PROCOAGULANT PROPERTIES OF AMNIOTIC FLUID

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Amniotic fluid embolism is a rare but dramatic obstetric event because of its unusually rapid fatal outcome. The patients who survive the first few hours develop coagulation defects (Anderson, 1967; Bhattacharya et al, under publication), i.e. depletion of several coagulation factors and activation of fibrinolytic system Beller et al (1963). The procoagulant properties (Weiner et al, 1950; Rendlestein et al, 1951; Phillips and Davidson, 1972) of amniotic fluid are relevant in this connection. The present paper describes our studies on those properties of amniotic fluid.

Material and Methods

Amniotic fluid samples from 50 pregnant women between 14 to 40 weeks of gestation and without any complications were studied. The dates of last menstrual period of these women were known and they had normal regular menstrual cycles.

Amniotic fluids at 14 to 22 weeks' gestation were obtained at hysterotomy done for medical termination of pregnancy. The sacs were delivered intact and the fluid aspirated by autoclaved syringe to avoid contamination with maternal blood. Specimens of amniotic fluid from 24 to 37 weeks of gestation were taken by amniocentesis. Later specimens were obtained from patients having labour induced by artificial rupture of membranes.

Plasma recalcification time, kaolin cephalin clotting time, prothrombin time and Russel viper venom time tests were done as described by Denson (1966). Factor VII + X deficient substrate plasma was prepared from buffalo plasma filtered through fine charcoal (Denson, 1966). Factor XI deficient plasma was prepared as described by Eichelberger (1965). Factor V deficient plasma was prepared as described by Denson (1966). Factor VIII and IX deficient plasma were separated from blood collected from severe haemophilia A and B patients, respectively.

Platelet aggregation with A.D.P. was tested by naked eye. 0.2 ml. of platelet rich plasma was taken in a tub to which was added 0.1 ml. of adenosine diphosphate (10³M). The time was noted when the platelet storm became visible Denson (1966).

Effect of amniotic fluid was tested by adding equal amount of amniotic fluid to
normal or deficient plasma samples and then doing the various tests.

Observations

Effect of Amniotic Fluid on Recalcification Time of Plasma

The recalcification time of the normal plasma varied between 102-145 seconds with a mean value of 117 ± 19.2 seconds. On addition of whole (uncentrifuged) amniotic fluid to these plasma samples, the recalcification time was shortened in all the specimens nearly to half of the original value. The range varied between 30 to 65 seconds with a mean of 51.6 ± 12.7 seconds. Similarly, on addition of centrifuged supernatant amniotic fluid to the normal plasma the recalcification times were shortened in all the specimens tested. Their ranges varied from 33 to 86 seconds with a mean of 56.7 ± 14.3 seconds. Statistically, the shortening of plasma recalcification time by the addition of uncentrifuged amniotic fluid as well as centrifuged amniotic fluid was highly significant. Although there appeared to be a little lesser fall by centrifuged amniotic fluid as compared to that of uncentrifuged amniotic fluid, on statistical analysis no significant difference was noted (Table 1).

Table II shows results obtained when 10 different samples of amniotic fluid were added to plasma deficient in one or more of the following:

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Effect of Amniotic Fluid on the Recalcification Time (PRT) of Normal Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material</td>
<td>PRT (Sec.)</td>
</tr>
<tr>
<td>1. Normal plasma samples (n = 40)</td>
<td></td>
</tr>
<tr>
<td>2. Normal plasma + amniotic fluid (uncentrifuged) (n = 40)</td>
<td></td>
</tr>
<tr>
<td>3. Normal plasma + amniotic fluid (supernatant) (n = 40)</td>
<td></td>
</tr>
</tbody>
</table>

P values: 2 Vs 1 <0.001
3 Vs 1 <0.001
3 Vs 2 >0.1

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>Effect of Amniotic Fluid on Recalcification Time of Deficient Plasma Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material</td>
<td>PRT (Sec.)</td>
</tr>
<tr>
<td>1. Factor XI-deficient plasma (PRT = 180 sec.) + amniotic fluid (n = 10)</td>
<td></td>
</tr>
<tr>
<td>2. Factor IX-deficient plasma (PRT &gt;300 sec.) + amniotic fluid (n = 10)</td>
<td></td>
</tr>
<tr>
<td>3. Factor VIII-deficient plasma (PRT &gt;300 sec.) + amniotic fluid (n = 10)</td>
<td></td>
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</tbody>
</table>
more clotting factors. The addition of amniotic fluid brought the recalcification times of plasma deficient in factor VIII, factor IX, or factor XI to approximately the same value as that of normal plasma after addition of amniotic fluid.

**Effect of Amniotic Fluid on the Prothrombin Time of Plasma.**

The prothrombin time of normal plasma ranged between 17.4 to 20 seconds with a mean value of 18.3 ± 0.5 seconds. After the addition of whole (uncentrifuged) amniotic fluid the prothrombin time was in the range of 12-13 seconds with a mean value of 16.19 ± 2.15 seconds. On addition of centrifuged amniotic fluid the range of prothrombin time varied from 12-24 seconds with a mean of 17.32 ± 2.32 seconds. Although the differences of mean values were not much, on statistical analysis the shortening of plasma prothrombin time was significant by the addition of uncentrifuged as well as centrifuged amniotic fluid.

The prothrombin time of factor VII/X-

**TABLE III**

**Effect of Amniotic Fluid on Prothrombin Time and RVV Time of Normal Plasma**

<table>
<thead>
<tr>
<th>Material</th>
<th>PT (Mean ± SD)</th>
<th>Range</th>
<th>RVV Time (Mean ± SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normal plasma (n = 40)</td>
<td>18.3 ± 0.5</td>
<td>17.4 - 20.0</td>
<td>19.0 ± 2.6</td>
<td>17 - 20.2</td>
</tr>
<tr>
<td>2. Normal plasma + uncentrifuged A.F. (n = 40)</td>
<td>16.2 ± 2.1</td>
<td>12.0 - 23.0</td>
<td>18.2 ± 2.6</td>
<td>16.2 - 20.1</td>
</tr>
<tr>
<td>3. Normal plasma + supernatant A.F. (n = 40)</td>
<td>17.3 ± 2.3</td>
<td>12.0 - 24.0</td>
<td>18.0 ± 2.5</td>
<td>16.0 - 20.4</td>
</tr>
</tbody>
</table>

P value

- 1 vs 1 <0.001 >0.1
- 3 vs 1 <0.02 >0.01
- 3 vs 2 <0.05 >0.05

**TABLE IV**

**Effect of Amniotic Fluid on Prothrombin Time (PT) and RVV Time of Deficient Substrate Plasma**

<table>
<thead>
<tr>
<th>Material</th>
<th>PT Mean</th>
<th>Range</th>
<th>RVV Time Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Factor VII + X deficient plasma (PT = 90'; RVVT = 41&quot;) + A.F. (n = 10)</td>
<td>28.3</td>
<td>30-83</td>
<td>36.1</td>
<td>34-39</td>
</tr>
<tr>
<td>2. Factor V deficient plasma (PT = 60'; RVVT = 106&quot;) + A.F. (n = 10)</td>
<td>104.3</td>
<td>100-109</td>
<td>112</td>
<td>105-118</td>
</tr>
</tbody>
</table>
deficient plasma was 90 seconds. After addition of 10 different samples of amniotic fluids, the prothrombin time showed a range of 50-63 seconds with a mean value of 58.30 seconds.

The prothrombin time of plasma having factor V deficiency was 80 seconds. On addition of different samples of amniotic fluids, the prothrombin times were prolonged and their range varied from 100-109 seconds with a mean value of 104.33 seconds.

Effect of Amniotic Fluid on the R.V.V. Time of Plasma

The R.V.V. time of control plasma showed a range between 17 to 20.2 seconds with a mean value of 19 seconds. After addition of uncentrifuged amniotic fluid the mean was 18.16 seconds with a range of 16 to 20.1 seconds. On addition of centrifuged amniotic fluid the range varied between 16 and 20.4 seconds with a mean of 17.99 seconds.

On statistical analysis the difference was not significant with uncentrifuged amniotic fluid while it was significant with centrifuged amniotic fluid.

The R.V.V. cephalin time of substrate plasma deficient in VII + X was 41". After addition of amniotic fluid it ranged between 34-39 seconds with a mean of 36.1 seconds. The R.V.V. time of factor-V deficient plasma was 112 seconds. After addition of amniotic fluid, it was not shortened in any of the specimens.

Procoagulant activity of amniotic fluid at different periods of gestation

The shortening of recalcification time by amniotic fluid samples at varying period of gestation was statistically analysed. The regression coefficient (r) was found to be -0.43, indicating significant reverse correlation i.e. more the period of gestation at which the amniotic fluid was collected, shorter the recalcification time. In other words, the procoagulant activity of amniotic fluid increases with the period of gestation.

Platelet Aggregating Effect of Amniotic Fluid

Table V clearly shows that centrifuged amniotic fluid does not have platelet aggregating activity. Neither there is any inhibiting effect of amniotic fluid on platelet aggregating activity of A.D.P.

<table>
<thead>
<tr>
<th>Material</th>
<th>Platelet aggregation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Platelet-rich plasma (PRP) + amniotic fluid (A.F.) (n = 5)</td>
<td>No aggregation in 10 min—</td>
</tr>
<tr>
<td>2. P.R.P. + adenosine diphosphate (n = 5)</td>
<td>26.4 sec. 23.2—28.6</td>
</tr>
<tr>
<td>3. P.R.P. + adenosine diphate + A.F. (n = 5)</td>
<td>27.2 sec. 22.8 — 30.2</td>
</tr>
</tbody>
</table>

was not significant with uncentrifuged amniotic fluid while it was significant with centrifuged amniotic fluid.

Anti-heparin Activity of Amniotic Fluid

Two samples of amniotic fluid were tested for antiheparin activity. The addition of amniotic fluid shortened the recalcification time of heparin added plasma. When heparin (3.5 units/ml) was added to the normal plasma the recalcification time was prolonged to 365 seconds in the
first sample and to 300 seconds in the second sample. On addition of amniotic fluid the recalcification time were shortened to 175 to 135 seconds respectively in first and second samples.

Discussion

The procoagulant activity in the amniotic fluid has been clearly demonstrated in this study as well as the previous ones (Weiner et al, 1950; Rendelstein et al, 1951; Phillips and Davidson, 1972). The results further indicate that addition of amniotic fluid bypasses the coagulation factors XI, IX and VIII in the intrinsic system and that its action is at a further lower level of the coagulation cascade.

As regards the extrinsic system of blood coagulation, the prothrombin time as well as R.V.V. time was reduced to a very small extent after the addition of amniotic fluid. These observations are in full accordance with those of Rendelstein et al (1951) and Phillips and Davidson (1972) and would indicate that the amniotic fluid acts in a manner very akin to tissue thromboplastin.

The addition of amniotic fluid corrected only partially the recalcification time as well as the prothrombin time of factor VII + X deficient substrate plasma. The mean prothrombin time of 90 seconds was reduced to 33.30 seconds. On the other hand, the R.V.V.—cephalin time of similar substrate plasma samples was reduced to a very small degree i.e. from a mean of 40 seconds to 36.1 seconds. Since the action of Russel Viper Venom does not require factor VII in contradistinction to that of tissue thromboplastin, the difference in the activity by these two tests on VII/X deficient substrate plasma would indicate that amniotic fluid bypasses most of the activity of factor VII and that its main action is on the activation of factor X.

Phillips and Davidson (1972) also had similar results with prothrombin time and R.V.V.—cephalin time using substrate plasma deficient in factor VII and X. They also used substrate plasma deficient in factor VII only and demonstrated marked lowering of recalcification time as well as R.V.V. time. On the other hand, with factor X deficient plasma the addition of amniotic fluid brought about rather an increase in R.V.V. time.

Since factor V is below factor X in the coagulation scheme, the substrate plasma deficient in the former would not be affected by the addition of amniotic fluid if its major action was the activation of factor X. Our experiments brought out exactly the same results. Similarly Rendelstein et al (1951) did not find the correction of prothrombin time of old plasma deficient in factor V.

Experiments with whole amniotic fluid (uncen rifuged) and with supernatant of centrifuged amniotic fluids showed little difference in procoagulant activity in these two types of samples. It can thus be inferred that the procoagulant activity is mostly present in the soluble form. Phillips and Davidson (1972) fractionated the lipid contents of the amniotic fluid. They demonstrated the presence of phosphatidyl ethanoplamine and these lipids could very well be expected to shorten the recalcification time.

It is probable that a part of thrombokinase like activity comes from the disposed material of epithelial cells of embryonic skin and amnion. A part of similar activity may also come from fetal urine in the amniotic fluid Rendelstein et al (1951). An indirect evidence to this effect comes from our observation that
the coagulant activity of amniotic fluid increased as pregnancy progressed.

The clear supernatant amniotic fluid after centrifugation does not possess any platelet aggregating activity neither any inhibiting material to A.D.P. was demonstrable. However, since particulate material is present in abundance in amniotic fluid, that may be of crucial significance in initiating platelet aggregation ultimately leading to disseminated intravascular coagulation McKay (1959).

The procoagulant activity of the amniotic fluid alone may not be sufficient to induce disseminated intravascular coagulation. Phillips and Davidson (1972) calculated the factor X activator activity of the amniotic fluid in terms of R.V.V. equivalent and taking a clue from experiments in rats with R.V.V. infusion Rasano and Davidson (1969). They thought that on "weight to weight basis approximately 10 litres of amniotic fluid would need to be infused into the maternal circulation to produce an activation of human coagulation system equivalent to that elicited by 100 ugm of R.V.V. in a rat", an amount which when injected leads to severe coagulation derangements but the rat survives. Schneider (1957) also pointed out that estimated lethal volume for instantaneous infusion into a human is so large as to preclude serious considerations. Nevertheless the platelet aggregating activity of the particulate material present in amniotic fluid together with the procoagulant activity in the clear fluid can certainly be a formidable combination.

It has been repeatedly shown in experimental animals that clear fluid failed to produce harmful effect on the animals but the fluid containing meconium did result in severe respiratory distress and cor pulmonale (Muirhead and Montgomery, 1951; Schneider, 1953). The particulate content of the meconium may be of great significance.

The procoagulant activity of amniotic fluid is probably physiologically important in maintaining the hemostasis at the time of normal placental separation. Female patients with factor XI deficiency seldom have postpartum hemorrhage. On the contrary, those with factor V deficiency have frequent problem of postpartum bleeding Phillips and Little (1962). This indicates the role of thrombokinase like activity of amniotic fluid in normal local hemostasis during parturition.

Summary

Amniotic fluid from 50 women with varying period of gestation was tested. The amniotic fluid is shown to possess potent procoagulant activity which increases with the advancement of pregnancy. Various experiments with deficient plasma substrate showed that its probable mode of action is activation of factor X. The amniotic fluid also possesses antiheparin activity but the clear supernatant fluid is devoid of any action on platelet aggregation.

References


