Parameters of Plasmin System during Normal Pregnancy, Labor and Puerperium, and Relation of Sex Hormones to Plasmin System*

By

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As to the change of factors relating to the plasmin system during pregnancy and puerperium, some conflicts have been found in the literature. This paper deals with the results of systemic analysis of this enzyme system, and the relation between sex hormones and the factors is discussed.

A marked elevation of factors of plasmin system during pregnancy was observed in fibrinogen, spontaneous caseinolytic activity of the euglobulin fraction, total plasmin and antiplasmin, but only a slight rise of fibrinolytic and fibrinogenolytic activities of the euglobulin.

The administration of progesterone to human females produced marked increases in total plasmin, antiplasmin and spontaneous fibrinolytic activity of the euglobulin, on the other hand, irregular changes in fibrinogen and spontaneous caseinolytic activity of the euglobulin.

Estrogen administration was responsible for an increase of spontaneous caseinolytic activity of the euglobulin and decreases in antiplasmin and spontaneous fibrinolytic activity of the euglobulin. However, essentially no change in fibrinogen and total plasmin was observed by the estrogen administration.

These findings suggest that the close relation exists between sex hormones and plasmin system.

Many reports concerning the change of factors of plasmin system during pregnancy, in particular with obstetrical hypofibrinogenemia, have been presented. However, only a few studies have reported systemic analysis of parameters of the plasmin system during pregnancy and puerperium. In addition, some conflicts have been found in the literature as to the plasmin system, especially the antiplasmin level during pregnancy.

This paper describes the change of factors of the plasmin system during pregnancy and puerperium, and the effect of estrogen and progesterone on plasmin system.

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MATERIALS AND METHODS

1. Test samples of plasma were obtained from women at various stages of pregnancy and puerperium by centrifuging a mixture of 1 vol. of 3.8% sodium citrate and 9 vols. of venous blood. Control plasma was collected by the same procedure from non-pregnant healthy female adults.

2. As sex hormones, progesterone (Proluton Depot-Shering A.G., in which 125 mg of 17-alpha-oxyprogesterone capronate is contained) and estrogen (Ovahormone Depot-Tecizo Co., which contains 10 mg of estradiol propionate) were used.

3. Fibrinogen in plasma was determined colorimetrically.

4. Total plasmin was determined on the SK(streptokinase)-activated euglobulin fraction by a caseinolytic method.

5. Spontaneous caseinolytic, fibrinolytic and fibrinogenolytic activities were determined on the euglobulin fraction, without any addition of activating procedure, by incubating with 2% casein (at 37°C for 20 hours) and 0.1% fibrinogen and fibrin (at 37°C for 18 hours), respectively. Because of the experimental purpose of this study, the three steps of blanks as described in the 8th report of this series were not prepared for the assay of fibrinolytic activity, but only one blank was used as in the 1st report.

6. The details of assay method of antiplasmin were described in the previous report. Plasma is preincubated with SK for 2 minutes and then further incubated with casein (I). SK-activated euglobulin was preincubated with inhibitor (euglobulin-eliminated supernate) for 2 hours in the presence of ethylamine, a stabilizer of plasmin, and then further incubated with casein (II). The differences between total plasmin and activity obtained by (I), and total plasmin and activity of (II) can express immediate and slow inhibitors to plasmin.

7. Spontaneous fibrinolytic activity of plasma was determined by a doubling dilution method.

RESULTS

I. Fibrinogen

Fibrinogen level was increased markedly as pregnancy advanced, but it did not return to a normal level during puerperal stage. In all 5 cases examined

<table>
<thead>
<tr>
<th>Table I. Fibrinogen Concentration during Pregnancy, Labor and Puerperium</th>
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<tbody>
<tr>
<td><strong>Stage</strong></td>
</tr>
<tr>
<td>Fibrinogen (mg%)</td>
</tr>
<tr>
<td>Percentage</td>
</tr>
<tr>
<td>Numbers examined</td>
</tr>
</tbody>
</table>

* I: non-pregnant female adult; II, III, IV: first, second, third trimesters of pregnancy; V: during labor; IV: immediately after delivery; VII, VIII: 3, 7 days after delivery
approximately 1 month after the delivery, the level of fibrinogen ranged within normal limit (Table I and Fig. 1).

2. Total plasmin

Table II and Fig. 2 show the total plasmin activity increased as pregnancy advanced, while after delivery the activity decreased in a rapid manner.

3. Spontaneous proteolytic activities of the euglobulin fraction

Spontaneous caseinolytic activity of the euglobulin increased gradually and reached a maximum at the second stage of labor, then returned to normal limits 3 days following delivery (Table V and Fig. 3).

Both spontaneous fibrinolytic and fibrinogenolytic activities of the euglobulin fraction gradually increased with the progress of pregnancy (Table III and Fig. 3), then decreased gradually with marked individual fluctuation after the delivery.

4. Antiplasmin activity

Immediate inhibitor and slow inhibitors to plasmin changed their activity almost similarly to that of total plasmin (Table II and Fig. 2).

5. Fibrinolytic activity in plasma

None of positive fibrinolytic activity in plasma determined by the doubling dilution method was observed in 280 normal pregnant women.
TABLE II. Activities of Total Plasmin, Immediate and Slow Inhibitors during Pregnancy, Labor and Puerperium

<table>
<thead>
<tr>
<th>Stage</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total plasmin (10^4 x units/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average activity</td>
<td>10.5</td>
<td>15.5</td>
<td>22.4</td>
<td>22.1</td>
<td>23.4</td>
<td>21.5</td>
<td>17.1</td>
<td>16.9</td>
</tr>
<tr>
<td>Range</td>
<td>6.2-14.7</td>
<td>7.7-22.1</td>
<td>10.6-31.9</td>
<td>9.0-34.7</td>
<td>14.7-30.8</td>
<td>10.1-34.5</td>
<td>8.4-43.0</td>
<td>9.5-22.9</td>
</tr>
<tr>
<td>Percentage activity</td>
<td>100</td>
<td>148</td>
<td>213</td>
<td>210</td>
<td>222</td>
<td>205</td>
<td>163</td>
<td>151</td>
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<tr>
<td>Numbers examined</td>
<td>25</td>
<td>24</td>
<td>37</td>
<td>66</td>
<td>31</td>
<td>27</td>
<td>21</td>
<td>13</td>
</tr>
<tr>
<td>Immediate inhibitor (10^-3 x units/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average activity</td>
<td>5.8</td>
<td>19.9</td>
<td>15.1</td>
<td>14.1</td>
<td>15.0</td>
<td>13.9</td>
<td>12.6</td>
<td>11.7</td>
</tr>
<tr>
<td>Range</td>
<td>1.3-12.9</td>
<td>3.2-27.4</td>
<td>8.2-22.6</td>
<td>3.2-32.0</td>
<td>6.3-21.6</td>
<td>7.4-22.1</td>
<td>1.1-19.6</td>
<td>10.9-12.9</td>
</tr>
<tr>
<td>Percentage activity</td>
<td>100</td>
<td>222</td>
<td>259</td>
<td>292</td>
<td>259</td>
<td>240</td>
<td>217</td>
<td>200</td>
</tr>
<tr>
<td>Numbers examined</td>
<td>11</td>
<td>15</td>
<td>25</td>
<td>44</td>
<td>21</td>
<td>13</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Slow inhibitor (10^-3 x units/ml)</td>
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<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Average activity</td>
<td>5.4</td>
<td>19.3</td>
<td>14.1</td>
<td>14.6</td>
<td>14.7</td>
<td>13.5</td>
<td>11.7</td>
<td>10.4</td>
</tr>
<tr>
<td>Range</td>
<td>3.0-7.0</td>
<td>8.2-28.2</td>
<td>5.6-30.2</td>
<td>24.6-34.2</td>
<td>7.8-32.0</td>
<td>6.8-24.1</td>
<td>13.2-20.2</td>
<td>4.8-13.2</td>
</tr>
<tr>
<td>Percentage activity</td>
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<td>228</td>
<td>262</td>
<td>268</td>
<td>273</td>
<td>250</td>
<td>217</td>
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<td>10</td>
<td>10</td>
<td>21</td>
<td>10</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

Figures in Roman style are expressed by the same fashion as in Table I.

Fig. 2. Changes in total plasmin (o-o), immediate inhibitor (o-o) and slow inhibitor (o-o) during pregnancy, labor and puerperium.

Figures in both abscissa and ordinate are expressed by the same fashion as in Fig. 1.

6. Influence of progesterone on factors of plasmin system

Depot progesterone (125 mg of 17-alpha-oxyprogesterone capronate) was administered intramuscularly to females with or without ovarian function.
Parameters of Plasmin System

Table III. Spontaneous Proteolytic Activities of the Euglobulin Fraction during Pregnancy, Labor and Puerperium

<table>
<thead>
<tr>
<th>Stage</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caseinolytic activity (PU)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Average activity</td>
<td>4.9</td>
<td>5.9</td>
<td>7.6</td>
<td>10.6</td>
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<td>15.5</td>
<td>5.8</td>
<td>4.5</td>
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<tr>
<td>Range</td>
<td>0.2-9</td>
<td>0.1-33</td>
<td>0.3-600</td>
<td>1-600</td>
<td>4-720</td>
<td>2-700</td>
<td>2-320</td>
<td>3-22</td>
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<tr>
<td>Percentage activity</td>
<td>100</td>
<td>120</td>
<td>155</td>
<td>210</td>
<td>449</td>
<td>316</td>
<td>120</td>
<td>92</td>
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<td>Numbers examined</td>
<td>16</td>
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<td>46</td>
<td>134</td>
<td>24</td>
<td>25</td>
<td>30</td>
<td>28</td>
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<tr>
<td>Fibrinolytic activity (μg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average activity</td>
<td>44.2</td>
<td>54.2</td>
<td>55.2</td>
<td>59.1</td>
<td>68.2</td>
<td>57.1</td>
<td>58.5</td>
<td>56.4</td>
</tr>
<tr>
<td>Range</td>
<td>35-60</td>
<td>40-87</td>
<td>58-77</td>
<td>34</td>
<td>108</td>
<td>3-710</td>
<td>46-72</td>
<td>37-79</td>
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<td>Percent activity</td>
<td>100</td>
<td>123</td>
<td>118</td>
<td>134</td>
<td>155</td>
<td>129</td>
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<td>Numbers examined</td>
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<td>13</td>
<td>45</td>
<td>85</td>
<td>30</td>
<td>14</td>
<td>11</td>
<td>4</td>
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<tr>
<td>Fibrinogenolytic activity (μg)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average activity</td>
<td>41.2</td>
<td>49.6</td>
<td>60.6</td>
<td>62.1</td>
<td>65.7</td>
<td>63.3</td>
<td>61.9</td>
<td>66.3</td>
</tr>
<tr>
<td>Range</td>
<td>30-60</td>
<td>30-85</td>
<td>46-80</td>
<td>38-104</td>
<td>42-90</td>
<td>48-69</td>
<td>46-83</td>
<td>56-87</td>
</tr>
<tr>
<td>Percent activity</td>
<td>100</td>
<td>120</td>
<td>147</td>
<td>151</td>
<td>160</td>
<td>154</td>
<td>150</td>
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<tr>
<td>Numbers examined</td>
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<td>57</td>
<td>93</td>
<td>31</td>
<td>13</td>
<td>11</td>
<td>3</td>
</tr>
</tbody>
</table>

Figures in Roman style are expressed by the same fashion as in Table I.

Fig. 3. Changes in spontaneous caseinolytic (---), fibrinolytic (-----) and fibrinogenolytic (o---o) activities in the euglobulin fraction.

Figures in both abscissa and ordinate are expressed by the same fashion as in Fig. 1.

The factors of plasmin system were determined at given times after the administration and compared with those of preadministration values. The total plasmin (Fig. 5), antiplasmin (Fig. 6) activities and spontaneous fibrinolytic activity in the euglobulin fraction (Fig. 8) were markedly increased 3 to 7 days after the administration with relatively wide individual variations. No specific
Fig. 4. Influence of progesterone (P) and estrogen (E) administered to human females on fibrinogen concentration.
Figures in ordinate represent fibrinogen concentration calculated individually by regarding preadministration value as 100%. (*-----*, average value)

Fig. 5. Influence of progesterone (P) and estrogen (E) administered to human female on total plasmin (proteolytic activity of SK-activated euglobulin).
Figures are expressed by the same fashion as in Fig. 4.

Tendency was noted in fibrinogen (Fig. 4) and caseinolytic activity of the euglobulin (Fig. 7).

7. Influence of estrogen on factors of plasmin system

Depot estrogen (10 mg of estradiol propionate) was administered in the same manner as progesterone. The average activity was markedly decreased in antiplasmin (Fig. 6) and fibrinolytic activity of the euglobulin fraction (Fig. 8). They reached a minimum value 1 to 3 days after the administration and then returned to near the preadministration values with wide individual variation.
Fig. 6. Influence of progesterone (P) and estrogen (E) administered to human females on antiplasmin (immediate inhibitor). Figures are expressed by the same fashion as in Fig. 4.

Fig. 7. Influence of progesterone (P) and estrogen (E) administered to human females on spontaneous caseinolytic activity of the euglobulin fraction. Figures are expressed by the same fashion as in Fig. 4.

The spontaneous caseinolytic activity of the euglobulin fraction (Fig. 7) was markedly enhanced. No change in total plasmin (Fig. 5) and variable fluctuation in fibrinogen (Fig. 4) were observed.

No case of conversion from negative to positive in fibrinolysis determined by the doubling dilution method was noted in both groups of progesterone and estrogen administration.

**DISCUSSION**

Reports concerned with fibrinolytic activity during pregnancy give widely divergent results. Margulis et al.\(^5\) and Niesert\(^6\) noted that there is a greater incidence of fibrinolysis in the puerperium than in the antenatal period. Mac-
Fig. 8. Influence of progesterone (P) and estrogen (E) administered to human females on spontaneous fibrinolytic activity of the euglobulin fraction. Figures are expressed by the same fashion as in Fig. 4.

Farlane et al.\textsuperscript{7} mentioned no evidence of proteolytic activity in 137 cases of normal women during pregnancy. The authors also noted none of fibrinolytic state, by using the doubling dilution method, in 280 cases of normal pregnancy. Biezinski and Moore\textsuperscript{8} observed a marked decrease in the activity in the last month of pregnancy and labor, and a quick return to non-pregnancy level immediately after delivery. The high incidence of positive fibrinolysis during early pregnancy and the puerperium reported by Biezinski and Moore may be due to their special technique for the detection of fibrinolytic activity. They used a clot procedure presented by Fearnley and Lackner\textsuperscript{9} which is based on the fact that the fibrinolytic activity is quickly destroyed at room temperature but can be preserved by keeping the plasma at ice temperature. However, one should note the fact that the proteolytic activity of plasma increases when plasma is stored in an ice chest. Therefore, the result obtained by such cold procedure may represent not only its own activity but also artificially induced fibrinolytic activity.
The spontaneous proteolytic activities of the euglobulin fraction were elevated as pregnancy advanced and then approached normal levels after delivery. This state was observed markedly in the caseinolytic activity, but unremarkably in the fibrinogenolytic and fibrinolytic ones. Gillman et al.\textsuperscript{10} noted that fibrinolytic activity measured by Von Kaulla's euglobulin lysis time decreased significantly during the early months of pregnancy and remained low until the second stage of labor. As detectable amounts of antiplasmin and fibrinogen in a greater variety of concentration are contained in the euglobulin fraction, the euglobulin lysis time would not completely represent the fibrinolytic activity of blood. When one utilizes the euglobulin fraction as a tool of determination of fibrinolytic activity, such contamination with these substances in the euglobulin fraction must be considered. However, more accurate results can be obtained by artificially adding fixed amount of substrate as in the authors' method.\textsuperscript{2,3}

An elevation of total plasmin during pregnancy was also noted by Elsner,\textsuperscript{11} however, the elevation was not as marked as the authors' result. This may be due to a difference of the calculation method of the caseinolytic activity of SK-activated euglobulin fraction.

As to the concentration of antiplasmin, Elsner\textsuperscript{11} and Ruckstuhl et al.\textsuperscript{12} noted a decrease, Biezenski\textsuperscript{13} did not observe the change, while Macfarlane et al.\textsuperscript{7} and the authors observed an increase. The assay method of antiplasmin employed by Elsner,\textsuperscript{11} Ruckstuhl et al.\textsuperscript{12} and Biezenski,\textsuperscript{13} in which plasma or serum itself was used as antiplasmin source, seems to be unable to represent the real antiplasmin level. This is because the antiplasmin level obtained by their method must be influenced with one's own plasminogen, which is markedly increased during pregnancy, in plasma or serum. If both plasminogen and antiplasmin are simultaneously increased or decreased in the same proportion, antiplasmin level never be changed unless the euglobulin-eliminated plasma or serum is used. It is, therefore, more accurate to use the euglobulin-eliminated plasma or serum as an antiplasmin source, in which most plasminogen or plasmin is removed.

Gillman et al.\textsuperscript{10} suggested that the endocrine changes during pregnancy may be responsible for an alteration in plasma fibrinolytic activity. The experiment presented here showed that the administration of progesterone gave increases of the total plasmin, antiplasmin and spontaneous fibrinolytic activity of the euglobulin fraction, but irregular changes in fibrinogen and caseinolytic activity of the euglobulin. Estrogen was responsible for an increase of spontaneous caseinolytic activity and decreases in antiplasmin and spontaneous fibrinolytic activity of the euglobulin. No change in total plasmin and variable fluctuation in fibrinogen were also observed by the estrogen administration. It is interesting to note that caseinolytic activity did not change concomitantly with fibrinolytic activity in the euglobulin by giving sex hormones. This may confirm the authors' opinion of proteolytic enzyme multiplicity in the euglobulin fraction.\textsuperscript{14} As to
the synthetic progesterone (Enovid) in playing occurrence of thromboembolic
diseases, conflicting views have been presented. In 1961, Phillips et al. noted that oral administrations of exogeneous estrogen preparations and of norethindrone appear to cause an increase in profibrinolysin, fibrinogen and antifibrinolysin.

During pregnancy, a sharp rise of estrogen occurs after the hundredth day of
pregnancy and the rise continues up to delivery, then falls. On the other hand, when fertilization occurs, pregnadiol excretion is maintained with the range in the first trimester from 5 to 17 mg per 24 hours. Between 12th and 14th weeks there is a progressive rise to a peak approximately 2 or 3 weeks prior to delivery. Following this the excretion level falls rapidly, disappearing within a few days after delivery.

A close relationship can be seen between changes of plasmin system (total plasmin and antiplasmin) observed by the experimental administration of progesterone and those observed in pregnant and puerperium women. There is also an entirely similar pattern between estrogen level and spontaneous caseinolytic activity of the euglobulin during pregnancy and puerperium. It is, therefore, possible to conclude that these sex hormones play an important role in controlling the plasmin system during pregnancy. However, one cannot neglect the presence of other controlling factors.

As is generally believed, the pregnant blood is a hypercoagulable state, in which an increase of factors I, II, V, VII and VIII, shortening of the clotting

![Diagram](https://example.com/diagram.png)

**Fig. 9.** Balance between coagulation and plasmin systems during pregnancy.
Parameters of plasmin System

...time of the whole blood, prothrombin time, thrombotest and recalcification time are observed. Accordingly, intravascular clotting which is often observed in obstetrical shock, may occur even in normally pregnant vessels to a slight degree. The increased total plasmin and heparin or heparin-like substance which is determined by a metachromatic procedure of Kikuchi\(^\text{18}\) during pregnancy are thought to be a reserve power to dissolve the fibrin clot when intravascular clotting or thrombosis occurs. On the other hand, the increased antiplasmin may play an inhibiting role to the occurrence of pathologic manifestations of fibrinogenolysis in the circulation.

Thus, the balance between coagulation and plasmin systems can be schematized as presented in Fig. 9.

References

4) Maki, M. ibid., in press.