Acta Medica Okayama

Volume 16, *Issue* 4 1962 *Article* 2

AUGUST 1962

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Studies on identification of menstrual blood stain by fibrin-plate method. I. A study on the incoagulability of menstrual blood*

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Abstract

As the results of present study on plasmin in menstrual blood by means of the Fibrin-Plate Method, a large amount of plasmin as much as dilution of 1: 100 or 1:1,000 of the menstrual blood serum, has been found in a natural form and it is deduced that the incoagulability of menstrual blood is the result of the plasmin formation in the same blood serum. Further, it has been recognized that this plasmin is found in a rather large quantity in the blood of the second menstrual day and it is decreased by the fifth day. In addition, the plasmin of menstrual blood is contained in the globulin fraction of the same serum, especially markedly in β-globulin fraction. On the other hand, it has also been clarified that in the circulating blood during menstruation no plasmin is present in natural state and that a large quantity of inactive plasminogen still exists in menstrual blood.

*PMID: 13977381 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 16, 192—200 (1962)

STUDIES ON IDENTIFICATION OF MENSTRUAL BLOOD STAIN BY FIBRIN-PLATE METHOD

I. A STUDY ON THE INCOAGULABILITY OF MENSTRUAL BLOOD

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Received for publication, August 15, 1962

Incoagulability of menstrual blood is a well-known fact but as for its exact cause opinions are varied. Some of the outstanding hypotheses are theories of the wasting of fibrinogen, thrombin deficiency, digestion by trypsine, the formation of some anticoagulant, the formation of hormones, and fibrinolysis due to fibrinolysin (plasmin). Among them the last one, fibrinolysis, is at present the most promising theory.

It seems that incoagulability of menstrual blood is intimately associated with plasmin, for this substance is actually present in a considerable amount in menstrual blood. Therefore, the incoagulability of menstrual blood has been studied by means of Fibrin-Plate Method[1] in order to solve this problem.

MATERIALS AND METHODS

The material for this study was the menstrual blood collected in sterilized vessel with the aid of a speculum at the time of discharge from the uterine cavity in three normal women once a day for five consecutive days during menstruation, and this procedure was repeated for 4—5 months. For the control the circulating blood drawn from median cubital vein in men and women was used. Particularly the circulating blood from women included that collected once each pre- and postmenstrual stages and also once a day during the menstruation, totalling 7 or 8 collections during a menstrual cycle.

For the Fibrin-Plate Method a petri dish of the diameter of 4.5 cm. with lid was used. In a dish 2 ml. of barbiturate buffer and 1 ml. of fibrinogen solution were put and 0.02 ml. of thrombin solution was added. This was mixed sufficiently by shaking gently for 3—5 seconds. When a white gelatin-like plate was obtained, it was left in an incubator at 37°C. for 30 minutes before using it.

The fibrinogen solution was prepared from bovine serum in the following manner. After adding 0.1 volume of 2.5% potassium oxalate monohydrate solution to bovine blood the mixture was centrifuged at 3,000 r. p. m. for 10 minutes and to 100 ml. of the supernatant so obtained 6 g. of tricalcium phosphate was added and let it mix for 20 minutes. The mixture was again centrifuged for 20 minutes at 3,000 r. p. m. and its supernatant was diluted with cold distilled water to a total volume of 200 ml.. While stirring this solution, 80 ml. of saturated ammonium sulfate solution was added a little at a time and then a white precipitate separated out. The white precipitate obtained after centrifugation was dissolved in 50 ml. cold physiological saline solution, and to this 100 ml. cold distilled water was again mixed. With further addition of 60 ml. saturated ammonium sulfate solution the mixture was reprecipitated and by centrifuging for 5 minutes precipitate was obtained. The fibrinogen solution was finally obtained by washing this precipitate once with cold distilled water and by dissolving it in 40 ml. diethyl barbiturate buffer.

The diethyl barbiturate buffer solution was prepared by mixing 662 ml. of 0.1 M sodium diethyl barbiturate, 338 ml. 0.1 M HCl and 320 ml. distilled water (pH 7.8).

The thrombin solution was in the concentration of 100 units of thrombin (the product of Mochida Pharmaceutical Co. Ltd., Japan) per one ml. of physiological saline solution.

Plasmin in the menstrual blood was estimated as follows. Dilutions of 1: 50, 1 : 100, 1 : 500, 1 : 1,000, 1 : 2,000, and 1 : 4,000 of menstrual blood serum were used and then a drop of each diluted serum was placed on the fibrin plate by a capillary pipette, covered with the lid and the plate was placed in an incubator at 37°C.. At the termination of 2, 4. 8, and 24 hour-incubation the dissolution of the fibrin plate was observed. The one which shows dissolution already after two hours was recorded as $+++$, that after 4 hours as $++$, and that after 8 hours as $+$. Namely, this estimation does not represent the extent of the dissolution of the fibrin plate. Thus the ones showing dissolution later than 24 hours were all judged as negative.

As for the estimation of plasmin in the circulating blood (control), dilutions of 1 : 1, 1 : 5, 1 : 50, 1 : 100, 1 : 40,000, 1 : 80,000, 1 : 160,000, 1 : 320,000, and 1: 640,000 of blood serum were used (natural fibrinolysis, without SK), while the inactive plasmin (plasminogen) was used in the form of a mixture of diluted serum and Streptokinase (SK) (100 mg % Varidase solution, American Lederle Co.) in the proportion of 3 : 1, and this mixture was estimated in a similar manner (with SK).

With the purpose to determine in which fraction the plasmin of menstrual blood serum was contained, Kobayashi's electrophoretic apparatus (Natsume Seiki,

Japan) was used with Toyo filter paper No. 51, 26 cm. × 16 cm., and developed it at 7.5 mA., 200 V. for 10 hours. After the developing, the filter paper was cut into two longitudinal halves and the one was stained with bromphenol blue for detection of protein, while the other half was cut into small pieces and placed on the fibrin plate as in the case with the serum of menstrual blood for the observation of its reaction.

RESULTS

It has been found that the serum of circulating blood either taken during menstruation or pre- or postmenstrual period does not bring about the dissolution of fibrin plate. Namely, in the circulating blood plasmin cannot at all be recognized in the form as it is. However, when SK was added to it, being activated it dissolved the fibrin plate up to the dilution of 1 : 320,000 or 1 : 640,000 of circulating blood (Table 1 and Figs. 1—3).

Table 1. Fibrinolytic activity of circulating blood (without SK)

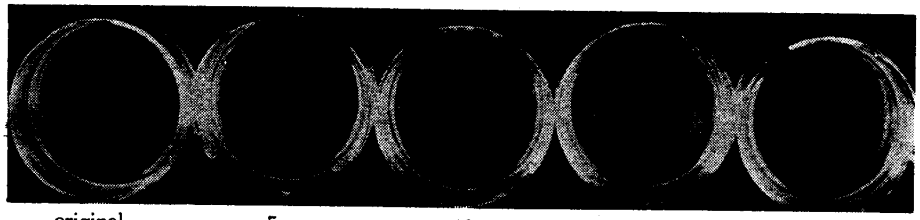
Dilution		original	5	10	50	100
Premenstrual stage		—	—	—	—	—
during menstruation	1st day	—	—	—	—	—
	2nd day	—	—	—	—	—
	3rd day	—	—	—	—	—
	4th day	—	—	—	—	—
	5th day	—	—	—	—	—
Postmenstrual stage		—	—	—	—	—
Normal man		—	—	—	—	—

No fibrinolytic activity can be observed in the blood drawn in menstrual, pre- and postmenstrual stages as well as in that of normal man.

Plasmin in the circulating blood of normal men likewise shows similar values as just described (Table 1 and Fig. 4). On the other hand, in the menstrual blood from normal women, at the dilution of 1 : 500 for the blood of the first menstrual day, at 1 : 500 or 1 : 1,000 for the second day blood, at 1 : 500 or 1 : 1,000 for the third day, at 1 : 100 or 1 : 500 for the fourth day, and at the dilution of 1 : 100 for the fifth day, the dissolution of fibrin plate can be recognized. This plasmin value is rather high on the second day of menstruation but it tends to decline later on (Table 2 and Fig. 5).

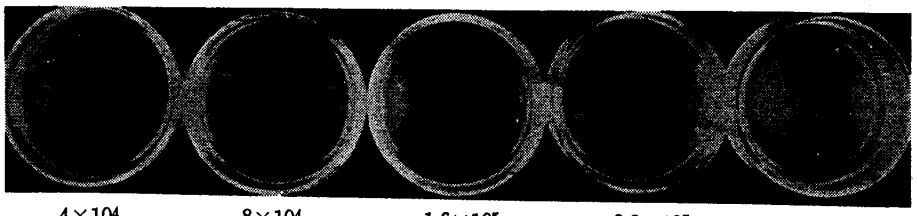
By the electrophoretic analysis it has been demonstrated that the developed paper of the same menstrual blood corresponding to serum globulin brings about the dissolution of fibrin plate, especially this is marked in the portion correspond-

a) without SK



original 5 10 5×10 10^2

b) with SK



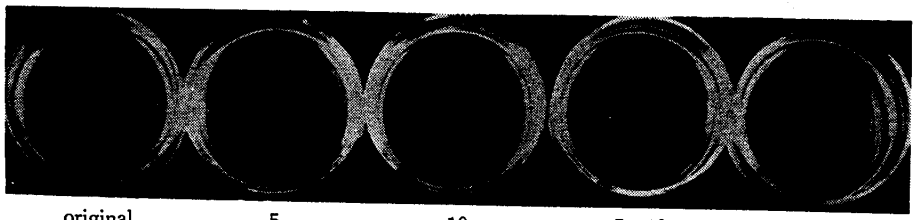
4×10^4 8×10^4 1.6×10^5 3.2×10^5 6.4×10^5

Fig. 1. Fibrinolytic activity of circulating blood in the woman (premenstrual stage. 8 hrs. after the test)

a) No dissolution of fibrin plate can be recognized in the serum without Streptokinase (SK).

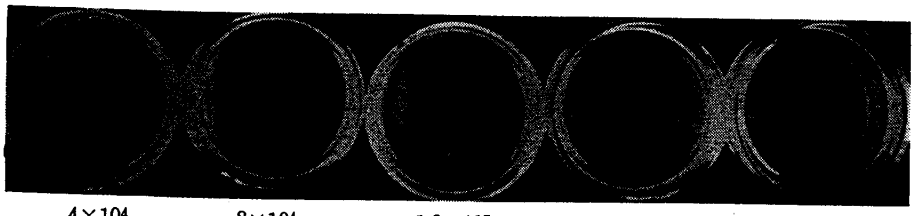
b) In the serum with SK up to the dilution of 1 : 320,000 the dissolution of fibrin plate can be seen.

a) without SK



original 5. 10 5×10 10^2

b) with SK



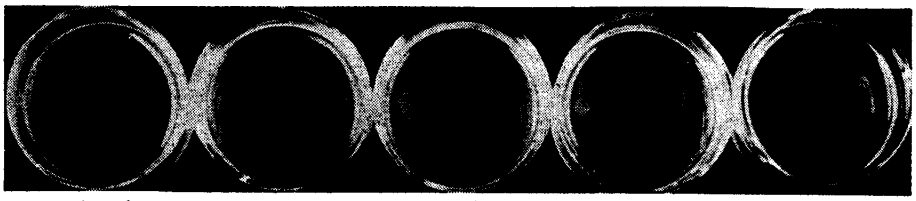
4×10^4 8×10^4 1.6×10^5 3.2×10^5 6.4×10^5

Fig. 2. Fibrinolytic activity of circulating blood in the woman during menstruation (on the second day of menstruation. 8 hrs. after the test)

a) No dissolution of fibrin plate with the serum free of SK.

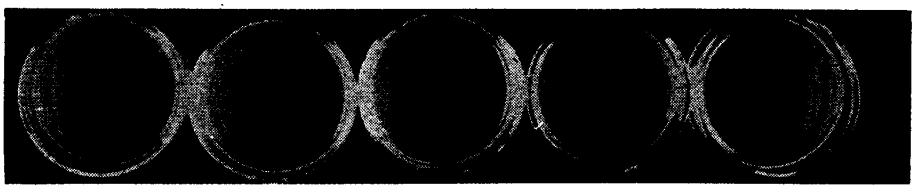
b) Dissolution of fibrin plate can be observed in the serum with SK up to the dilution of 1 : 640,000.

a) without SK



original 5 10 5×10 10^2

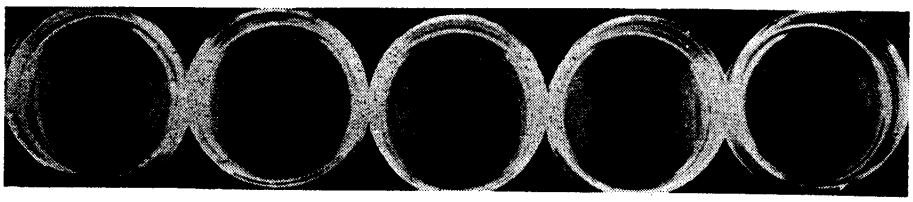
b) with SK



4×10^4 8×10^4 1.6×10^5 3.2×10^5 6.4×10^5

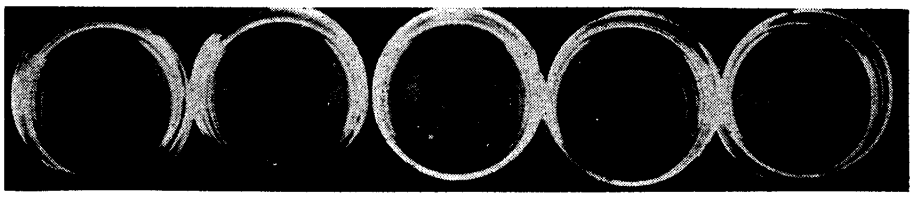
Fig. 3. Fibrinolytic activity of circulating blood in the woman (postmenstrual stage. 8 hrs. after the test)
a) No dissolution of fibrin plate in the serum without SK.
b) Dissolution of fibrin plate can be observed in the serum with SK up to the dilution of 1 : 320,000.

a) without SK



original 5 10 5×10 10^2

b) with SK



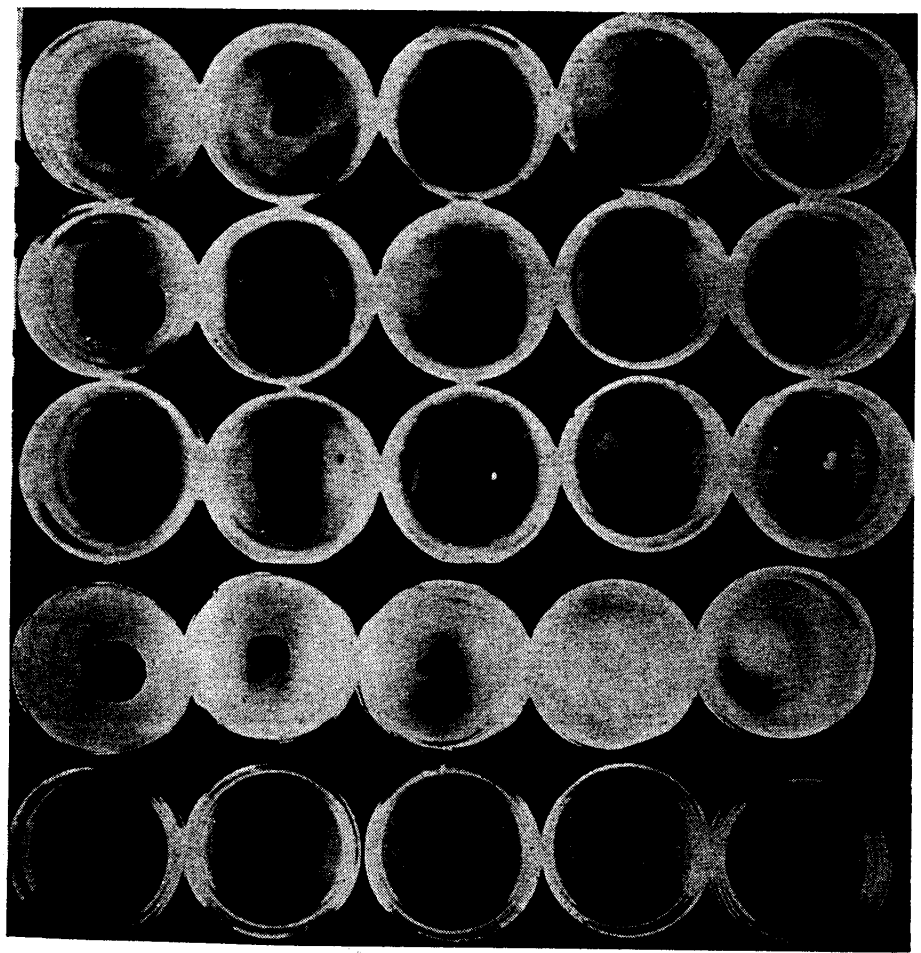
4×10^4 8×10^4 1.6×10^5 3.2×10^5 6.4×10^5

Fig. 4. Fibrinolytic activity of circulating blood in normal man (8 hrs. after the test)
a) No dissolution of fibrin plate in the serum without SK.
b) Dissolution of fibrin plate can be seen in the serum with SK up to the dilution of 1 : 640,000.

Table 2. Fibrinolytic activity of menstrual blood (without SK)
Maximum dilution at which a positive dissolution occurs.

	lst day	2nd day	3rd day	4th day	5th day
1	500	500	500	100	100
2	500	1, 000	1, 000	500	100
3	500	1, 000	500	100	100

Menstrual blood serum up to the dilution of 1 : 100 or 1 : 1000 induces dissolution of the fibrin plate.



5×10 10^2 5×10^2 10^3 2×10^3

Fig. 5. Fibrinolytic activity of menstrual blood (without SK. 8 hrs. after the test)
Horizontally each series of pictures show the activity of the first day to fifth day of menstruation, from top to bottom.

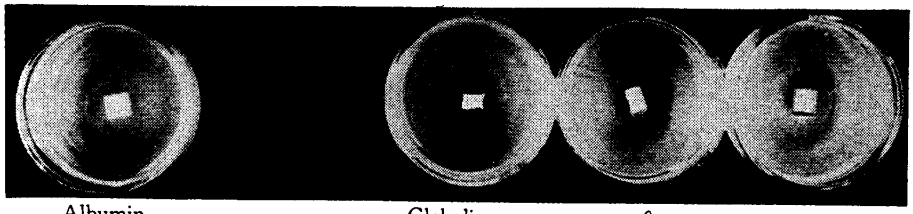


Fig. 6. The location of plasmin in the serum proteins of menstrual blood (8 hrs. after the test. Without SK).

ing to β-globulin. On the other hand, the part of the developed paper corresponding to albumin does not induce the dissolution of fibrin plate (Fig. 6).

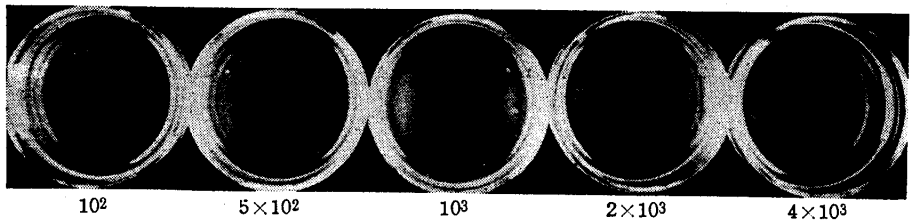
In addition, when SK is added to the menstrual blood serum, the plasmin value is found in the dilution of 1 : 10,000 or 1 : 20,000 of the same serum, revealing a marked decrease as compared with the circulating blood plasmin value of the same women (Table 3 and Fig. 7).

Table 3. Plasminogen in menstrual blood. Maximum dilution of serum at which a positive dissolution can be recognized (with SK).

	lst day	2nd day	3rd day	4th day	5th day
1	20, 000	20, 000	10, 000	10, 000	20, 000
2	20, 000	10, 000	10, 000	10, 000	20, 000
3	10, 000	20, 000	20, 000	20, 000	10, 000

The serum of menstrual blood in the dilution up to 1 : 10,000 or 1 : 20,000 shows dissolution of the fibrin plate if supplemented with SK.

a) without SK



b) with SK

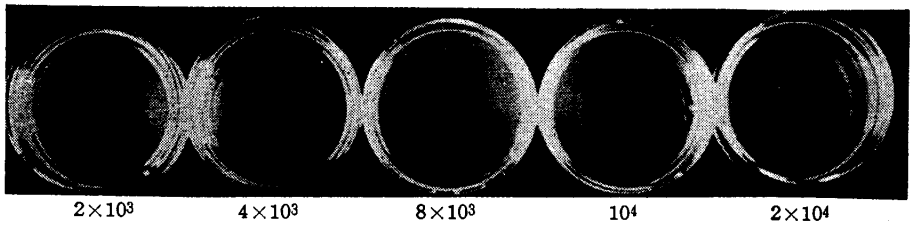


Fig. 7. Plasmin and plasminogen in the menstrual blood (of the second day blood. 8 hrs. after the test).

a) This series shows plasmin contained in the menstrual blood in natural state.

b) This series shows plasminogen activated by addition of SK and transformed as plasmin.

DISCUSSION

Concerning the factor that makes menstrual blood incoagulable there are many hypotheses presented by such investigators as SMITH and SMITH (1954)[2], MANO (1954)[3], ALBRECHTSEN (1956)[4], BELLER and GRAF (1957)[5], and YAMADA (1959)[6]. At present, fibrinolysis by plasmin is thought to be responsible for this phenomenon. For an example, ALBRECHTSEN (1956)[4] explained this phenomenon that due to destruction of the endometrium or the cell necrosis in the menstrual stage a tissue activator is discharged, and by activating plasminogen in the menstrual blood, plasmin is formed, which in turn brings about fibrinolysis, resulting in the incoagulability of the blood. Thus it is considered that, while menstrual blood is coagulable at first in the uterine cavity, it passes through the fibrinolytic processes in the uterine cavity,

tissue activator + plasminogen → plasminogen-activator → plasmin,

resulting in fibrinolysis. Namely, WHITEHOUSE (1914)[7] recognized that menstrual blood taken by a glass catheter inserted into the uterine cavity did coagulate but the blood discharged from the uterine cavity did not.

In the present study a considerable quantity of plasmin has been detected in menstrual blood by the Fibrin-Plate Method. Furthermore, it has been confirmed that this plasmin is found in a rather large amount in the second day menstrual blood, but it is decreased in the fifth day, and in addition, plasmin is contained in globulin fraction, especially markedly in β-globulin fraction while none in albumin fraction. The presence of such a large amount of plasmin in menstrual blood suggests, as ALBRECHTSEN mentioned, seems to have a great bearing on the incoagulability of menstrual blood.

On the other hand, SMITH and SMITH (1954)[2] contended that they found free plasmin in the circulating blood at menstrual stage and this ought to have some relation with the incoagulability of menstrual blood. YAMAZAKI (1954)[8], SUGIZAKI (1954)[9], and HORIGUCHI (1961)[10] agree to this opinion. Concerning this point, MACFARLANE and BIGGS (1946)[11], MANO (1954)[3], and YAMADA (1954)[6], all refuted this view. In the present study conducted by the Fibrin-Plate Method likewise no free plasmin has been detected in the circulating blood at menstrual stage. Namely, the appearance of plasmin during menstruation should be considered as a local phenomenon occurring in the uterine cavity.

By the present experiment it has been elucidated that the plasmin value in circulating blood during menstruation shows the dilution of 1 : 320,000 or 1 : 640,000 of serum when supplemented with SK, but when SK is added to the menstrual blood of the same subject, the plasmin value becomes the dilution of 1 : 10,000 or 1 : 20,000 of serum, and even then a large amount of inactive plasminogen is contained in the menstrual blood.

CONCLUSION

As the results of present study on plasmin in menstrual blood by means of the Fibrin-Plate Method, a large amount of plasmin as much as dilution of 1 : 100 or 1 : 1,000 of the menstrual blood serum, has been found in a natural form and it is deduced that the incoagulability of menstrual blood is the result of the plasmin formation in the same blood serum. Further, it has been recognized that this plasmin is found in a rather large quantity in the blood of the second menstrual day and it is decreased by the fifth day. In addition, the plasmin of menstrual blood is contained in the globulin fraction of the same serum, especially markedly in β-globulin fraction. On the other hand, it has also been clarified that in the circulating blood during menstruation no plasmin is present in natural state and that a large quantity of inactive plasminogen still exists in menstrual blood.

ACKNOWLEDGEMENT

The author wishes to express his profound thanks to Professor Y. Mikami for his valuable advices and encouragement throughout this study.

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