Prostaglandin F<sub>2α</sub> and E<sub>1</sub> in Plasma and Amniotic Fluid During Human Pregnancy and Labor

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Synopsis

In order to elucidate the significance of PGs in human labor, PGE<sub>1</sub> and F<sub>2α</sub> in biological fluid during human pregnancy and labor were measured by RIA newly developed.

Analytical studies demonstrated that the levels of PGF<sub>2α</sub> in maternal plasma were 3.7±2.5 ng/ml a few days before parturition, 2.0±0.9 ng/ml in the first stage of labor and 1.9±1.4 ng/ml at delivery. Thus the concentrations of PGF<sub>2α</sub> in maternal plasma showed no significant changes around parturition. On the other hand, the level of PGF<sub>2α</sub> in amniotic fluid represented a significant increase up to 44.27±32.81 ng/ml at delivery from 1.45±0.76 ng/ml before labor at 38-40 weeks of pregnancy (p<0.05), although it was uncertain whether this elevation was the cause or effect of uterine contraction.

The concentration of PGE<sub>1</sub> ranged from 2 to 14 ng/ml around parturition. This indicates that there was little remarkable difference between the levels of PGE<sub>1</sub> in plasma and amniotic fluid during the last month of pregnancy and labor.

Possible involvement of prostaglandins (PGs) in human labor has been discussed.

There are a lot of evidences supporting the suggestion that prostaglandins (PGs) may be one of the promising candidates to play a role in the initiation and maintenance of human labor. Much attention has been paid to the great ability of PGs, especially PGF<sub>2α</sub>, E<sub>1</sub> and E<sub>2</sub> to cause uterine contractions at various stages of pregnancy (Bygdeman et al., 1968). This led obstetricians to use PGs clinically as abortients (Kinoshita et al., 1971a; Wiqvist et al., 1972) and oxytocics (Kinoshita et al., 1971b; Anderson et al., 1972). It has, however, been difficult to document how PGs are concerned with pregnancy and labor, despite the accumulation of pharmacological and clinical data concerning the induction of uterine contraction.

Karim and his associates, using bioassay (Karim and Devlin, 1967), found increased concentration of PGF<sub>2α</sub> during labor in amniotic fluid, but not in plasma. However, bioassay systems used were not necessarily sensitive and specific enough to analyze PGs in biological samples. The development of more specific and relatively simple assay methods for PGs has, therefore, been urgently required and consequently several papers on radioimmunoassay systems (RIA) for PGE, F and A have been presented (Caldwell et al., 1971; Jaffe et al., 1973; Lindgren et al., 1974).

Little is, however, known about the levels of PGE<sub>1</sub> and F<sub>2α</sub> in maternal blood and amniotic fluid throughout pregnancy and labor. In the present paper, the ana-
lytical study of PGF\textsubscript{2\alpha} and E\textsubscript{1} during pregnancy and labor is reported, by our RIA newly developed.

**Materials and Methods**

**Extraction of PGs:**
Two ml of plasma or amniotic fluid was added with ³H-PGF\textsubscript{2\alpha} (approx. 1,000 cpm, 50 μCi/1.2 μg) for recovery and extracted with 2 volumes of methylalcohol (5:1). The resultant precipitate was re-extracted with 5 ml of chloroform-methanol (2:1). The extracts were subsequently partitioned between 20 ml each of petroleum ether and 90% methanol to remove less polar lipids. The 90% methanol fraction was evaporated to dryness in vacuo.

**Separation of PGs:**
One g of silicic acid was immersed in benzeneethyl acetate-methanol (60:40:0.5) (solvent 1) to make slurry. After adding the slurry to each glass column (i.d. 0.7×20 cm), the silicic acid was washed with 30 ml of solvent 1. Sample residues were dissolved in 1 ml of solvent 1, and were applied to the columns which were allowed to run dry. Prostaglandin fractions were obtained by developing the columns serially with 14 ml of solvent 1 (PGA and PGB), 14 ml of solvent 2 (benzene-ethyl acetate-methanol 60:40:3.5) (PGE), and 10 ml of solvent 3 (benzene-ethyl acetate-methanol 60:40:10) (PGF) (Fig. 1). The fractions were taken to dryness by evaporation in air at 40°C and dissolved in 1 ml of methanol. Half of the sample was transferred to 10 ml of toluene scintillation solution for counting to permit calculation of recovery. Another 0.5 ml of the sample was evaporated to dryness and dissolved in 0.1 ml of 0.1 M phosphate buffer (pH 7.4) containing 0.15 M NaCl and 0.1% gelatin (assay buffer).

**Antisera:**
PGF\textsubscript{2\alpha}- and PGE\textsubscript{1}-bovine serum albumin conjugates, as antigen, were prepared by carbodiimide reagent and supplied by Dr. Hirata of Ono Central Research Institute. Adult rabbits were immunized subcutaneously or intramuscularly once a week for four weeks, with an emulsified mixture of 1 ml each of complete Freund's adjuvant and physiological saline containing 1 mg of the conjugate.

**Radioimmunoassay system:**
A mixture of 0.1 ml each of tritiated prostaglandin (6,000 cpm, ³H-PGF\textsubscript{2\alpha} 50 μCi/1.2 μg or ³H-PGE\textsubscript{1} 50 μCi/1.2 μg) and of unknown sample or unlabeled prostaglandin (0.5–10 ng) in assay buffer was incubated for 1 hour at 37°C. Separation of antibody-bound from unbound ³H-PG was accomplished by adding to the reaction mixture 0.1 ml of dextran coated charcoal (Norit A, 5 g; dextran T 40, 0.5 g in 100 ml of assay buffer). After 10-min centrifugation at 4°C, 3,000 rpm, the supernatant (containing antibody bound ³H-PG) was immediately decanted into 10 ml of Bray's scintillation solution. Radioactivity was counted in an Aloka liquid scintillation counter Model 653.

**Plasma and amniotic fluid samples:**
One hundred and six plasma samples for PG analysis were collected from pregnant women during late pregnancy and labor. In addition, specimens of amniotic fluid, maternal venous plasma and cord plasma were obtained from patients at normal delivery. Amniotic fluid was obtained by amniocentesis from patients before labor.

![Fig. 1. Elution pattern for PGs separated by chromatography on silicic acid column.](image-url)
Table 1. Specificity of Antiserum against PGF$_{2\alpha}$-BSA

<table>
<thead>
<tr>
<th>PGs etc</th>
<th>Quantity on 50% Inhibition (ng)</th>
<th>Relative Cross Reaction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F$_{2\alpha}$</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>E$_1$</td>
<td>5.2</td>
<td>19.2</td>
</tr>
<tr>
<td>F$_{1\alpha}$</td>
<td>8.6</td>
<td>14.7</td>
</tr>
<tr>
<td>A$_2$</td>
<td>260</td>
<td>0.39</td>
</tr>
<tr>
<td>A$_1$</td>
<td>3000</td>
<td>0.03</td>
</tr>
<tr>
<td>Metabolite</td>
<td>≥</td>
<td>≤</td>
</tr>
</tbody>
</table>

Table 2. Specificity of antiserum against PGE$_1$-BSA

<table>
<thead>
<tr>
<th>PGs</th>
<th>Quantity on 50% Inhibition (ng)</th>
<th>Relative Cross Reaction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E$_1$</td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td>E$_2$</td>
<td>8.0</td>
<td>6.2</td>
</tr>
<tr>
<td>F$_{1\alpha}$</td>
<td>13.0</td>
<td>3.8</td>
</tr>
<tr>
<td>F$_{2\alpha}$</td>
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<td>0</td>
</tr>
<tr>
<td>A$_1$</td>
<td>≥</td>
<td>0</td>
</tr>
<tr>
<td>A$_2$</td>
<td>≥</td>
<td>0</td>
</tr>
<tr>
<td>B$_1$</td>
<td>≥</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 2. Cross reactions with antiserum to PGF$_{2\alpha}$ at a dilution of 1/600.

Fig. 3. Cross reactions with antiserum to PGE$_1$ at a dilution of 1/800.
PGF₁α, PGA₂, PGA₁ and urinary metabolite of PGF₂α, respectively and bound 50% of $^3$H-PGF₂α added at 600-fold dilution. On the other hand, an antiserum to PGE₁ had relative cross-reactivities of 6.2%, 3.8%, and 0% to PGE₂, PGA₁α, and other PGs, respectively and bound 50% of $^3$H-PGE₁ added at 800-fold dilution. Both antisera were, therefore, satisfactory enough for RIA in terms of specificity.

Validation of the assay method:
Accuracy was estimated by recovery experiments performed by measuring the levels of PGF₂α present in plasma to which known amounts had been added. The value measured did not differ by more than 20% from the expected value (Table 3). The same experiment demonstrated the validity of RIA for PGE₁ as well.

Table 3. Radioimmunoassay of known amounts of PGF₂α added to plasma

<table>
<thead>
<tr>
<th>Added PGF₂α (ng)</th>
<th>n</th>
<th>Plasma Volume</th>
<th>Found, mean $\pm$ SD (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>4</td>
<td>2.0</td>
<td>2.63 $\pm$ 0.64</td>
</tr>
<tr>
<td>1.0</td>
<td>3</td>
<td>2.0</td>
<td>3.73 $\pm$ 0.26</td>
</tr>
<tr>
<td>5.0</td>
<td>3</td>
<td>2.0</td>
<td>6.67 $\pm$ 0.58</td>
</tr>
<tr>
<td>10.0</td>
<td>4</td>
<td>2.0</td>
<td>11.01 $\pm$ 2.22</td>
</tr>
<tr>
<td>20.0</td>
<td>3</td>
<td>2.0</td>
<td>21.03 $\pm$ 3.49</td>
</tr>
</tbody>
</table>

Measurement of PGF₂α and PGE₁:
The standard curve for PGF₂α was shown in Fig. 4 using an antiserum at 600-fold dilution.

The levels of PGF₂α in plasma obtained before and after parturition were demonstrated in Fig. 5. The concentration of PGF₂α ranged from 1 to 6 ng/ml and no significant changes in the level of PGF₂α were found in plasma around parturition. The levels of PGF₂α in plasma were $3.7 \pm 2.5$ ng/ml ($n=10$) a few days before the initiation of labor and $2.0 \pm 0.9$ ng/ml ($n=10$) (mean $\pm$ SD) in the first stage of labor.

PGF₂α plasma levels followed serially from the near term to delivery in 10 patients are shown in Fig. 6. Half of the patients showed higher concentrations of PGF₂α a few days before parturition than those in the first stage of labor, whereas the other half did not represent such a trend. Thus the levels of PGF₂α in each patient plasma during the last month of pregnancy and labor did not show a uniform pattern.

The levels of PGF₂α in maternal peripheral blood, umbilical venous and arterial blood, retro-placental blood and amniotic fluid obtained at normal delivery ($n=10$) were $1.92 \pm 1.42$, $3.41 \pm 2.40$, $2.74 \pm 1.27$, $12.5 \pm 9.79$, and $44.27 \pm 32.80$ ng/ml (mean...
Fig. 6. PGF levels in peripheral plasma of ten patients throughout their course of normal pregnancy and delivery.

±SD), respectively (Fig. 7). The concentration of PGF$_{2\alpha}$ in amniotic fluid obtained before labor at 38–40 weeks of pregnancy was 1.45 ± 0.76 ng/ml (n=6) (Fig 8). The level of PGF$_{2\alpha}$ in amniotic fluid at delivery was significantly higher than those in amniotic fluid obtained without any labor pain and in maternal plasma (p<0.05).

The concentrations of PGE$_1$ in maternal plasma in the last month of pregnancy and labor were demonstrated in Fig. 9. There was no statistically significant difference among the mean values in each week of pregnancy and labor. The levels of PGE$_1$ were around 10 ng/ml in the last month of pregnancy.

Fig. 10 showed the concentration of PGE$_1$ in plasma of 8 cases before and after the initiation of labor. The results showed that there was a considerable individual variation in the concentration of PGE$_1$ around parturition and that the levels of PGE$_1$ had no correlation with the progress
of cervical dilatation.

The samples collected at delivery were also measured by RIA for PGE\(_1\). The measurement of PGE\(_1\) in amniotic fluid obtained during labor showed no increase in the concentrations, differing from those of PGF\(_{2\alpha}\). Namely, 4.4±1.8 ng/ml (n=3) in amniotic fluid during labor and 5.0±1.2 ng/ml (n=7) without uterine contractions were presented. The concentrations of PGE\(_1\) in umbilical artery, umbilical vein, maternal plasma, uterine vein and retroplacental blood obtained at delivery were 1.9±0.4, 3.8±2.3, 6.8±5.7, 1.8±0.4 and 3.0±0.8 ng/ml, respectively (n=7).

Thus, as far as the levels of PGE\(_1\) in plasma and amniotic fluid are concerned, there were no significant changes observed in relation to uterine contractions.
Discussion

There have recently been several reports that antibodies to several kinds of PGs were prepared and their usefulness was evaluated. Since specificity and simplicity of RIA are thought to depend ultimately on the serological specificity of the antigen-antibody reaction, the property of antisera decides the reliability of the assay.

Antisera to PGF sub 2 alpha reported in this paper had 19.2% of cross-reaction with PGE sub 1. The extracts from samples, therefore, were necessary to be subjected to column chromatography using silicic acid to separate PGF sub 2 alpha from E. In addition, our antisera had 14.2% of cross-reaction with PGF sub 1 alpha and, moreover, chromatographical separation of PGF sub 2 alpha from F sub 1 alpha on a silicic acid column was impossible. The data presented here concerning PGF sub 2 alpha levels, therefore, should be evaluated as total PGF values.

On the other hand, it has generally been impressed that it is very difficult to prepare the specific antisera to PGE sub 1, since dehydration of PGE to A may easily occur during conjugation procedure using carbodiimide (Levine et al., 1971) and further conversion of PGA to B may often take place after the injection into animals, of which plasma contains the isomerase catalyzing the reaction (Piper et al., 1970). However, the antisera to PGE sub 1 we prepared had the high degree of specificity to allow reliable determination of PGE sub 1 in biological fluid.

The study for validation of the assay system showed that our RIA was considered to be quite satisfactory with respect to precision, recovery and reproducibility.

It was evidenced from the data presented that there were no remarkable variations in the levels of PGF sub 2 alpha and E sub 1 in maternal plasma in relation to parturition. On the contrary, the significant elevation of PGF sub 2 alpha concentrations in amniotic fluid was clearly demonstrated only during labor. But it was uncertain whether this elevation was the cause or effect of uterine contractions.

Conflicting data have been reported by several investigators that PGF levels in peripheral plasma and serum during pregnancy were unchanged, increased slightly (Patrono, 1973), reached to a peak during the second trimester (Jutierrez-Cernosek et al., 1972), or showed the lowest values during the same period (Hennam et al., 1974). Moreover, there was a report that high levels of PGF sub 2 alpha in plasma during labor were observed (Brummer and Craft, 1973), whereas in another paper no correlation between plasma PGF sub 2 alpha levels and the stage of labor was reported (Johnson et al., 1975).

On the contrary, as to the levels of PGF sub 2 alpha in amniotic fluid, Salmon and Amy (1973), and Keirse et al. (1974) reported the similar results to ours. These data may suggest that PGF sub 2 alpha and E sub 1 in circulating blood are rapidly metabolized in lung and liver (Hamberg and Samuelsson, 1971; Granström, 1972) it seems to be very hard to detect the changes in the levels of the primary PGs, mainly PGF and E, in peripheral plasma and serum in relation to human labor. Meanwhile, it is conjectured that the accumulation of PGF sub 2 alpha in amniotic fluid might result partly from the slow degradation of PGF in amniotic fluid and partly from increased the production of PGF in relation to the uterine contractions.

The values of PGs measured by our RIA were of the order of nanogram, while the normal plasma and serum levels of PGs reported by other laboratories ranged from about 10 pg/ml to 10 ng/ml. These discrepancies seem to be attributed to the difference of antisera used and the variation in sampling technique. Besides, it should be pointed out that the concentrations of PGs may, in general, represent higher levels
than those expected due to the possible formation of PGs during the collection and isolation of plasma, since it has been shown that platelets are able to form endoperoxide intermediates rapidly, which are then converted into PGE and F (Smith and Willis, 1971; Smith et al., 1973).

It is, therefore, obvious that the estimation of the major metabolites, i.e. 15-keto-13, 14-dihydro PGF\(_{2\alpha}\), 15-keto-13, 14-dihydro PGE\(_2\) and the urinary metabolites may be more reliable for monitoring the production of PGF\(_{2\alpha}\) and E\(_2\). The report by Gréen et al. (1974) using gas-chromatography-mass spectrometry showed that the concentration of 15-keto-13, 14-dihydro PGF\(_{2\alpha}\) in plasma was increased to 10- to 30-fold during active labor compared with that in the last month of pregnancy preceding labor.

In conclusion, the present results reported here especially concerning PGF levels in amniotic fluid clearly indicate that PGF\(_{2\alpha}\) endogenously formed may be strongly involved in human labor, although the main sites of PG production have been unknown yet.

References


