

Acta Medica Okayama

Volume 15, Issue 5

1961

Article 4

OCTOBER 1961

The identification of menstrual blood by means of paper electrophoresis, a medico-legal study

Osamu Kamimura*

*Okayama University,

Copyright ©1999 OKAYAMA UNIVERSITY MEDICAL SCHOOL. All rights reserved.

The identification of menstrual blood by means of paper electrophoresis, a medico-legal study*

Osamu Kamimura

Abstract

Attempts were made to identify menstrual blood by means of paper electrophoresis with preparation of extracts of menstrual blood isolated under various conditions and mixed with human fibrin. Also similar analyses were conducted with blood aspirated from the median cubital vein of a woman during menstruation as well as from a man as the control, also with extracts of lochial blood from a woman after normal delivery, and of the blood obtained at artificial abortion. Animal fibrins (from rabbit, mouse, steer, and guinea pig) were also used to see the lytic action of the bloods. The following are the results of the present experiments. 1. The identification of menstrual blood by means of paper electrophoresis is a simple method in legal medicine and its electrophorogram is an excellent method to offer an evidence of proof for menstrual blood. 2. By this method it is possible to identify the menstrual bloodstain even after the lapse of time as much as 6 months. 3. It is possible to identify even putrefied menstrual bloodstain. 4. In the case where the material stained with menstrual blood is found in water, it is not possible to identify the menstrual blood by this method. 5. When the menstrual blood is heated at 60°C over 30 minutes, it becomes impossible to identify it by this method. 6. In the case of venous blood during menstruation fibrinolytic product can be detected only on the first day of menstruation, but since it appears only in trace, it is easy to differentiate it from menstrual blood. 7. As for lochial blood the fibrinolytic product can be detected only in the blood obtained on the first and second days of puerperium, but the amount being so slight that it can readily be distinguished from menstrual blood. 8. In the case of the blood obtained at artificial abortion fibrinolytic product appears just as much as in the case of menstrual blood, and thus it is impossible to differentiate it from menstrual blood by this method. 9. As for the use of human fibrin it is best to employ it while it is fresh, but the human fibrin up to 6 days old can be used. However, the older is the human fibrin the lesser the fibrinolytic product detectable. 10. In the case using animal fibrins mixed with the extract of menstrual blood some do produce fibrinolytic product in trace, but since there is a danger of also producing the fibrinolytic product-like substance in venous blood, it is advisable not to use animal fibrins.

*PMID: 14453450 [PubMed - OLDMEDLINE] Copyright ©OKAYAMA UNIVERSITY MEDICAL SCHOOL

Acta Med. Okayama 15, 317—326 (1961)

**THE IDENTIFICATION OF MENSTRUAL BLOOD BY
MEANS OF PAPER ELECTROPHORESIS,
A MEDICO-LEGAL STUDY**

Osamu KAMIMURA

*Department of Legal Medicine, Okayama University Medical School
Okayama (Director: Prof. Y. Mikami)*

Received for publication, May 25, 1961

In legal medicine identification of menstrual blood is often requested. At present we have several available methods of identifying menstrual blood e. g. a histochemical detection of glycogen containing vaginal epithelial cells found occasionally in the blood^{1,2}, identification of vaginal bacilli in the blood^{3,4}, serological tests^{5,6,7}, and the tests on fibrinolysin value⁸ and denatured fibrinogen⁹, but we can detect the vaginal epithelial cells and the vaginal bacilli not only in menstrual blood but vaginal secretion. Concerning serological tests the followers state that they can not detect the menstrual blood and fibrinolysin value test can identify the blood only in 5 days after the blood was discharged, detection of the denatured fibrinogen is good one among the methods mentioned above, but is used as preliminary test. Thus we still have hardly any reliable method of the identification.

Recently CULLIFORD¹⁰ reported a method to identify the blood extracts by paper electrophoresis, which were incubated with human fibrin at 37°C for 24 hours.

In the present paper the author describes some of his findings on his experiments in which he followed Culliford's method with a slight modification.

MATERIALS AND METHODS

Menstrual blood, the venous blood from menstruating woman, the lochial blood after normal delivery, and the blood obtained at artificial abortion served as the materials. For the control the blood from the median cubital vein of a man was used. For reactions fibrins obtained from normal human, rabbit, mouse, guinea pig, and steer were employed.

Fresh menstrual blood is absorbed to the balls of absorbent cotton or this ball is in turn wrapped with cotton cloth and by pressing the blood is absorbed to cotton cloth.

From these balls of cotton the blood extract is prepared at room temperature by soaking in 1.0 ml. distilled water 0.5 cm³. of the blood stained absorbent

cotton for 20 minutes.

The blood stained cotton cloth is divided into 3 groups, cotton cloth in the first group is used to make extract immediately, and that in the 2nd group is dried and left standing at room temperature for the periods of 1, 2, 3, 4, 5, and 6 months and then extracts are prepared, the cloth of the last group is left in tap water for 24 hours and after drying it is used for making extract.

Extracts are prepared from blood stained cotton cloth in all groups at room temperature by soaking 1.0 cm². of the cloth in 0.5 ml. distilled water respectively for 20 minutes but in the 2nd group for 60 minutes.

The extract obtained from the fresh blood stained cotton cloth (the 1st group) with distilled water is divided into 3 groups and the first group is used immediately, extract in the 2nd group is left standing in test tubes for one week at 37°C until it is putrefied as to emanate a foul smell, and the last group of extract is heated over a hot water bath at 60°C for 30 minutes.

The blood aspirated from the median cubital vein of a man is soaked in cotton cloth or in absorbent cotton, and extracts are prepared with distilled water as the control, and also this extract is treated just as the extract from menstrual blood stain.

Venous blood from the median cubital vein of a menstruating woman is absorbed to cotton cloth and after drying it the extract is prepared with 1.0 cm². of this dried blood stained cotton cloth by rinsing it in 0.5 ml distilled water.

The lochial blood of the woman after normal delivery is absorbed to balls of absorbent cotton from day to day and extract is prepared by soaking 0.5 cm³. of these blood stained cotton balls in 1.0 ml distilled water.

The blood obtained at artificial abortion performed on woman of the 2nd gravid month is absorbed to cotton cloth and extract is prepared with 1.0 cm². of the blood stained cloth by soaking it in 0.5 ml distilled water.

Normal blood of man, rabbit, mouse, guinea pig, and steer is centrifuged in dry centrifuge tubes for 10 minutes at 3,000 r. p. m. and fibrin clots in the components of serum and blood corpuscles are removed. The fibrin clots are washed with physiological saline solution and then with distilled water until the clots become white. Some of these are dried under sunlight and pulverized.

The fibrin isolated from about 3.0 ml. blood is added to each extract of blood (whose protein contents proved to be 3.0—5.0% as measured by Hitachi Hand Protein Refractometer) and the mixture is left standing at 37°C for 24 hours. After the reaction paper electrophoresis is performed. For the paper electrophoresis Kobayashi's electrophoretic apparatus (Natume) is employed. No. 51 of Toyo-Roshi, 26 cm. × 16 cm. in size serves as the filter paper and is used in horizontal position.

The electrodes are of silver, the electrode tank contains 1.0 per cent KCl

solution, the electrophoresis tank contains the Barbiton buffer solution (composed of 33.0 g. sodium diethyl-barbiturate, 19.5 g. sodium acetate, 205 ml. N/10 hydrochloric acid, dissolved in water as to make the total volume 3 l) adjusted to pH of 8.6.

Four lines of 2.0 cm. in length 1.0 cm. apart are drawn on the filter paper by a glass-pen dipped into the solution to be tested, and the electrophoresis is conducted for 16 hours at 80—120 V. and 7 mA. In this instance albumin spreads out about 8 cm. After removing the filter paper and drying it at 100°C for 30 minutes, it is stained with bromphenol blue and dried again at room temperature for 24 hours. This is made semi-transparent with paraffin at 80°C and measured by Kobayashi's papyrographic apparatus with a 590Å filter.

Estimation: In the papyrogram (by means of papyrography) it is found that: (1) the fibrinolytic product shows a distinct peak while there is a clear-cut valley in between hemoglobin and the peak, and the one that has a difference between the peak and valley of more than 10 per cent as compared with the optical density of the albumin peak is considered as (##); (2) the same as in the case of (##), the one that can be clearly distinguished as the peak of the fibrinolytic product but whose difference between the peak and valley is less than 10 per cent as compared with the optical density of the albumin peak is classified as (+); (3) the one that shows the peak of the fibrinolytic product but without any valley between it and the hemoglobin peak is classified as (+); (4) the one that shows a bulging at the crus of the cathode side of hemoglobin but showing no clear-cut peak is classified as (±); and (5) the one that reveals no change at all at the crus of the cathode side of hemoglobin is classified as (-).

RESULTS

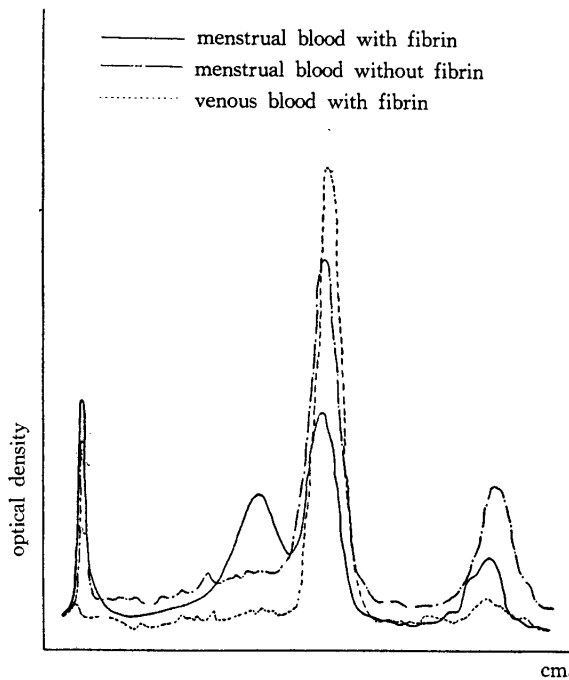
A comparative study of the methods: In the staining after paper electrophoresis Culliford used azocarmine B while the author employed bromphenol blue. After the paper electrophoresis performed with the use of the extracts from the fresh menstrual bloodstain and fresh human fibrin under the identically same conditions with the same reagents, one group was stained with azocarmine B while the other group by bromphenol blue. As the result it has been found that the fibrinolytic product can be clearly distinguished in the two stainings and that the valley between the fibrinolytic product and hemoglobin is far more distinct in the group stained with bromphenol blue (Fig. 2).

Experiments using the extract of fresh menstrual bloodstain: In the case of the extract from fresh menstrual bloodstain prepared as mentioned in the preparation, and mixed with fresh human fibrin, a line of spots appears on the cathode side of hemoglobin, which can be clearly distinguished from hemoglo-

bin, but in the case without the use of human fibrin no such a peak can be recognized. Moreover, since there appear no spots on the cathode side of hemoglobin in the case with the extract of venous bloodstain either with or without addition of human fibrin, it is possible to distinguish the menstrual bloodstain from the venous bloodstain.

The substance that appears on the cathode side of hemoglobin is thought to be fibrinogen or a fibrinogen-like substance (Fig. 1, 3).

Fig. 1. Papyrogram



Experiments with menstrual bloodstain left standing in room temperature for a long period of time: In the case with the extracts of the menstrual bloodstain prepared as in the preparation after being left standing in room temperature for the periods of 1, 2, 3, 4, 5, and 6 months, and mixed with fresh human fibrin there appear spots on the cathode side of hemoglobin just as in the case with the extract of fresh menstrual bloodstain mixed with human fibrin. However, in the case of these extracts without human fibrin as well as with the extract of venous bloodstain supplemented with fresh human fibrin no such spots can be recognized in either one of them. This fibrinolytic product shows no quantitative difference associated with the length of time the bloodstain has been left standing.

Experiments with putrefied extract of menstrual bloodstain: By the

paper electrophoresis of the putrefied extract isolated from menstrual bloodstain supplemented with fresh human fibrin, there appear the spots of fibrinolytic product and also similar spots can be seen at the same site even in the case of the putrefied extract of menstrual bloodstain without addition of human fibrin. On the other hand, in the case of putrefied extract of venous bloodstain either with or without fresh human fibrin there appears no such fibrinolytic product. Therefore, it is possible to identify menstrual bloodstain distinctly from venous blood even after its putrefaction.

Experiments with the extract of menstrual bloodstain which was immersed in water: In the case with the extract of menstrual bloodstain immersed in tap water and supplemented with fresh human fibrin no fibrinolytic product can at all be seen after the paper electrophoresis and hemoglobin is also indistinct.

With the extract of menstrual bloodstain, which has been heated at 60° C for 30 minutes: In the case of this extract no fibrinolytic spots can be observed even when supplemented with fresh human fibrin.

Comparative study of venous bloodstain at menstruation with menstrual bloodstain itself: In the case with the extract of the venous bloodstain from a woman on her first day of menstruation and mixed with fresh human fibrin, a minimal quantity of fibrinolytic product can be detected, but the amount being extremely small as compared with that of the extract from the menstrual bloodstain of the same woman taken on the same day, it can readily be distinguished from menstrual blood. In the case with the venous blood stain from a menstruating woman taken later than the second day of menstruation and mixed with fresh human fibrin no fibrinolytic product can at all be detected just as in the case of venous blood obtained from a man and mixed with human fibrin.

A comparative study of lochial bloodstain and menstrual bloodstain: In the case with the extract of lochial bloodstain obtained on the first day of the puerperium from a woman after normal delivery and mixed with fresh human fibrin, fibrinolytic product can be detected weakly, and the extract of such lochial bloodstain taken on the second day of the puerperium and prepared in the same manner shows it only slightly, but the quantities of the fibrinolytic product in both cases are extremely small as compared with that of the extract of menstrual bloodstain mixed with fresh human fibrin. In the extracts prepared from the lochial bloodstain taken later than the third day of the puerperium no fibrinolytic product can be detected. In the case with extract of the lochial bloodstain without addition of human fibrin likewise no fibrinolytic product can be recognized.

Comparison of bloodstain at artificial abortion and menstrual blood-

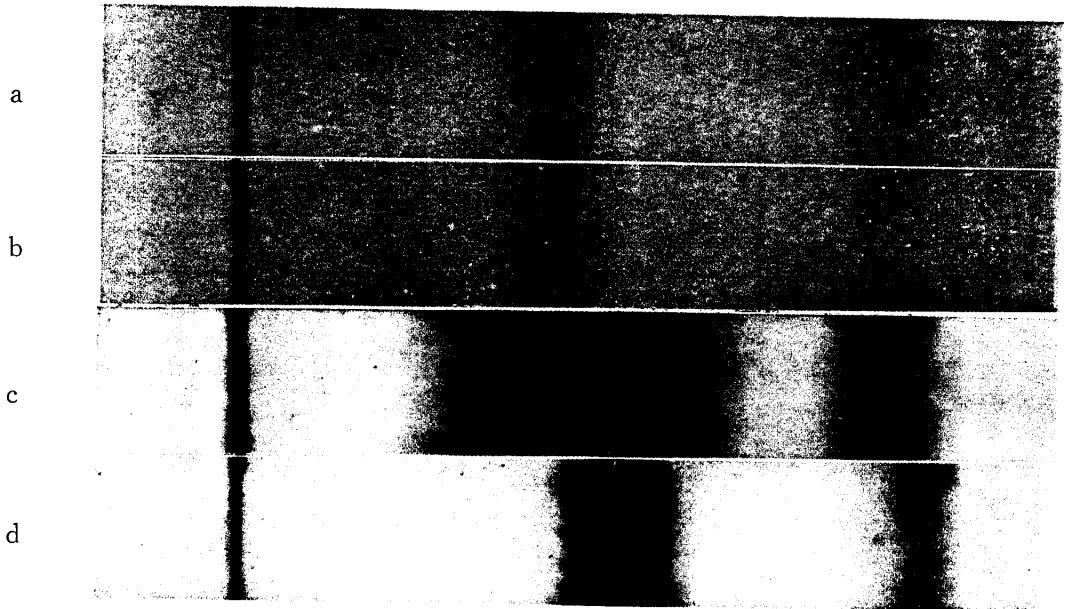


Fig. 2. Paper electrophoresis of the extract of fresh bloodstain mixed with fresh human fibrin
Factors: 7 mA/12.5 cm. 100—75V/22 cm. Barbiton buffer pH 8.6

Legends: a and b are stained by azocarmine B.

a: Venous bloodstain, b: Menstrual bloodstain,

c and d are stained by bromphenol blue.

c: Menstrual bloodstain, d: Venous bloodstain

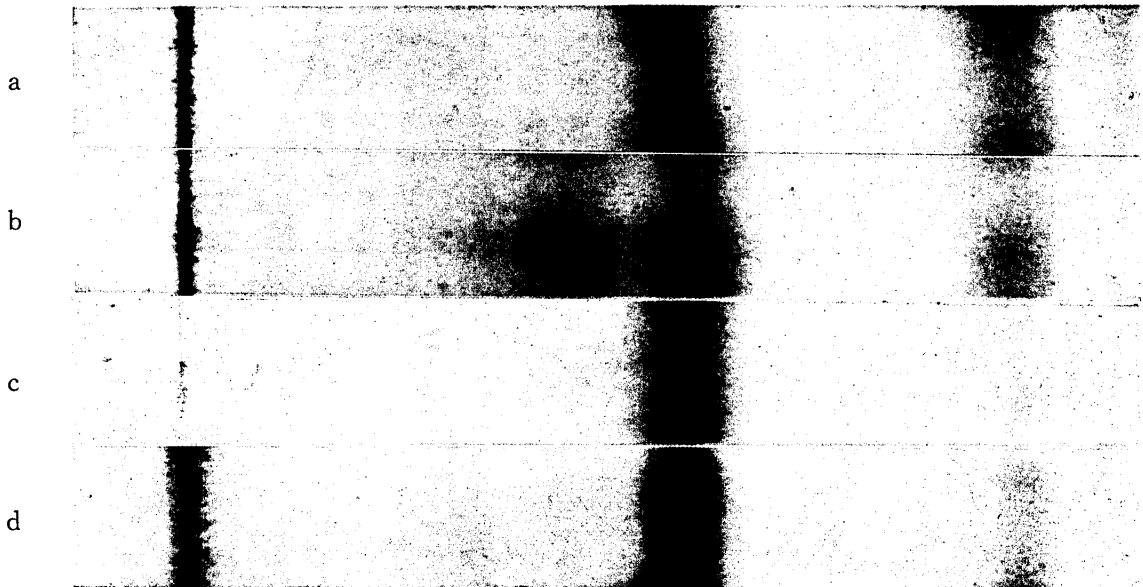


Fig. 3. Paper electrophoresis of the extract of fresh bloodstain mixed with or without fresh human fibrin

Factors: 7 mA/12.5 cm. 100—75V/22 cm. Barbiton buffer pH 8.6

Legends: a, b, c and d all stained with bromphenol blue.

- a: Menstrual bloodstain without fibrin
- b: Menstrual bloodstain with fibrin
- c: Venous bloodstain with fibrin
- d: Venous blood without fibrin

stain : When fresh human fibrin is added to the extract of the blood at artificial abortion, fibrinolytic product can be detected in the same degree as in the case with the extract of menstrual bloodstain mixed with fresh human fibrin. The results so far described above are illustrated in Table 1.

Table 1. Comparison of fibrinolytic product in the extracts of menstrual bloodstain under various conditions with extracts of various other bloodstains by means of paper electrophoresis

		Extract of menstrual bloodstain		Extract of venous bloodstain		Extract of other bloodstain	
		Without fibrin	With fibrin	Without fibrin	With fibrin	Without fibrin	With fibrin
Fresh		(-)	(+++)	(-)	(-)		
Old		(-)	(+++)	(-)	(-)		
Putrefied		(#)	(+++)	(-)	(-)		
Immersed in water		(-)	(-)	(-)	(-)		
Heated at 60°C for 30 min.		(-)	(-)	(-)	(-)		
Compared with venous blood at menstruation	1st day	(-)	(+++)	(-)	(-)	(-)	(+)
	2nd day	(-)	(+++)	(-)	(-)	(-)	(-)
Compared with lochial blood	1st day	(-)	(+++)	(-)	(-)	(-)	(+)
	2nd day	(-)	(+++)	(-)	(-)	(-)	(±)
	3rd day	(-)	(+++)	(-)	(-)	(-)	(-)
Compared with bloodstain at artificial abortion		(-)	(+++)	(-)	(-)	(-)	(+++)

Experiments with the use of dried and pulverized human fibrin : When dried human fibrin powder is added to the extracts of fresh menstrual bloodstain, fibrinolytic product can be detected moderately in the case with the pulverized human fibrin one and 2 days old, weakly with the fibrin of 3, 4, and 5 days old, only slightly with the fibrin 6 days old, and none at all with the fibrin older than 7 days.

Table 2. Quantities of fibrinolytic product as detected by paper electrophoretic method with the use of dried fibrin powder

Age of human fibrin (days after preparation)	immed. after	1	2	3	4	5	6	7	8
Quantities of fibrinolytic product	(+++)	(++)	(++)	(+)	(+)	(+)	(±)	(-)	(-)

Experiments with the use of animal fibrins: When the fresh fibrin isolated from rabbit blood is added to the extract of menstrual bloodstain, fibrinolytic product can be detected weakly, and only in a trace in the case of the extract of venous bloodstain. Using fresh mouse fibrin, the fibrinolytic product can be detected in a trace with the extract of menstrual bloodstain but none at all with the venous blood extract. With the use of fresh fibrin from guinea-pig blood, traces of fibrinolytic product can be observed both in the extracts of menstrual bloodstain and venous bloodstain. With fresh bovine fibrin it is detected weakly in both menstrual blood and venous blood extracts, but none with the use of dried bovine fibrin in either of these blood extracts.

Table 3. Detection of fibrinolytic product in the extracts of menstrual blood and venous blood with animal fibrins

extract \ fibrin	rabbit	mouse	guinea pig	steer (fresh)	steer (dried)
menstrual blood	(+)	(±)	(±)	(+)	(-)
venous blood	(±)	(-)	(±)	(+)	(-)

DISCUSSION

The fluidity of menstrual blood is a well-known fact, but as regards the causative factors of this fluidity opinions are divided. KRAUL¹¹ for an instance states that the coagulative element of menstrual blood plasma lacks fibrinogen and this is due to the lysis of fibrinogen by trypsin existing in the same blood plasma. MANO¹² and YAMADA¹³ also recognize the fibrinolytic action in the menstrual blood and they believe that this action originates from the myometrium. SMITH, O. W, and SMITH, G. V.¹⁴ likewise recognize the fibrinolytic action in the circulating blood during menstruation. Recently there are many who support the view that the fluidity of menstrual blood is dependent on fibrinogenolysis and fibrinolysis by plasmin and as for the mechanism of plasmin production it is believed that the plasminogen existing in human blood plasma is transformed into plasmin by the activating action of streptokinase-like substance. Consequently, it is natural to expect that so long as there exists plasmin in menstrual blood, fibrinolysis can be elicited by adding fibrin to the menstrual blood kept under optimal temperature and pH, thus giving rise to fibrinolytic product. On the basis of serological examinations of this fibrinolytic product SATOH¹⁵ considers it to be a denatured fibrinogen and he states that it has properties different from normal fibrinogen in that it does not salt out in the presence of neutral salts, it does not produce fibrin even when mixed with thrombin, and it is not easily soluble in distilled water. The substance produced in the present experiments by paper electrophoretic method, which is thought to be fibrinolytic product, ap-

pears around -2.00 when the mobility of hemoglobin and albumin is to be at -2.88 and -5.92 (as stated by ARMSTRONG¹⁶), respectively, and in addition, the site of fibrinolytic product changes somewhat with each electrophoresis. Judging from the facts and also from the fact that the mobility of fibrinogen at -2.06 and that it has affinity to filter paper, there is no evidence to uphold Satoh's contention that the fibrinolytic substance is fibrinogen.

According to TOWATARI¹⁷ the fibrinolytic action of menstrual blood decreases day by day and the identification of menstrual blood by means of estimating the fibrinolysin value is possible only with the lapse of 4~5 days after the obtaining of the blood sample. However, it is to be noted that by paper electrophoretic procedure the fibrinolytic product can be clearly detected even with the lapse of time as much as 6 months and that even the putrefaction of menstrual blood does not hinder the identification, which fact stands in a marked contrast to the other method.

In relation to the fibrinolytic activity of the venous blood during menstruation, KISHI¹⁸ by Caultier's method measured the quantities of amino acids produced from gelatin by the action of trypsin and states that the coagulability of circulating systemic blood is lowered during menstruation. YAMADA¹⁹, following the method of Satoh-Ueda, reports that he can not recognize any fibrinolytic activity in the circulating blood during menstruation. In the present experiments by paper electrophoretic method an extremely weak fibrinolytic activity was recognized in the circulating blood only on the first day of menstruation.

The fact that the incoagulable blood from asphyxiated body can not be differentiated from menstrual blood by paper electrophoretic method can be readily understood from the findings of the present experiments as well as from the idea that the causative factor of the fluidity in incoagulable blood from asphyxiated body and menstrual blood might be fibrinolysis.

The azocarmine B staining by Culliford and the bromphenol blue staining employed in the present experiments are believed to be excellent stainings, but in the author's own experiences the staining by bromphenol blue is superior in the detection of fibrinolytic product, and in addition, it is a less-costly dye.

CONCLUSION

Attempts were made to identify menstrual blood by means of paper electrophoresis with preparation of extracts of menstrual blood isolated under various conditions and mixed with human fibrin. Also similar analyses were conducted with blood aspirated from the median cubital vein of a woman during menstruation as well as from a man as the control, also with extracts of lochial blood from a woman after normal delivery, and of the blood obtained at artificial

abortion. Animal fibrins (from rabbit, mouse, steer, and guinea pig) were also used to see the lytic action of the bloods. The following are the results of the present experiments.

1. The identification of menstrual blood by means of paper electrophoresis is a simple method in legal medicine and its electrophorogram is an excellent method to offer an evidence of proof for menstrual blood.

2. By this method it is possible to identify the menstrual bloodstain even after the lapse of time as much as 6 months.

3. It is possible to identify even putrefied menstrual bloodstain.

4. In the case where the material stained with menstrual blood is found in water, it is not possible to identify the menstrual blood by this method.

5. When the menstrual blood is heated at 60°C over 30 minutes, it becomes impossible to identify it by this method.

6. In the case of venous blood during menstruation fibrinolytic product can be detected only on the first day of menstruation, but since it appears only in trace, it is easy to differentiate it from menstrual blood.

7. As for lochial blood the fibrinolytic product can be detected only in the blood obtained on the first and second days of puerperium, but the amount being so slight that it can readily be distinguished from menstrual blood.

8. In the case of the blood obtained at artificial abortion fibrinolytic product appears just as much as in the case of menstrual blood, and thus it is impossible to differentiate it from menstrual blood by this method.

9. As for the use of human fibrin it is best to employ it while it is fresh, but the human fibrin up to 6 days old can be used. However, the older is the human fibrin the lesser the fibrinolytic product detectable.

10. In the case using animal fibrins mixed with the extract of menstrual blood some do produce fibrinolytic product in trace, but since there is a danger of also producing the fibrinolytic product-like substance in venous blood, it is advisable not to use animal fibrins.

REFERENCES

1. MERKEL, H.: Über den Glykogengehalt des Scheidenepithels, seine diagnostische Bedeutung und deren kritische Bewertung. *Dtsch. z. gerichtl. Med.* 4, 1—8, 1924
2. SAKAMOTO, F.: Medico-legal study on epithelial cells containing glycogen storage. *J. Kurume Med. Assoc.* 20, 1763—1780, 1957 (in Japanese)
3. BERG, S. P.: Methoden zum Nachweis von Genitalsekretspuren. *Dtsch. z. gerichtl. Med.* 42, 605—613, 1954
4. OTA, S., JINNAI, Y., KUDO, K. & FUKUSHIMA, M.: Utilization of *Bacillus vaginalis* in the domain of the legal medicine. *Science & crime detection.* 16, 179—183, 1957 (in Japanese)
5. SUDO, J.: Examination of menstrual blood. *J. Kurume med. Assoc.* 20, 810—819, 1957

- (in Japanese)
6. SAKAMOTO, S.: Study on proving intrapartum hemorrhage (Lochia) J. Kurume Med. Assoc. 21, 434—455, 1958 (in Japanese)
 7. HARADA, R.: Studies on specificity of fibrinogen. J. Kurume med. Assoc. 23, 3297—3314, 1960 (in Japanese)
 8. YAMADA, T.: Studies on menstrual blood. Jap. J. legal med. a. criminol. 25, 68—90, 1959 (in Japanese)
 9. KAMIJO, M.: A study on the detection of the menstrual blood. Jap. J. legal med. 11, 700—725, 1957 (in Japanese)
 10. CULLIFORD, B. J.: Identification of menstrual blood in dried stain form, by an electrophoretic method. J. forinc. med. 6, 112—115, 1953
 11. KRAUL, L.: Über die Ungerinnbarkeit des Menstrualblutes. Zbl. f. Gyne. 49, 471—473, 1925
 12. MANO, H.: Studies on incoagulability of menstrual blood. Nagoya igaku. 68, 852—866, 1954 (in Japanese)
 13. YAMADA, T.: Studies on menstraual blood. Jap. J. legal med. & criminol. 25, 68—90, 1959 (in Japanese)
 14. SMITH, O. W. & SMITH, G. V.: A fibrinolytic enzyme in menstruation and late pregnancy toxemia. Science. 102, 253—254, 1954
 15. SATOH, T.: Causation of fluid blood in the cadaver and advocacy of the role of "Prefibrin" during the secondary stage of blood coagulation. J. faculty med. Shinshu univ. 4, 35—70, 1954 (in Japanese)
 16. MIZUSHIMA, S., & AKAHORI, S.: Chemistry of Proteins (Kyoritu Shuppan) 3, 352, 1954 (in Japanese)
 17. TOWATARI, K. Differentiation between menstrual blood and circulatory blood by measuring the fibrinolysin value. J. Kurume med. Assoc. 21, 1853—1869, 1958 (in Japanese)
 18. KISHI, M.: A study about the condition of menstrual blood. J. Kurume med. assoc. 22, 4185—4206, 1959 (in Japanese)
 19. YAMADA, T.: Studies on menstrual blood. Jap. J. legal med. & criminol. 25, 68—90, 1959 (in Japanese)