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# A Coagulation Anomaly: Plasma Being Made Non-clottable by Adding Thrombin, and Clottable by Thromboplastin-calcium or Toluidine Blue-thrombin*

By

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Plasma samples obtained from 3 cases of a severe hemorrhagic disorder with hypofibrinogenemia during pregnancy were not clotted by simple addition of thrombin but clotted with thromboplastin-calcium or toluidine blue-thrombin. Laboratory results revealed severe coagulation defects and elevated fibrinolytic activity. In these cases, fibrinogen concentration determined by a procedure of centrifugation after heating at 56°C was markedly higher than that obtained by adding thromboplastin-calcium or toluidine blue-thrombin. The facts suggest that a significant amount of digest products of fibrinogen by plasmin, which are still coagulable by heating and have an anticoagulant activity, must be present in the patients' plasma.

In 1963, Maki[1] noted a case of hypofibrinogenemia associated with abruptio placentae in which the plasma was not coagulable by adding thrombin but coagulable in the presence of thromboplastin-calcium. The author, at that time, could not explain the mechanism of such a coagulation defect. However, recent progress in the study of digest products of fibrinogen or fibrin by plasmin (DPF) has made it possible to clarify the manner[2-6]. This paper describes the mechanism of this coagulation defect with 3 illustrative cases.

## CASE REPORT

Case 1.

A 32-year-old housewife, 24-week gestation, para i, gravida ii, was admitted to Hirosaki University Hospital with the complaint of uncontrollable bleeding from the gums. Approximately 12 hours after the onset of symptoms, various manifestations of abruptio placentae such as acute anemia, abdominal pain, disappearance of fetal movement and fetal heart sounds, enlargement of the uterine size and

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* The 13th report on Pathophysiology of Plasmin System

TABLE I. Laboratory Results

| Test | Case 1 | Case 2 | Case 3 | Control |
|---|---|---|---|---|
| Clotting time of whole blood (min) | 30 | 10 | | 6-9 |
| Clot lysis time (min) | 60 | 180 | | 60×24×7 |
| Platelets ($\times 10^3$) | 81 | 60 | | 200-300 |
| Bleeding time (min) | 60 | 9.5 | 119 | 2-3 |
| Thrombin time (sec) | ∞ | ∞ | ∞ | 10-13 |
| Prothrombin time (sec) | 180 | 30 | 16 | 10-12 |
| Toluid.-thromb time (sec) | | 18 | 45 | 5-8 |
| Recalc. time (sec) | | 120 | 630 | 120-180 |
| Heparin tol. test (min) | | 41 | 180 | 20-30 |
| Fibrinogen (mg%) | | | | |
| determined by heating | | 175 | 150 | |
| by prothromb.-Ca. | 36.1 | 58.8 | 40.0 | |
| by toluid.-thromb. | | 70.2 | 60.8 | |
| by thrombin | 0 | 0 | 0 | 390 |
| Spontaneous proteolytic activity of euglobulin | | | | |
| Caseinolysis ($\mu$g) | 43 | | | 21.9 |
| Fibrinogenolysis ($\mu$g) | 60 | | 52 | 68.2 |
| Fibrinolysis ($\mu$g) | 50 | | 72 | 65.7 |
| Total plasmin ($\times 10^{-3}$U) | 6.1 | 8.0 | 9.3 | 23.4 |
| Erythrocytes sed. rate | | | | |
| 1-hour rate (mm) | 4 | 6 | 10 | 30-60 |
| 2-hour rate (mm) | 8 | 16 | 20 | 40-100 |

continuous uterine bleeding were noted. The laboratory results prior to the initiation of therapeutic procedures are summarized in Table I. The patient was diagnosed as abruptio placentae with hypofibrinogenemia. As the patient's cervix uteri was rigid with no dilatation, Porro's operation was performed after the clotting and bleeding time were returned to a normal level by the administration of epsilon aminocaproic acid, dexamethasone and a total of 3800 ml of fresh blood. The uterus showed a typical picture of Couvalaire's apoplexie uteroplacentaire, and marked purpura was noted on the intestinal wall, peritoneum and omentum. Dark brown intestinal contents, perhaps mixed with blood, were observed through the intestinal wall. Fig. 1 shows the uterus removed with fetus indicating a separation of the normally implanted placenta. The fact that only a small hematoma was observed in the retroplacental space can be explained by assuming that the non-clotting blood in this space escaped through the vagina without forming solid hematoma, because of the severe coagulation defect and or elevated fibrinolysis. The volume of hemorrhage totaled 3,000 ml. She was discharged uneventfully after 17 days hospitalization. The details of this cases were reported elsewhere[7].

Case 2.

A 28-year-old housewife, para ii, gravida iii, with no family or personal

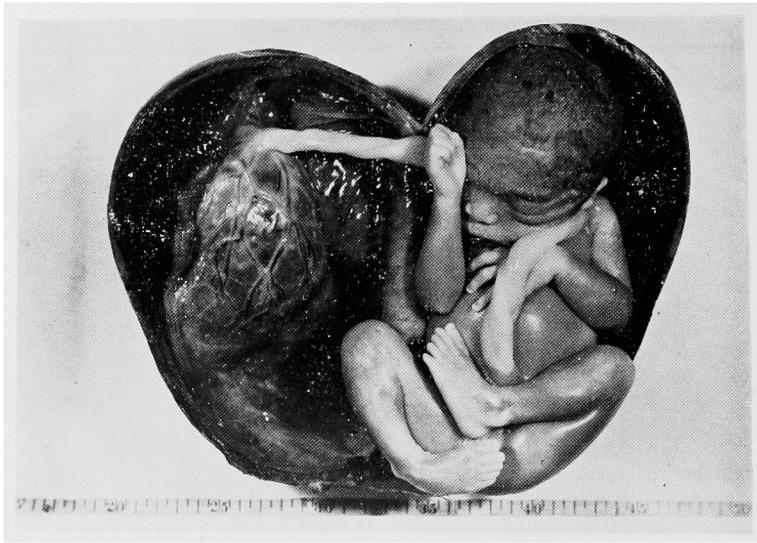

Fig. 1. Premature separation of the normally implanted placenta with fetus of breech presentation.

history of hemorrhagic diathesis, was admitted as an emergency patient in shock with continuous uterine and gingival bleeding. Such symptoms as paleness, low blood pressure, abnormally enlarged uterus, painful abdomen, disappearance of fetal heart sounds and fetal movement with difficulty in palpating fetal parts, suggested the patient having abruptio placentae. The laboratory results are presented in Table I. The vaginal examination on admission showed 3 cm dilatation of the cervix, occiput presentation of the fetus and no palpable placental tissue in the lower segment of the uterus. After normalization of clotting and bleeding time by giving 12 g of epsilon aminocaproic acid, 800 ml of fresh blood and 2 g of fibrinogen, artificial rupture of the membrane followed by forceps operation was performed when the cervix uteri was dilated to approximately 5 cm. Following artificial rupture of the membrane, approximately 2,000 ml of non-coagulable blood was observed flowing out. A few small coagulable elements were noted following the placenta delivery. Total volume of the hemorrhage was 3,000 ml. She had an uneventful puerperal course and was discharged in good condition.

Case 3.

A 20-year-old, para 0, gravida 1, female at 13-week gestation with no family or personal history of hemorrhagic diathesis was admitted with a diagnosis of hyperemesis gravidarum. A curettage under intravenous anesthesia with barbiturate was uneventfully performed to interrupt the pregancy. A massive hemorrhage totaling 1200 ml began 4 hours following the procedure with no hemo-

static effect provided by the administration of 10 mg of vitamin $K_1$, 0.2 mg Methergin (methylergometrin maleate), 50 mg Adona (derivative of adrenochrome), 1 vial of Reptilase and 4 g of epsilon aminocaproic acid. Her bleeding time was markedly prolonged (119 min.). Recurettage revealed no retained placental fragments. Effective hemostasis was achieved by further addition of epsilon aminocaproic acid, 1 g of fibrinogen and 400 ml fresh blood. Thereafter no pathologic bleeding was observed and she was discharged.

*Laboratory results of clotting and plasmin systems*

The results of these cases are summarized in Table I with average values of pregnant females at term. Clotting time of the whole blood (Lee-White), bleeding time (Duke), prothrombin time (Quick's one stage method), recalcification time, heparin tolerance test, toluidine blue-thrombin time and thrombin clotting time (Hougie) were slightly or markedly prolonged in these cases as compared with control values. It was very interesting that in these patients fibrinogen was not detected by simple addition of thrombin, but became detectable in the presence of thromboplastin-calcium. A significant increase of fibrinogen was counted by a determination method of Foster *et al.*[8] Spontaneous fibrinolytic and caseinolytic activities of the euglobulin fraction[9] were singificantly elevated. Total plasmin[9] in Cases 1 and 2 was markedly decreased, while in Case 3 it was slightly decreased. Furthermore, platelets were also diminished.

*Explanation of the coagulation anomaly*

It is very interesting to note that blood samples of these patients were unclottable by adding excess thrombin, but were clottable in the presence of thromboplastin-calcium. The whole blood formed a very soft clot after a long incubation time and then the clot lysed rapidly. Usually, prolongation of the thrombin clotting time to a non-coagulable state is observed when plasma lacks fibrinogen or contains an excess of anticoagulant such as heparin or DPF. The thrombin clotting time of heparinated plasma is corrected by such neutralizing agents as toluidine blue, protamine sulfate or polybrene.

Fig. 2 shows that the thrombin clotting time measured on normal plasma containing various amounts of exogeneously added heparin. The thrombin clotting time of the heparinated plasma was completely corrected to normal by adding toluidine blue in so far as the heparin concentration was not excessive. Addition of calcium to the heparinated plasma could shorten the clotting time, but only to a slight degree.

Fig. 3 shows that the thrombin clotting time of DPF added plasma could be shortened by the addition of toluidine blue, but could not be shortened to the level of control plasma. This suggests that the activity of DPF may not be interfered with by adding toluidine blue. Prolongation of thrombin clotting

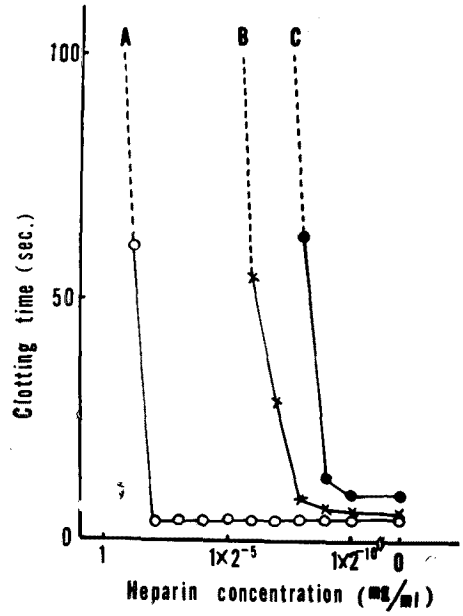


Fig. 2. Thrombin clotting time of heparinated plasma (C) in the presence of calcium (B) and toluidine blue (A).

The thrombin clotting time was measured by adding 0.1 ml of thrombin to A mixture (0.1 ml each of plasma, heparin and saline), B (0.1 ml each of plasma, heparin and 1/40 M calcium chloride) and C (0.1 ml each of plasma, heparin and 0.1% toluidine blue).

with DPF, however, might be markedly enhanced in the presence of heparin. Fletcher, Alkjaersig and Sherry noted that the addition of protamine sulfate to plasma containing DPF does not correct the coagulation defect. Therefore, the shortening of thrombin clotting time by the addition of toluidine blue may be due to a neutralizing effect on heparin or heparin-like substance which is normally present in the circulating blood, but not on DPF. On the other hand, as noted previously, the thrombin clotting time of fibrinogen solution containing DPF is markedly shortened in the presence of calcium. Accordingly, the phenomenon in which the patients' plasma is non-clottable by adding thrombin, while clottable with thromboplastin-calcium, could be partly explained by assuming a low level of calcium in the clotting system in which lowered fibrinogen and perhaps anticoagulants such as heparin or DPF are present.

The fact that the toluidine blue-thrombin clotting time of the patients' plasma was 18 (Case 2) and 45 (Case 3) seconds, whereas the plasma was not clotted by simple adition of thrombin, suggests the presence of an anticoagulant in the patients' plasma.

Since human plasma contains a significant amount of anticoagulant which is

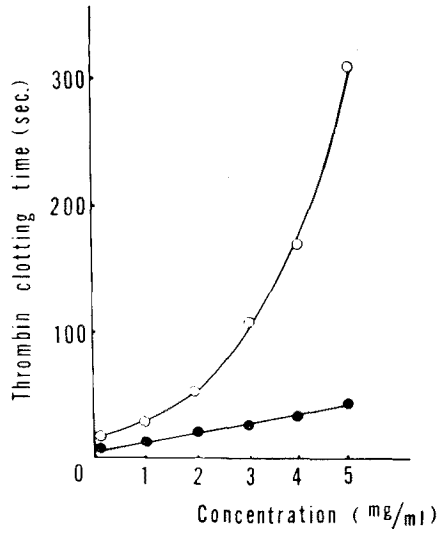


Fig. 3. Thrombin clotting time measured on DPF-plasma in the presence (-•—•-) or absence (-o—o-) of toluidine blue.

The clotting time was measured on 0.1 ml of plasma to which 0.1 ml of purified DPF (1, 2, 3, 4 and 5 mg per ml of veronal buffer) and 0.1 ml of thrombin (1 unit) were added.

neutralized by adding toludine blue, protamine sulfate or polybrene, diferential diagnosis of a coagulation defect due to heparin or DPF is difficult by means of the neutralizing test. In order to detect the coagulation anomaly due to DPF, at present time, it is necessary to determine the plasma fibrinogen in the following 3 steps: (1) determination of fibrinogen by adding thrombin, (2) adding thrombin plus neutralizing agents and (3) by the heating procedure of Foster, DeNatale and Dotti[8]. By adding thrombin to plasma, fibrinogen is completely determined if the plasma does not contain an excess of anticoagulant such as heparin. If plasma contains an excess anticoagulant, accurate fibrinogen value can be obtained by adding thrombin plus neutralizing agents. As DPF still has coagulability by heating at 56°C, fibrinogen level obtained by heat procedure represents not only naturally occurring fibrinogen but also DPF. Foster *et al.*, who described the determination method of fibrinogen by means of centrifugation after heating, noted that variable results were obtained in plasma from patients with jaundice. As is generally known, an elevation of fibrinolytic activity is often noted in patients with jaundice, therefore, this variability may be a result of DPF release in the plasma. A marked higher fibrinogen level obtained by the method of heating procedure could be explained by the presence of DPF in the plasma.

## DISCUSSION

One of the most important causes of hemorrhagic disorders occurring during pregnancy or postpartum is hypofibrinogenemia. A few cases of hemorrhagic disorder following pregnancy, associated with the presence of an endogeneous anticoagulant have also been reported for example, by Dreskin and Rosenthal[10] and by Biggs and Macfaralane[11], in which the anticoagulant concerned inactivated antihemophilic globulin. A release of a heparin-like anticoagulant was reported in dogs suffering from anaphylactic shock, irradiated dogs and some patietns with hemorrhagic diathesis. However, DPF was not studied in these cases. On the other hand, Kikuchi[12] noted a decrease of heparin or heparin-like substance, which was determined chemically by a metachromatic method, in patients with abnormal postpartum hemorrhage. Baker and Jacob[13] noted a hemorrhagic diathesis occurring during pregnancy with a coagulation anomaly which was reversible by protamine sulfate or toluidine blue, but did not resemble hyperplasminemia in the respect of thromboplastin and thrombin generation tests. Sharp, *et al.*[14] suggested that in the early stages of this syndrome poor activity of fibrinogen to thrombin may be an important feature of the coagulation defect. Beller *et al.*[15] noted a hemorrhagic disorder combined with amniotic fluid embolism due to qualitative change of fibrinogen resulting from elevated fibrinogenolysis, and termed this situation as dysfibrinogenemia or recently, as dysfibrinemia. As in the authors' cases the thrombin clotting time was partially corrected by adding neutralizing agent as was noted also in a case reported by Baker and Jacob[13], where the presence of heparin or heparin-like substance could not be completely neglected. However, 3-step determination of fibrinogen made it possible to clarify the presence of DPF.

One may say that it is possible that hemorrhagic disorder due to hyperplasminemia is complex in its nature, namely, not only a decrease of such clotting factors as factors I, II, V and VIII and platelets but also a release of DPF may relate to the hemorrhage. Since the thrombin clotting time of plasma with DPF is partially corrected by adding neutralizing agents, these substance may be helpful for the treatment of this syndrome, as Baker and Jacob described. However, DPF itself is not neutralized by these agents, so such a therapeutic procedure does not seem to be a rational therapy. At the present time, there is no adequate therapy of this situation. One may wait until DPF disappears or is inactivated spontaneously (average 50% clearance time of DPF is 9 hours) and prevent further formation of DPF by the administration of antiplasmin.

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