

## Physiology of fibrinolysis

# Clinical physiology of the fibrinolytic enzyme system

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Although much of the interest which has arisen in fibrinolysis in recent years has been in respect of the activation of this system in therapeutic attempts to dissolve thrombi in man, nevertheless inevitably much fundamental knowledge of this mechanism has been collected as a consequence of this developing therapeutic interest. Any original work described in this communication comes from my collaborators in Aberdeen or Glasgow; as the work comes from clinical departments the observations referred to are in respect of studies in man.

### Historical Introduction

It has been known for many years that human blood possesses fibrinolytic activity. John Hunter writing in 1794 in 'A treatise on the blood, inflammation and gun-shot wounds' records that in 'animals killed by lightning or electricity' or in animals 'who are run very hard, and killed in such a state' the blood does not clot. A partial explanation for this phenomenon was found in 1906 by Morawitz who noted that the blood from victims of sudden death contained no fibrinogen and could destroy the fibrinogen or fibrin of normal blood. Denis (1838) observed that the blood clots obtained in wet cupping redissolved in less than 24 hours. Green (1887) noted that fibrin prepared from ox blood, then dissolved in saline, could not be clotted again by thrombin. Hedin in 1903 found spontaneous fibrinolytic activity in the globulin fraction of ox blood. Macfarlane (1937) demonstrated that in man fibrinolytic activity in the blood could be provoked by a surgical operation.

### COMPONENTS OF THE FIBRINOLYTIC ENZYME SYSTEM

These are plasminogen, plasmin, activators, and inhibitors.

### Plasminogen

The evidence is that conversion to plasmin is a proteolytic phenomenon and four aspects seem reasonably well established: (1) activation is an enzymic reaction; (2) activation is irreversible; (3) splitting of a limited number of peptide bonds is involved; and (4) activators of plasminogen possess the property of splitting lysine and arginine esters and it seems likely that lysine and arginine bonds are split during the activation process, uncovering active sites.

### ACTIVATORS OF PLASMINOGEN

#### Blood activator

Plasminogen activator activity in the blood is responsible for physiological plasma fibrinolysis. Trace quantities of activator are present in normal plasma and increased amounts can be detected after exercise, emotional stress, surgical operations and other trauma, adrenaline administration, and nicotinic acid injection. There are differing grades of response amongst individuals to standard stimuli. *In vitro* plasma activator is labile at room temperature, less labile at 4°C, and apparently stabilized by fibrin formation. *In vivo*, plasma activator probably has a half-life of about 15 minutes.

The source of plasma activator is uncertain but it probably arises in part at least from vascular endothelium, a cholinergic effector mechanism perhaps being involved. It is well established that the rendering of a limb ischaemic by the application of a sphygmomanometer cuff followed by release results in blood from the ischaemic limb having increased activator activity. Whether anoxia is the stimulus for this, however, is uncertain because work done by Cunningham and collaborators (to be published) has failed to demonstrate a rise in activator level, when subjects are rendered hypoxic.

*Tissue activator*

Most tissues contain both an activator readily soluble in saline and a much less soluble activator probably protein bound which can be extracted with potassium thiocyanate. The insoluble plasminogen activator has been named fibrinokinase, to support the concept of active synthesis of activator in the tissues themselves. More activator is present in the supernatant than can be extracted from the cells of a culture, suggesting that activator is released from actively metabolizing cells rather than being a product of cell destruction.

Not only is plasminogen activator activity present in the walls of blood vessels, especially veins, but in tissues it is concentrated round blood vessels, particularly veins and venules, the arteries being less active.

Plasminogen activation activity can be isolated from almost all body tissues with the exception of liver and placenta. The failure to demonstrate activator in placenta may well relate to the physiology of the fibrinolytic enzyme system in pregnancy. Tissue activator activity has been reported to be localized in the lysosomal fraction of the cells. Tissues especially rich in activator include prostate, uterus, thyroid, and lung.

*Activator in secretions*

Plasminogen activator is found in milk, tears, saliva, and seminal fluid, but not in sweat; this may possibly have a function in maintaining the patency of small excretory ducts.

**Fibrinolysis and Repair Processes**

The widespread distribution of plasminogen activator in the tissues supports the concept of fibrinolysis having an extravascular as well as intravascular role. During the process of healing after myocardial infarction high concentration of tissue activator is found in the connective tissues, activity appearing to be correlated with organization, being localized in newly formed vessels.

**Fibrinolytic Activity of Urine**

Urokinase is a colourless protein, with a molecular weight of possibly 54 000, but this is debated; it is a proteolytic enzyme and activates plasminogen by first order kinetics, probably by splitting lysine and/or arginine bonds. Urokinase probably has a physiological role in maintaining the patency of the urinary tract by promoting lysis of fibrinous deposits.

Urokinase can be found in the supernatant from human kidney tissue culture. The relationship between urokinase and plasma activator is confused.

Human plasma activator, milk activator, and tissue activator (adrenal cortex and ovarian tissue) when examined are found immunologically to be different from urokinase.

In addition to the activators described above there is also an activator which can be extracted from red cells (Semar, Skoza, and Johnson, 1969).

*Autocatalytic activation*

If the reaction is stabilized in 50 per cent glycerol the plasmin itself can produce activation of activator-free plasminogen.

**Coagulation-dependent Activation of Fibrinolytic Activity**

Kaolin-activated plasma has a much shorter lysis time than intact (non-contact-activated plasma) when tested after acidification and lowering of ionic strength. This kaolin-induced generation of fibrinolytic activity requires factor XII (Hageman factor) and plasminogen and at least one further plasma factor which has been termed Hageman factor cofactor (HF cofactor) (Ogston, Ogston, Ratnoff, and Forbes, 1969). The euglobulin lysis time of intact normal plasma can be shortened from 140 minutes to 10 minutes by kaolin activation. If plasma is treated with glass of specific particle size and this then removed by centrifugation the supernatant plasma is deficient in an activity needed for activation-dependent fibrinolytic activity. Such glass-treated plasma contains normal concentrations of factor XII and of plasminogen, fibrinogen, and antiplasmin. This phenomenon is due to the removal of the Hageman factor cofactor.

**Factor XIII**

Urea-soluble clots are more sensitive to lysis than those formed in the presence of factor XIII.

**Inhibitors of the Plasminogen-plasmin System**

These are of two types, those inhibiting plasminogen activation and those which inhibit plasmin. Although there are methodological difficulties in differentiating anti-activator from antiplasmin activity, there is reasonable evidence that normal plasma possesses anti-activator activity.

Attention should be drawn to the labile activator-inhibitor which develops in serum as a consequence of coagulation occurring. This has been described by Bennett (1970) and may well be a phenomenon of considerable importance. This is investigated in a system rich in fibrinogen and plasminogen and inhibitor of urokinase activity is tested. A very

marked difference between plasma and serum can be seen, the serum concentration being much higher than that in plasma. The inhibitor is present in serum and requires for its development conditions similar to those necessary for coagulation to occur by intrinsic thromboplastin formation. The presence of large numbers of platelets increases the amount of inhibitor formed. This inhibitor may be advantageous to the organism to ensure the persistence of fibrin deposited in injured tissues in the initiation of the healing process.

#### Physiological Variations in Fibrinolytic Activity

Emotional stress and exercise can cause increased plasma activator levels. There is a circadian rhythm in blood fibrinolysis with more activity by day than by night and some variation throughout the day. Activator activity is diminished in the obese and markedly so at the end of pregnancy. Exercise, obesity, and pregnancy are physiological variants.

The relationship between anoxia and fibrinolysis is not clear. It is established that fibrinolytic activity is increased in ischaemic limbs and venous occlusion has been shown to increase the fibrinolytic activity of blood within the occluded segment. These changes have been ascribed to anoxia but in volunteers, studied by Cunningham and his colleagues (1970), rendered acutely hypoxic or hyperoxic by breathing high and low oxygen-containing mixtures respectively, euglobulin lysis activity was unchanged.

Racial differences may also be important. Studies made in East Africa found greater fibrinolytic activity and plasminogen levels in African medical students as compared with those of European controls (Mackay, Ferguson, and McNicol, 1970).

#### EXERCISE

It is well known that exercise increases fibrinolytic activity. The precise mechanism, however, is uncertain. Injection of nicotinic acid is known to cause a brisk fibrinolytic response but the mechanism rapidly fails to respond to repeated injection; however, when response to nicotinic acid has ceased, there is still a response to exercise indicating that the effector mechanism must differ. The reverse phenomenon can also be shown (McNicol and Douglas, 1965).

In observations from Aberdeen the levels of the blood components of the fibrinolytic enzyme system were measured during and after a four-hour period of exercise in eight subjects (Bennett, Ogston, and Ogston, 1968). The mean plasminogen activator level rose progressively over the first three hours with little further change during the fourth hour of exercise. Following the period of exercise there

was a rise in the circulating anti-activator which was still present 20 hours after completion of the exercise. There was no alteration in the mean blood level of plasminogen, fibrinogen, antiplasmin, or the serum inhibitor of plasminogen activation during or following the exercise.

#### WEIGHT CHANGE

In observations also made in Aberdeen plasma fibrinolysis was diminished in obese subjects, some of whom had increased levels of fibrinogen (Ogston and McAndrew, 1964; Bennett *et al*, 1966). Plasminogen levels were normal. Obese persons showed an increased fibrinolysis during rapid weight loss induced by dietary restriction. Similar dietary restriction in lean persons also increased fibrinolysis.

#### PREGNANCY

Although we accept pregnancy as a physiological state, there are very marked changes compared with the non-pregnant state.

Plasminogen and fibrinogen concentrations rise throughout pregnancy and return to normal in the puerperium (Bonnar, McNicol, and Douglas, 1969a). Activator levels, as reflected in the euglobulin lysis times, are markedly inhibited as pregnancy advances and the levels return to normal with extraordinary rapidity when the placenta has been delivered. This inhibition may be a mechanism to secure maximal haemostasis at the placental site.

Fibrinogen degradation products have been measured in pregnancy and throughout the gestation period low levels are present which increase in the puerperium, presumably due to the removal of fibrin from the placental site. As activator level rises when delivery of the intrauterine contents has been completed, when the level of fibrinogen degradation products in the blood follows (Bonnar, Davidson, Pidgeon, McNicol, and Douglas, 1969).

Pregnancy and its complications raises the issue as to the 'normal concentrations' of components of the fibrinolytic enzyme system. In a study of the coagulation and fibrinolytic mechanisms in abruptio placentae Bonnar, McNicol, and Douglas (1969b) demonstrated that levels of fibrinogen and plasminogen, acceptable as normal in the non-pregnant state, may represent a very significant fall for the pregnant patient—for example, suffering from abruptio placentae.

The remarkable speed with which the fibrinolytic mechanism can respond under physiological circumstances can also be seen in pregnancy. When the placenta has been delivered the level of activator can return to normal non-pregnant levels within one hour (Bonnar, McNicol, and Douglas, 1970).

The site of blood sampling has also been shown

to be of major importance in studies on the pregnant patient. In patients undergoing caesarean section, blood taken from the uterine vein at the time of placental separation was compared with peripheral

vein blood. There was considerable activator activity in the uterine vein blood but negligible amounts in the peripheral vein blood (Bonnar, Prentice, McNicol, and Douglas, 1970).

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