin Plate method. Acta Physiol. Scand., 27, 371

Ledent, J., De Geeter, L., 1959 — Un cas de fibrinolyse prostatique. Acta Urol. Belg., 27, 160

Lee, L.W., 1962 — Experiences with hemorrhagic blood disease complicating urological surgery with particular reference to prostatic surgery. Nebraska State Med. J., 47, 63

Lendrum, A.C., 1961 — Further observations on fibrinous vasculosis. Nederl. T. Geneesk., 105, 1359

Lendrum, A.C., 1964 — Histological Appearances of Fibrin. In: Fibrinogen and Fibrin Turnover of Clotting Factors, Trans. Conf. Int. Comm. Blood Clotting Factors, Gleneagles, Scotland, July 1963. Thromb. Diath. Haemorrh., Suppl. 13, p.153

Leng-Levy, J., David-Chausse, J., Julien, J., Riviere, P., 1959 — Syndrome hémorragique au cours du cancer de la prostate. J. Méd. Bordeaux, 4, 479

Lewis, /

Lewis, J.H., Ferguson, J.H., 1950 — Studies on a proteolytic enzyme system of the blood. II. Fibrinolysokinase activators for profibrinolysin. J. Clin. Invest., 29, 1059

Lhoiry, J., Fayet, H., 1954 — A propos de deux observations (chirurgicale et obstétricale) d'incoagulabilité sanguine. Rev. Hémat., 9, 201

Lieberman, J., 1961 — The nature of the fibrinolytic enzyme defect in hyaline-membrane disease. New Engl. J. Med., 265, 363

Lillie, R.D., 1965 — Histopathologic Technique and Practical Histochemistry. 3rd Edition, p. 38 McGraw Hill: London

Limbourg, P., 1889 — Ueber Lösung und Fällung von Eiweisskörpern durch Salze. Hoppe-Seyler's Zeit. Physiol. Chem., 13, 450

Lincoln, A.F., Moorman, J.A., Schultz, R.L., 1957 — Fibrinolysis following Thoracic Surgery. Surg. Gynec. Obstet., 105, 541

Lombardo, L.J., 1957 — Fibrinolysis following prostatic surgery. J. Urol., 77, 289

Lombardo, /

Lombardo, L.J., 1958 — Studies in the prevention of fibrinolysis occurring in prostatic surgery. J. Urol., 79, 507

Luck, R.J., 1965 — In: The Scientific Basis of Surgery, p.336. Edited by W.T. Irvine. Churchill: London

Lundquist, F., 1952 — Studies on the Biochemistry of Human Semen. Acta Physiol. Scand., 25, 178

Lundquist, F., Thorsteinsson, T., Buus, O., 1955 — Purification and Properties of Some Enzymes in Human Seminal Plasma. Biochem. J., 59, 69

Lynn Evans, I., 1964 — Changes in fibrinolytic activity during surgical procedures. J. Clin. Path., 17, 369

Macfarlane, R.G., 1937 — Fibrinolysis following operation. Lancet, 1, 10

Macfarlane, R.G., 1964 — The Development of Ideas on Fibrinolysis. Brit. Med. Bull., 20, 173

Macfarlane, R.G., Biggs, R., 1946 — Observations on Fibrinolysis. Spontaneous activity associated with surgical operations, trauma, etc. Lancet, 2, 862

Macfarlane, /

Macfarlane, R.G., Pilling, J., 1946 — Observations on fibrinolysis. Plasminogen, Plasmin and antiplasmin content of human blood. Lancet, 2, 562

Macfarlane, R.G., Pilling, J., 1947 — Fibrinolytic Activity of Normal Urine. Nature, 159, 779

McFarlane, A.S., Todd, D., Cromwell, S., 1964 — Fibrinogen catabolism in humans. Clin. Sci., 26, 415

McKay, D.G., 1965 — Disseminated intravascular coagulation. An intermediary mechanism of disease. Hoeber Medical Division, Harper and Row: New York

McKay, D.G., Mansell, H., Hertig, A.T., 1953 — Carcinoma of the body of the pancreas with fibrin thrombosis and fibrinogenopenia. Cancer, 6, 863

McNicol, G.P., Fletcher, A. P., Alkjaersig, N., Sherry, S., 1961a — Impairment of hemostasis in the urinary tract : The role of urokinase. J. Lab. Clin. Med., 58, 34

McNicol, G.P., Fletcher, A. P., Alkjaersig, N., Sherry, S., 1961b — The use of Epsilon amino-caproic acid, a potent inhibitor of Fibrinolytic activity, in the Management of Postoperative hematuria. J. Urol., 86, 829

Mainwaring, /

Mainwaring, D., Keidan, S.E., 1965
Fibrinolysis in Haemophilia: The Effect of E-Amino-Caproic Acid.
Brit. J. Haemat., 11, 682

Mairano, C., Rigotti, E., 1960
Alcuni particolari aspetti della fibrinolisi nella diagnostica del carcinoma prostatico.
Minerva Urol., 12, 148

Maki, M., Beller, F.K., 1966
Comparative Studies of Fibrinolytic Inhibitors in Vitro.
Thromb. Diath. Haemorrh., 16, 668

Malcolm, D., O'Connor, J.J., 1963
Generalized fibrinolytic bleeding following cystoscopy in a patient with carcinoma of the prostate.
J. Urol., 90, 458

Mann, T., 1963
Biochemistry of the prostate gland and its secretion.
Nat. Cancer Instit. Monogr., 12, 235

Mann, T., 1964
Biochemistry of Semen and of the Male Reproductive Tract.
Methuen : London

Manrique, R., Hörder, M.-H., 1965
Schwere Hämorrhagische Diathese als erstes Zeichen eines metastasierenden Prostata-karzinoms.
Med. Klin., 60, 1393

Marchal, /

Marchal, G., Duhamel, G., Samama, M., Flandrin, G., 1963

Fibrinolyse aiguë révélatrice d'un cancer de la prostate avec métastases osseuses. Efficacité de l'acide E-amino-caproique.
Bull. Soc. Méd. Hôp. Paris, 114, 143

Marchal, G., Escalier, A., Duhamel, G., Leroux, M.E., Nivet, M., Samama, M., 1959

Sur deux cas de fibrinolyse au cours de cancers de la prostate avec métastases osseuses.
Sem. Hôp. Paris, 35, 2618

Marchal, G., Frileux, C., Bilsky-Pasquier, G., Weiss, M., Cornet, P., Samama, M., Larrieu, P., 1959

Fibrinolyse mortelle au cours d'une anastomose porto-cave pour cirrhose compliquée d'hématémèses récidivantes.
Presse Med., 67, 1851

Marder, M., Weiner, M., Shulman, P., Shapiro, S., 1949

Afibrinogenemia occurring in a case of malignancy of the prostate with bone metastases.
New York State J. Med., 49, 1197

Marx, R., Clemente, P., Werle, E., Appel, W., 1959

Zum Problem eines Antidotes in der internen Thrombo-therapie mit Fibrinolytika.
Blut., 5, 367

Marx, A.J., Gueft, B., Moskal, J.F., 1965

Prostatic Corpora Amylacea. A Study With the Electron Microscope and Electron Probe.
Arch. Path., 80, 487

Mathey, /

Mathey, J., Daumet, P., Soulier, J.-P., Le Bolloch, A.G., Fayet, H., 1950
Hémorragies graves au cours d'interventions thoraciques par incoagulabilité sanguine dûe à la fibrinolyse. Mem. Acad. Chir., 76, 977

Mavor, G.E., Galloway, J. M.D., 1969
Iliofemoral Venous Thrombosis. Brit. J. Surg., 56, 45

Meuwissen, T., Zaman, C.L., 1956
Prostaatcarcinoom met hemorragische diathese. Nederl. T. Geneesk., 100, 1697

Miller, J.M., Meisel, H.J., Jackson, D.A., Collier, C. S., 1959
Fibrinolysis in a patient with carcinoma of the prostate. J. Urol., 81, 672

Milstone, H., 1941
A factor in normal human blood which participates in streptococcal fibrinolysis. J. Immunol., 42, 109

Mole, R.H., 1948
Fibrinolysin and the fluidity of the blood post mortem. J. Path. Bact., 60, 413

Moore, R.A., 1936
Morphology of prostatic corpora amylacea and calculi. Arch. Path., 22, 24

Moore, R.A., Hanzel, R.F., 1936
Chemical composition of prostatic corpora amylacea and calculi. Arch. Path., 22, 41

Morawitz, /

Morawitz, P., 1906 — Über einige postmortale Blutveränderungen. Beitr. Z. Chem. Physiol. Path., 8, 1

Morgagni, J.B., 1769 — The Seats and Causes of Diseases. Vol. 3, Book 4, Letter 53, Article 26, p.185 London : Millar and Cadell

Morgan, Morton, Parker 1965 — Cell and Tissue Culture by John Paul, 3rd Edition, p.90 Livingstone : Edinburgh

Müllertz, S., 1952 — The Effect of Sodium Citrate on the Fibrinolytic Activity of Plasmin and Trypsin. Arch. Biochem., 41, 235

Müllertz, S., 1955 — Formation and Properties of the Activator of Plasminogen and of Human and Bovine Plasmin. Biochem. J., 61, 424

Müllertz, S., Lassen, M., 1953 — An Activator System in Blood Indispensable for Formation of Plasmin by Streptokinase. Proc. Soc. Exp. Biol. Med., 82, 264

Naeye, R.L., 1962 — Thrombotic State after a Hemorrhagic Diathesis, a Possible Complication of Therapy with Epsilon-Amino-caproic Acid. Blood, 19, 694

Naimi, /

Naimi, S., Goldstein, R., Proger, S., 1963 — Studies of Coagulation and Fibrinolysis of the Arterial and Venous Blood in Normal Subjects and Patients with Atherosclerosis. Circulation, 27, 904

Neft, L.G., Dugdale, M., Biggs, A.W., Raines, S.L., 1961 — Treatment of abnormal bleeding associated with prostatic disease by intravenous fat emulsion. J. Urol., 85, 329

Nestel, P.J., 1959 — Fibrinolytic activity of the blood in intermittent claudication. Lancet, 2, 373

Niewiarowski, S., Kowalski, E., 1957 — Formation of an Antithrombin-Like Anticoagulant during Proteolysis of Fibrinogen. Bull. Acad. Pol. Sci. Ser. Sci. Biol., 5, 169

Nilsson, I.M., Krook, H., Sternby, N.-H., Söderberg, E., Söderström, N., 1961 — Severe Thrombotic Disease in a Young Man with Bone Marrow and Skeletal Changes and with a High Content of an Inhibitor in the Fibrinolytic System. Acta Med. Scand., 169, 323

Nilsson, I.M., Niléhn, J.-E., Cronberg, S., Nordén, G., 1966 — Hypofibrinogenaemia and Massive Thrombosis. Acta Med. Scand., 180, 65

Nolf, P., 1904 — De la nature de l'hypoleucocytose propeptonique. Arch Int. Physiol., 1, 242

Nolf, /

Nolf, P., 1905 — Des modifications de la coagulation du sang chez le chien aprés extirpation du foie. Arch. Int. Physiol., 3, 1

Nolf, P., 1908a — Contribution à l'etude de la coagulation du sang (3e mémoire). Les facteurs primordiaux, leur origine. Arch. Int. Physiol., 6, 1

Nolf, P., 1908b — Contribution à l'etude de la coagulation du sang (4e mémoire). La formation de la fibrine. Arch. Int. Physiol., 6, 115

Nolf, P., 1921 — Action coagulante du chloroforme sur le plasma de chien. Arch.Int.Physiol., 18, 549

Nolf, P., 1922a — La thrombine du sérum chloroformique de chien et les venins de serpent. Arch. Int. Physiol., 19,227

Nolf, P., 1922b — Injection au chien de sérum chloroformique homologue. Arch. Int. Physiol., 19, 292

Norman, P.S., 1958 — Studies of the plasmin system. II. Inhibition of plasmin by serum or plasma. J. Exp. Med., 108, 53

Nour-Eldin, /

Nour-Eldin, F., Draisey, T.F., 1963 — Hepatic and cardiac necrosis in a patient with prostatic carcinoma given E-amino-caproic acid. J. Clin. Path., 16, 61

Ohler, W.G.A., Fischer, J., Enders, W., Kikillus, B., 1966 — Hypofibrinogenämische hämorrhagische Diathese bei Prostatakarzinom. Deut. Med. Wschr., 91, 119

Okamoto, S., 1954 — Patent Specification 770693: The Patent Office, London, p.9, Oct. 21. Mitsubishi Kasei Kogyo Co.Ltd.

Okamoto, S., Okamoto, U., 1962 — Amino-methyl-cyclohexane-carboxylic acid : AMCHA. A new potent inhibitor of the fibrinolysis. Keio J. Med., 11, 105

Opie, E.L., Barker, B.I., 1907 — Leucoprotease and anti-leucoprotease of mammals and of birds. J. Exp. Med., 9, 207

Pandolfi, M., Astrup, T., 1967 — A Histochemical Study of the Fibrinolytic Activity. Cornea, Conjunctiva, and Lacrimal Gland. Arch. Ophthal., 77, 258

Pandolfi, M., Nilsson, I.M., Robertson, B., Isacson, S., 1967 — Fibrinolytic Activity of Human Veins. Lancet, 2, 127

Pellman, /

Pellman, C.M., Ridlon, H.C., Phillips, L.L., 1966 — Manifestation and management of hypofibrinogenemia and fibrinolysis in patients with carcinoma of the prostate. J. Urol., 96, 375

Penn, S.R., Walker, J.H., 1954 — Defective blood coagulation following pulmonary surgery. New Engl. J. Med., 250, 764

Permin, P.M., 1947 — Properties of the Fibrinokinase-Fibrinolysin System. Nature, 160, 571

Permin, P.M., 1949 — Undersøgelser over fibrinolytiske Enzymer. Thesis, Copenhagen : Store Nordiske Videnskabsboghandel.

Phillips, L.L., Skrodelis, V., 1958 — A comparison of the fibrinolytic enzyme system in maternal and unbilical-cord blood. Pediatrics, 22, 715

Phillips, L.L., Skrodelis, V., Furey, C.A., 1959 — The fibrinolytic enzyme system in prostatic cancer. Cancer, 12, 721

Plauchu, M., Potton, F., Brizard, F., Rouchon, M.-Th., 1955 — Purpura thrombocytopénique mortel révélateur d'un cancer de la prostate. Commentaires à propos d'une nouvelle observation. Lyon Méd., 42, 327

Ploug, /

Ploug, J., Kjeldgaard, N.O., 1957 — Urokinase. An activator of plasminogen from human urine. I. Isolation and properties. Biochim. Biophys. Acta, 24 278

Prentice, C.R.M., McNicol, G.P., Douglas, A.S., 1969 — In vitro and in vivo studies with urokinase. Communication read at Meeting, Scottish Soc. Exper. Med., Edinburgh, May 31, 1969. Publication pending.

Prout, G.R., Siegel, M., Cliffton, E.E., Whitmore, W.F., 1956 — Hemorrhagic diathesis in patients with carcinoma of prostate. J.A.M.A., 160, 840

Ramioul, H., 1960 — Syndrome hémorragique au cours d'un cancer prostatique. Discussion de l'éventualité d'une fibrinolyse. Acta Urol. Belg., 28, 93

Rapaport, S.I., Chapman, C.G., 1959 — Coexistent Hypercoagulability and Acute Hypofibrinogenemia in a Patient with Prostatic Carcinoma. Amer. J. Med., 27, 144

Rasmussen, J., Albrechtsen, O.K., 1960a — Characterisation of the Fibrinolytic Components in the Human Prostate. Scand. J. Clin. Lab. Invest., 12, 261

Rasmussen, J., Albrechtsen, O.K., 1960b — Fibrinolytic activity in Human Seminal Plasma. Fertil. Steril., 11, 264

Rasmussen, /

Rasmussen, J., Albrechtsen, O.K., Astrup, T., 1958
The Fibrinolytic Activity in the Human Prostate and in Seminal Fluid.
Proc. 6th Congr. Europ. Soc. Haemat., 2, 494
Basel : Karger

Rasmussen, J., Ladehoff, A., 1959
Fibrinolytic Activity in Blood Plasma after Prostatectomy for Benign Hypertrophy.
Proc. 7th Congr. Europ. Soc. Haemat., London, 1959; part II, p. 902
Basel : Karger, 1960

Rasmussen, J., Schwartz, M., 1957
Metastaserende cancer prostatae med haemorrhagisk diathese forårsaget af fibrinolyse.
Ugeskr. Laeger, 119, 1086

Ratnoff, O.D., 1952
Studies on a proteolytic enzyme in human plasma. VII. A fatal hemorrhagic state associated with excessive plasma proteolytic activity in a patient undergoing surgery.
J. Clin. Invest., 31, 521

Roos, J., 1957
Blood coagulation as a continuous process.
Thromb. Diath. Haemorrh., 1, 471

Rosenmann, M., 1920
Über Fibrinolyse.
Biochem. Zeit, 112, 98

Roy, /

Roy, A., 1957 — Egg Yolk-Coagulating Enzyme in the Semen and Cowper's Gland of the Goat. Nature, 179, 318

Rulot, H., 1904 — Intervention des leucocytes dans l'autolyse de la fibrine (Fibrinolyse de Dastre). Arch. Int. Physiol., 1 152

Runnström, J., Lindvall, S., Tiselius, A., 1944 — Gamones from the Sperm of Sea Urchin and Salmon. Nature, 153, 285

Sack, E., Spaet, T.H., Gentile, R.L., Hudson, P.B., — Reduction of Postprostatectomy Bleeding by Epsilon-Amino-caproic Acid. New. Engl. J. Med., 266, 541

Salmon, J., 1961 — Mise en évidence de produits de fibrinolyse ou de fibrinogénolyse dans le sang humain normal. C.R. Soc. Biol. (Paris), 155, 1154

Salomon, L., Stefanini, M., 1954 — Control of Bleeding Manifestations in "Fibrinolytic States" by Corticotropin and Cortisone. Clin. Res. Proc., 2, 33

Sato, S., Ishibashi, Y., Endo, T., Watanabe, T., Nakajima, K., 1959 — Clinical use of E-amino-N-caproic acid on metropathia hemorrhagica. Keio J. Med., 8, 267

Schmid, /

Schmid, J., 1951 — Die Blutgerinnung in Theorie und Praxis. Wien.

Schmutzler, R., Fürstenberg, H., 1966 — Fibrinolyse und Blutverlust nach Prostata-operationen und deren Beein flussbarkeit durch Antifibrinolytika. Deut. Med. Wschr., 91, 297

Schumacher, G.F.B., Pearl, M.J., 1968 — Alpha-Antitrypsin in Cervical Mucus. Fertil. Steril., 19, 91

Scott, E.V.Z., 1956 — Prostatic enzymes and abnormal bleeding. J. Urol., 75, 116

Scott, E.V.Z., Matthews, W. F., Butterworth, C.E., Frommeyer, W.B., 1954 — Abnormal plasma proteolytic activity. Diagnosis and treatment. Surg. Gynec. Obstets., 99 679

Seale, R.A., Jampolis, R.W., Bargen, J.A., 1951 — Clotting defect in the presence of metastatic carcinoma of prostate. A case report. Surg. Clin. N.Amer., 31, 1111

Serre, H., Izarn, P., Simon, L., 1963 — Grand syndrome hémorragique par fibrinolyse au cours d'une ostéose prostatique métastatique. Rev. Rhum., 30, 32

Sharp, A.A., 1964 — Pathological Fibrinolysis. Brit. Med. Bull., 20, 240

Sherry, /

Sherry, S., 1954 — The fibrinolytic activity of streptokinase activated human plasmin. J. Clin. Invest., 33, 1054

Sherry, S., Fletcher, A.P., Alkjaersig, N., 1959 — Fibrinolysis and Fibrinolytic Activity in Man. Physiol. Rev., 39, 343

Sigstad, H., 1961 — Hemoragisk diathese ved prostata-lidelser med fibrinolyse og fibrinogenopeni. Behandlung med epsilon-amino-kapronsyre. Farmakoterapi, 17, 73

Smith, M.J.V., 1966 — Prostatic Corpora Amylacea. Monogr. Surg. Sci., 3, 209

Soulier, J.P., Mathey, J., Le Bolloch, A.G., Daumet, P., Fayet, H., 1952 — Syndromes hémorragiques mortels avec incoagulabilité totale par défibrination et avec fibrinolyse. I. Au cours des exérèses pulmonaires. Rev. Hematol., 7, 30

Stefan, H., Chrobák, L., Nerad, V., Groh, J., 1959 — Hypofibrinogenämie und Fibrinolyse als Blutungsursache beim Prostatakarzinom. Zeit. Urol., 52, 735

Stefanini, M., 1952 — Fibrinolysis and "Fibrinolytic Purpura". Blood, 7, 1044

Stefanini, /

Stefanini, M., 1958 — Fibrinolysis as Mechanism of Hemorrhagic Tendency. Acta Haemat., 20, 85

Stefanini, M., Dameshek, W., 1955 — The Hemorrhagic Disorders : A clinical and therapeutic approach. Grune and Stratton : New York.

Stefanini, M., Gendel, B.R., 1953 — Influence of Fibrinolysin on the Survival "in vivo" of Various Coagulation Factors and Favorable Effect of Cortisone in "Fibrinolytic Purpura". Clin. Res. Proc., 1, 5

Steichele, D.F., Herschlein, H.J., 1961 — Zur antifibrinolytischen Wirkung des Trypsin-Kallikrein-Inaktivators. Med. Welt, 42, 2170

Sterns, L.P., Lillehei, C.W., 1967 — Effect of epsilon aminocaproic acid upon blood loss following open-heart surgery : an analysis of 340 patients. Canad. J. Surg., 10, 304

Stockard, C.R., Papanicolaou, G.N., 1919 — The vaginal closure membrane, copulation, and the vaginal plug in the guinea-pig, with further considerations of the oestrous rhythm. Biol. Bull. Woods Hole, 37, 222

Stocker, H., Maier, C., 1964 — Chronische Fibrinolyse bei Prostatakarzinom. Schweiz. Med. Wschr., 94, 1373

Storm, /

Storm, O., 1955 — Fibrinolytic activity in human tears. Scand. J. Clin. Lab. Invest., 7, 55

Surós, J., Foz, M., 1965 — Carcinoma de prostata. Diatesis hemorragica por hiperfibrinolisis. Med. Clin. (Barc.), 44, 259

Swan, H.T., Kerridge, D.F., 1965 — Fibrinolysis and carcinoma of the prostate. J. Clin. Path., 18, 330

Swan, H.T., Wood, K.F., Daniel, O., 1957 — Dangerous bleeding associated with carcinoma of prostate. Brit. Med. J., 1, 495

Tagnon, H.J., 1942 — The significance of fibrinolysis in the mechanism of coagulation of blood. Science, 95, 334

Tagnon, H.J., 1953 — Enzymes in clinical medicine. Recent Developments in the Practical Application of Enzymes to Clinical Medicine. New Engl. J. Med., 249, 650

Tagnon, H.J., Palade, G.E., 1950 — Activation of proplasmin by a factor from mammalian tissue. J. Clin. Invest., 29, 317

Tagnon, H.J., Petermann, M. L., 1949a — Activation of Proplasmin by a Tissue Fraction. Proc. Soc. Exp. Biol. Med., 70, 359

Tagnon, /

Tagnon, H.J., Petermann, M. L., 1949b — A Proplasmin Activator from Mammalian Tissue. J. Clin. Invest., 28, 814

Tagnon, H.J., Whitmore, W. F., Schulman, P., Kravitz, S.C., 1953a — The significance of fibrinolysis occurring in patients with metastatic cancer of the prostate. Cancer, 6, 63

Tagnon, H.J. Schulman, P., Whitmore, W.F., Leone, L.A., 1953b — Prostatic Fibrinolysin. Study of a Case Illustrating Role in Hemorrhagic Diathesis of Cancer of the Prostate. Amer. J. Med., 15, 875

Thomas, K., Goerne, M.H.G., 1914 — Über die Herkunft des Kreatins im tierischen Organismus. II. Mitteilung. Das Verhatten der E-Guanido-, E-Ureido- und E-Amino-n-capronsäure im Organismus des Kaninchens. Hoppe-Seyler's Zeit. Physiol. Chem., 92, 163

Tice, D.A., Reed, G.E., Clauss, R.H., Worth, M.H., 1963 — Hemorrhage due to fibrinolysis occurring with open-heart operations. J. Thorac. Cardiovasc. Surg., 46, 673

Tillett, W.S., Garner, R.L., 1933 — The fibrinolytic activity of hemolytic streptococci. J. Exp. Med., 58, 485

Tjioe, D.Y., Oentoeng, S., 1968 — The Viscosity of Human Semen and the Percentage of Motile Spermatozoa. Fertil. Steril., 19, 562

Tocantins, /

Tocantins, L.M., Lindquist, J.N., 1947 — Thromboplastic Activity of the Urine. Proc. Soc. Exp. Biol. Med., 65, 44

Tocantins, L.M., Reid, W.O., Silver, M.J., Kazal, L.A., 1964 — Current problems in hemostasis. Ann. N.Y. Acad. Sci., 115, Article 1, 21

Todd, A.S., 1958 — Fibrinolysis Autographs. Nature, 181, 495

Todd, A.S., 1959 — The histological localisation of fibrinolysin activator. J. Path. Bact., 78, 281

Todd, A.S., 1961 — On the fibrinolytic activity of tissues. M.D. Thesis, University of Durham.

Todd, A.S., 1964 — Some topographical observations on fibrinolysis. J. Clin. Path., 17, 324

Todd, A.S., 1969a — Local factors in thrombolysis. J. Clin. Path., 22, 368

Todd, A.S., 1969b — Fibrinolytic activity in small blood vessels. Communication read at Scientific Meeting, British Microcirculation Society, Glasgow, September 18, 1969

Todd, /

Todd, A.S., Nunn, A., 1967 — Fibrinolytic activity in tissues and thrombi. Proc. First International Symposium on Tissue Factors in the Homeostasis of the Coagulation-Fibrinolysis System, Firenze, Maggio, p.57

Turpie, A.G.G., Forbes, C. D., McNicol, G.P., 1967 — Idiopathic Gangrene in African Children. Brit. Med. J., 3, 646

Tyler, A., 1939 — Extraction of an egg membrane-lysin from sperm of the giant keyhole limpet (Megathura crenulata). Proc. Nat. Acad. Sci.,U.S.A., 25, 317

Tyler, A., O'Melveny, K., 1941 — The role of Antifertilizin in the fertilization of sea-urchin eggs. Biol. Bull., Woods Hole, 81, 364

Tympanidis, K., Astrup, T., 1968 — Fibrinolytic Activity of Rat, Rabbit and Human Sperm Cells. Proc. Soc. Exp. Biol. Med., 129, 179

Urlus, J.H.L., 1962 — Fibrinolysis in the Surgical Clinic. J. Int. Coll. Surg., 38, 38

Verstraete, /

Verstraete, M., Vermylen, J., Amery, A., Vermylen, C., 1966 — Thrombolytic therapy with Streptokinase using a Standard Dosage Scheme. Brit. Med. J., 1, 454

Vlaardingerbroek, W.M., 1960 — Fibrinogeentekort bij prostaatcarcinoom. Nederl. T. Geneesk., 104, 1330

Vogel, R., Trautschold, I., Werle, E., 1968 — Natural Proteinase Inhibitors, p.104.- Academic Press : London.

von Kaulla, K.N., 1947 — Betrachtungen zur post-narkotischen Fibrinolyse. Schweiz. Med. Wschr., 77, 313

von Kaulla, K.N., 1952 — Discussant in : Pepsinogen in Human Seminal Fluid. Lundquist, F., Seedorff, H.H. Nature, 170, 1115

von Kaulla, K.N., 1953 — Über Beziehungen zwischen dem fibrinolytischen Ferment des Blutes und dem des Sperma. Zbl. Gynaek., 75 1068

von Kaulla, K.N., 1956 — Methods for Preparation of Purified Human Thromboplastin and Fibrinolysokinase from Urine. Acta Haemat. (Basel), 16, 315

von Kaulla, K.N., 1960 — Designated Discussion, p.87, Proc. Conference Thrombolytic Agents, Hematology Study Section, P.H.S., April 8-9, Chicago, Ill.

von /

von Kaulla, K.N., Shettles, L.B., 1953 — Relationship between human seminal fluid and the fibrinolytic system. Proc. Soc. Exp. Biol. Med., 83, 692

von Kaulla, K.N., Shettles, L.B., 1954 — Beitrag zur Kenntnis des proteolytischen Fermentsystems in menschlichen Spermaplasma, Mucus cervicalis, Tubarschleimhout und liquor folliculi. Klin. Wchnschr., 32, 468

von Kaulla, K.N., Shettles, L.B., 1956 — Thromboplastic Activity of Human Cervical Mucus and Ovarian Follicular and Seminal Fluids. Fertil. Steril., 7, 166

von Kaulla, K.N., Swan, H., 1958 — Clotting deviations in man during cardiac bypass : fibrinolysis and circulating anticoagulant. J. Thorac. Surg., 36, 519

von Rokitansky, C., 1852 — A Manual of Pathological Anatomy, translated by George E. Day, London : Sydenham Society, Vol. 4, p. 261

Vreeken, J., 1965 — Pathologische intravasale bloedstolling. Nederl. T. Geneesk., 109,897

Vulpé, /

Vulpé, M., Usher, D., Leblond, C.P., 1956 — Influence of the extirpation of male accessories on the occurrence of a "soft calculus" in the urinary bladder of the rat. Endocrinology, 59, 257

Wagner, B.M., Kim, H.S., 1963 — Histochemical studies of fibrinoid substances and other abnormal tissue proteins ; VI. Comparative reactivity of corpora amylacea and mucous secretions. Ann. N.Y. Acad. Sci., 106, 804

Walker, G., 1910 — A Special Function Discovered in a Glandular Structure hitherto supposed to form a part of the Prostate Gland in Rats and Guinea-pigs. Bull. Johns Hopkins Hosp., 21, 182

Warren, B.A., 1964 — Fibrinolytic activity of vascular endothelium. Brit. Med. Bull., 20, 213

Watson, C.J., Schultz, A.L., Wikoff, H.M., 1947 — Purpura following estrogen therapy, with particular reference to hypersensitivity to (Diethyl) stilbestrol and with a note on the possible relationship of purpura to endogenous estrogens. J. Lab. Clin. Med., 32, 606

Weil, /

Weil, A.J., Rodenburg, J.M., 1960 — Immunological Differentiation of Human Testicular (Spermatocele) and Seminal Spermatozoa. Proc. Soc. Exp. Biol. Med., 105, 43

Weiner, M., Redisch, W., Steele, J.M., 1958 — Occurrence of Fibrinolytic Activity Following Administration of Nicotinic Acid. Proc. Soc. Exp. Biol. Med., 98, 755

Welborn, J.K., Brennan, M. J., Hathaway, J.C., 1964 — Acute Fatal Fibrinolysis with Gastric Carcinoma. Amer. J. Surg., 108, 344

Wendt, H., Perlick, E., Seyffarth, G.H., 1957 — Zur Blutung und Nachblutung bei der Prostatektomie. Langenbecks Arch. Deut. Zeit. Chir., 286, 322

Whitehouse, H.B., 1914 — The physiology and pathology of uterine haemorrhage. Lancet, 1, 877

Woolf, N., 1961 — The distribution of fibrin within the aortic intima. An immunohistochemical study. Amer. J. Path., 39, 521

Ying, S.H., Day, E., Whitmore, W.F., Tagnon, H.J., 1956 — Fibrinolytic Activity in Human Prostatic Fluid and Semen. Fertil. Steril., 7, 80

Yudin, S.S., 1936 — Transfusion of cadaver blood. J.A.M.A., 106, 997

Zeh, /

Zeh, E., Ott, H., 1954 — Klinische Gesichtspunkte zur Entstehung der erworbenen Afibrinogenämie und Fibrinogenopenie mit hämorrhagischer Diathese. Acta Haemat. (Basel), 12, 106

Zimmermann, G., 1846 — Quoted by Fermi, C., in: Zeit. Biol., 28, 229

Zucker, M.B., 1964 — Unexplained bleeding in operations for neoplasias. Ann. N.Y. Acad. Sci., 115, Article 1, 225

ACKNOWLEDGEMENTS

ACKNOWLEDGEMENTS

The investigations which form the basis of this thesis would not have been possible but for the encouragement I received from many people.

In particular, I am deeply indebted to Dr. Alistair S. Todd for his continued interest and support, and I am grateful to Mrs. Anne Nunn for technical advice.

I wish to thank Professor D.M. Douglas for laboratory facilities, Mr. Stanley Soutar for access to biopsy material from his cases, Dr. David Piercy for his interest, Miss Mary Benstead, medical artist, for her assistance, and Dr. C. Cameron for the supply of human fibrinogen.

I am especially grateful to Mr. Tom King for his invaluable help in preparing the photomicrographs, and to Miss Nan Smith for typing the script.

I also thank the Board of Management, Dundee General Teaching Hospitals, for a research grant.