The fibrinolytic activity of menstrual blood

By

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Several hypotheses have been proposed in order to explain the inability of normal menstrual blood to clot spontaneously. Neither the alkaline secretion of the uterine cervix nor the acid secretion of the vagina are responsible for the fluidity as suggested by some authors (for references see King, 1921). It is now generally accepted that the fluidity of menstrual blood is caused by the absence of fibrinogen and fibrin and that this is due to a fibrinolytic activity in the discharge. Opinions have varied concerning the nature of the fibrinolytic agents involved.

Investigations over the last few years have shown the fibrinolytic system in the organism to be very complicated (for references see Astrup, 1956). The fibrinolytic enzyme (plasmin, fibrinolysin) normally exists in the blood as a precursor (plasminogen, profibrinolysin). The transformation of plasminogen into plasmin is brought about by activators. Such activators are contained in different tissues (Astrup & Permin, 1947). Furthermore normal human blood contains a precursor of a plasminogen activator. The transformation of this precursor into a plasminogen activator is caused by certain active agents (lysokinas). Streptokinase acts as a lysokinsase (Müllertz & Lassen, 1953) and certain tissues contain tissue lysokinases (Astrup & Sterndorff, 1956 b). The interactions involved are presented in Fig. 1.

![Diagram of fibrinolytic system]

Fig. 1.
proactivator

lysokinsase → plasminogen
activator → plasmin
The present paper reports experiments on the fibrinolytic activity of menstrual blood. The experiments were performed in order to elucidate the nature of the fibrinolytic system in menstrual blood in the hope of finding the underlying cause of the fibrinolysis.

MATERIALS AND METHODS

Menstrual discharge was obtained from 9 normal women by means of a rubber vaginal diaphragm. The blood was collected after 4–8 hours and potassium oxalate added as an anticoagulant. After centrifugation the plasma was investigated immediately or stored at –20°C until used.

Streptokinase («Varidase») was kindly obtained from the Lederle Laboratories. The stock solution contained 1 mg. per ml. and was diluted 1 to 25 times in barbital buffer before use. The barbital buffer was Michaelis diethylbarbiturate buffer 0.05-M; pH 7.3, containing 0.1-M NaCl. Total μ = 0.15.

Bovine plasminogen was prepared as described by Astrup & Sterndorff (1953). The plasma was precipitated isoelectrically as described by Milstone (1941) in order to remove inhibitory proteins, and the precipitate was redissolved in the original volume of barbital buffer. The euglobulin fraction obtained was investigated by the fibrin plate method (Astrup & Müllertz, 1952). The bovine fibrin in the standard fibrin plates contains large amounts of plasminogen. The activity recorded by this method is therefore caused by the plasminogen activators as well as by the plasmin in the samples applied to the plates. By heating the standard fibrin plate to 85°C in 35 minutes the plasminogen is destroyed (Lassen, 1952). This method was used in order to estimate the content of plasmin only in the menstrual plasma.

Dilutions curves obtained by applying different concentrations of the plasma (with barbital buffer) to the plates were prepared. The fibrinolytic activity was recorded as the diameter product (in mm²) of the lysed zones after 20 hours digestion at 37°C (average of 3 determinations).

RESULTS

Variable and in some cases considerable lysis was obtained when samples of isoelectrically precipitated menstrual plasma were applied to the standard fibrin plates. Addition of diluted streptokinase (1/3 vol.) to the samples produced in all cases an increase in the activity on the standard fibrin plates while streptokinase alone had no effect (Fig. 2). Digestion of the substrate was obtained when the samples were applied to heated fibrin plates (Fig. 3). These results demonstrate the presence of a fibrinolytic enzyme of varying and in some cases large amounts of an activator of plasminogen as well as of considerable amounts of a precursor of a plasminogen activator.

Addition of streptokinase (1/3 vol.) to the samples produced no or only very small increases in the activity on the heated fibrin plates, whereas the activity was increased considerably by the addition of bovine plasminogen
Activity of serial dilutions of isoelectrically precipitated menstrual plasma with barbital buffer on standard fibrin plates before (curve A) and after (curve B) addition of streptokinase. *Abscissa:* Concentration of plasma in % of stock solution. *Ordinate:* Activity as product of 2 diameters (mm²) of the lysed zones.

![Activity graph](image)

**Fig. 2.**

Activity of serial dilutions of isoelectrically precipitated menstrual plasma on heated fibrin plates. Menstrual plasma (0.75 ml.) + barbital buffer (0.25 ml.) = menstrual plasma (0.75 ml.) + streptokinase (0.25 ml.) (curve a). Menstrual plasma (0.75 ml.) + bovine plasminogen (0.25 ml.) (curve b). Menstrual plasma (0.75 ml.) + bovine plasminogen + streptokinase) (0.25 ml.) (curve c). Streptokinase: no activity. *Abscissa and ordinate:* see Fig. 2.

![Activity graph](image)

**Fig. 3.**

(1/3 vol.) (Fig. 3). This activity was further increased by the addition of streptokinase (1/3 vol.) containing plasminogen 30 mg. per ml.). These results indicate the presence of no or only very small amounts of plasminogen in the samples and confirm the previous results that considerable amounts of an activator of plasminogen and large amounts of a precursor of a plasminogen activator were present.

In one case menstrual discharge was collected on the first and second days...
Activity of serial dilutions of isoelectrically precipitated menstrual plasma on standard fibrin plates. Menstrual plasma from the first day of menstruation (curve a). Menstrual plasma from the second day of menstruation (curve b). Abscissa and ordinate: see Fig. 2.

of menstruation. A comparison between the activities in these two samples as estimated on the standard fibrin plates indicates that the amount of plasminogen activator decrease during menstruation (Fig. 4).

In order to characterize the plasminogen activator present in menstrual discharge we have studied its stability at different temperatures and pH values. After regulating the pH value (using 0.1-N HCl, 0.1--N NaOH and different buffers (citrate, phosphate, borate)) and heating for 30 minutes at 37°C, 50°C, 70°C and 100°C the samples were cooled to 0°C in iced water, neutralized and immediately applied to the standard fibrin plates in order to estimate the remaining activity (Fig. 5). The activator was stable at all pH values at 37° and 50°. At 70° it was stable at very acid reaction (pH 2) but deteriorated at higher

Fibrinolytic activity of isoelectrically precipitated menstrual plasma on standard fibrin plates after heating for 30 min. at 37°C, 50°C, 70°C, and 100°C. 

Abscissa: pH values of the samples during heating. Ordinate: Activity as product of 2 diameters (mm²) of the lysed zones (average of 3 determinations).
values and was completely destroyed above pH 4. At 100° a small effect was still left at pH 2. The remaining activities were not due to plasmin as no or only very small activities were recorded when the samples were applied to heated fibrin plates. Consequently the activities recorded on the standard fibrin plates were due to a plasminogen activator and not to plasmin.

**DISCUSSION**

*Bell* (1910) was the first to report the absence of fibrinogen in haematocolpos fluid and similar results were obtained in normal menstrual blood by *Cristea 

*Whitehouse* (1914) reported that blood obtained from uterine cavity during menstruation always clotted first and then underwent lysis. These findings drew attention to the fibrinolytic properties of menstrual discharge. Fibrinolytic activity in menstrual blood has since been reported several times. *Kross* (1923, 1924) found fibrinolytic activity in the uterine discharge from rats. He also observed that a mixture of normal blood with haematocolpos fluid underwent lysis after coagulation. *Andor & Waldbauer* (1928) reported an increased splitting of proteins in menstrual blood. Fibrinolytic activity in menstrual blood has also been reported by *Lozner, Taylor & Taylor* (1942), *Huggins, Vail & Davis* (1943), *Smith & Smith* (1945 a) and *Smith* (1947). The methods used by most of these authors make it possible to determine whether fibrinogen and fibrin is destroyed, but no information has been obtained concerning the fibrinolytic agents involved in the process.

Several investigations have been performed in order to elucidate the nature of the fibrinolytic process. The presence of fibrinolytic activity in the circulating blood during menstruation was reported by *Smith & Smith* (1945 b), *Willson & Munnell* (1946) and *Marx & Rovatti* (1952). However, this phenomenon might have no relation to the fibrinolytic activity in menstrual discharge and furthermore there is no agreement concerning these findings (*Macfarlane & Biggs*, 1946). Blood obtained from small incisions made in collum uteri during menstruation clotted spontaneously and did not undergo lysis (*Kross*, 1924). Consequently the factors involved in the fibrinolytic process are most probably localised in the endometrium. Several investigations have revealed the existence of fibrinolytic agents in the human endometrium and these agents have been considered the cause of the fibrinolytic activity in the menstrual blood. However, no agreements exists concerning the nature of these fibrinolytic agents. We found that human endometrium contains an activator of plasmogen. This
activator was of the stable type and was present in increasing amounts in the premenstrual phase and during certain abnormal types of uterine bleeding (for references see Albrechtsen, 1956).

The results presented here have revealed the existence of an activator of plasminogen in menstrual discharge. According to present knowledge two different kinds of plasminogen activators exists in the human organism. One is a stable tissue activator of plasminogen which is contained chiefly in various tissues. A second labile plasminogen activator can be formed from a precursor normally present in the blood by addition of streptokinase. The different stabilities of these two plasminogen activators at different temperatures and pH values make it possible to determine whether a fibrinolytic process is caused by one or the other of these two activators. The stabilities of the activator in menstrual blood at different temperatures and pH values correspond with the values obtained for the stable tissue activator of plasminogen contained in pig hearts (Astrup & Sterndorff, 1956) and in the normal human endometrium (Albrechtsen, 1956). The values differ from the stabilities obtained for the labile plasminogen activator formed in blood by addition of streptokinase (Müllertz, 1956). These results indicate that the activator contained in menstrual blood is derived from the endometrial tissue.

Addition of streptokinase to the menstrual plasma caused an increase in the activity on the standard fibrin plates and no increase on the heated plates. This indicates that large amounts of a precursor of a plasminogen activator were present in the menstrual blood. This is not surprising as such a precursor is known to exist as a normal component of blood. However, there seems to be no relation between this plasminogen proactivator and the plasminogen activator present in the menstrual discharge, which apparently entirely originates in the tissue.

The estimations from the heated fibrin plates have revealed the presence of a fibrinolytic enzyme (most probably plasmin) in menstrual blood. By addition of streptokinase to the menstrual plasma a transformation of the plasminogen proactivator into plasminogen activator is obtained (as shown in the experiments on the standard fibrin plates). Consequently the slight increase in the activity obtained by addition of streptokinase to the samples when applied to the heated fibrin plates indicates that only very small amounts of plasminogen are present in the menstrual plasma. The conclusions drawn from these experiments must therefore be that all or almost all plasminogen (which normally is present in blood plasma) has been transformed to plasmin in the menstrual plasma as a result of the action of a plasminogen activator of the stable type. These findings apparently explain the fluidity of the menstrual discharge.

Investigations by Whitehouse (1914) have shown that menstrual blood clots in the uterine cavity. This is possibly due to the existence of thromboplastic agents in the endometrium. A resolution of these fibrin clots must be of im-
portance for the removal of the blood from the cavity. Such a resolution can easily be performed by plasmin produced by the effect of activators on plasminogen. Frequently menstrual blood from normal women contains small clots of blood (Whitehouse, 1914). Furthermore it is known clinically that even large clots of blood can be present in the uterine discharge during certain abnormal bleedings (proliferative bleedings, endometrial hyperplasia). Although the endometrium in the premenstrual phase and in such pathological conditions contains large amounts of the tissue activator it is possible that the amount of the activator in these cases has been insufficient to cause a complete transformation of the plasminogen in the clots into plasmin and thus produce a resolution of the large fibrin clots formed during such vigorous bleeding. Another possibility is that only insufficient amounts of plasmin are formed in such conditions because of the too small amounts of plasminogen in the blood.

The amount of the plasminogen activator in menstrual blood decreases after the first day of menstruation. This is in accordance with the assumption that the activator is derived from the endometrium. Most of the endometrial tissue is discharged from the uterine cavity during the first day of menstruation.

SUMMARY

1. Human menstrual discharge contains an activator of plasminogen, a fibrinolytic enzyme (plasmin), large amounts of a precursor of a plasminogen activator, and no plasminogen.

2. The activator of plasminogen in menstrual discharge is similar to the plasminogen activator present in the human endometrium and in other tissues. It differs in stability from the plasminogen activator formed in blood by addition of streptokinase.

3. The presence of this activator explains the absence of fibrin in normal menstrual discharge and is the cause of the fluidity of menstrual blood.

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