Foetal membranes and attached decidua vera were removed from placenta collected at the time of elective caesarean section performed before the onset of labour. The tissues were washed twice in ice-cold 0.15 M NaCl. The amnion tissue was peeled away from the chorion laeve, and the decidua vera was removed from the chorion laeve by scraping. A portion of the villous tissue of placenta was cut from the maternal surface. Specimens of each tissue were homogenized in ice-cold 50 mM-phosphate buffer, pH 7.4 (buffer: tissue ratio, 1:3 w/v), with a Polytron PT10 homogenizer at a setting of 6 for 30 s. Each homogenate was successively centrifuged at 1000 g for 20 min and 105,000 g for 1 h. The resulting supernatant fraction (cytosol) was used for assays of prostaglandin synthase in 1.0 ml of 50 mM-phosphate buffer, pH 7.4, with or without test cytosol. The reaction was started by addition of sodium arachidonate (final concn. 61 μM), and the tubes were aerated with gentle shaking. After 20 min, the reaction was stopped by adding 0.5 ml of 0.4 M citric acid, and the mixture was centrifuged at 2000 g for 30 min to remove denatured proteins. Experiments were conducted with arachidonic acid and bovine seminal-vesicle prostaglandin synthase (Miles Laboratories, Elkhart, IN, U.S.A.). The incubation mixture consisted of 4 μg of synthase in 1.0 ml of 50 mM-phosphate buffer, pH 7.4, with or without test cytosol. The reaction was started by addition of sodium arachidonate (final concn. 61 μM), and the tubes were aerated with gentle shaking. After 20 min, the reaction was stopped by adding 0.5 ml of 0.4 M citric acid, and the mixture was extracted with 7.0 ml of ethyl acetate. The organic layer was removed, evaporated to dryness under N2, and the residue dissolved in 1.0 ml of phosphate buffer. Prostaglandin E2, prostaglandin F2α, and 6-oxoprostaglandin F1α, a metabolite of prostacyclin, were measured by specific radioimmunoassays (Mitchell & Flint, 1978; Mitchell, 1978).

Table 1. Effect of cytosol from various intra-uterine tissues on prostaglandin biosynthesis by bovine seminal-vesicle prosta-
glandin synthase

<table>
<thead>
<tr>
<th>Tissue source</th>
<th>Dose of cytosol (μl)</th>
<th>Prostaglandin E2 (pmol)</th>
<th>Prostaglandin F2α (pmol)</th>
<th>6-Oxoprostaglandin F1α (pmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amnion</td>
<td>100</td>
<td>1.1 (1.3)</td>
<td>1.3 (1.4)</td>
<td>1.1 (1.1)</td>
</tr>
<tr>
<td>Chorion laeve</td>
<td>600</td>
<td>1.2 (1.0)</td>
<td>2.4 (1.9)</td>
<td>1.6 (1.1)</td>
</tr>
<tr>
<td>Decidua vera</td>
<td>600</td>
<td>1.3 (0.8)</td>
<td>3.4 (1.7)</td>
<td>1.9 (1.4)</td>
</tr>
<tr>
<td>Placenta</td>
<td>100</td>
<td>1.4 (0.8)</td>
<td>1.6 (1.0)</td>
<td>1.2 (1.5)</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>1.2 (0.8)</td>
<td>1.9 (1.3)</td>
<td>1.3 (1.4)</td>
</tr>
</tbody>
</table>

Endogenous inhibitors of prostaglandin biosynthesis in human amniotic fluid and pregnancy plasma

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It has been demonstrated that blood plasma or serum of adult, but not foetal, mammals potently inhibits the biosynthesis of prostaglandins (Saeed et al., 1977; DiRosa et al., 1979). These findings have led to the conclusion that human plasma contains one or more circulating endogenous inhibitors of prostaglandin synthase (Saeed et al., 1977; Collier et al., 1982). In more recent reports describing the activity of such endogenous inhibitors in pregnant sheep plasma (Mitchell et al., 1981; Collier et al., 1982) it was suggested that there is a further pregnancy-associated inhibitor. Indeed, Robinson et al. (1978) have postulated that endogenous inhibitors of prostaglandin synthase may act to suppress prostaglandin biosynthesis tonically during pregnancy and hence prevent abortion and premature delivery.

The results obtained with various cytosols (boiled and unboiled) tested both at low and high concentration for the stimulation of prostaglandin synthase system of bovine seminal vesicles are summarized in Table 1. It is evident that the presence of unboiled cytosol in the reaction mixture enhanced the production of prostaglandins, especially that of prostaglandin F2α and 6-oxoprostaglandin F1α. This effect of the cytosol was dose-dependent and sensitive to boiling. Among the cytosols of various intrauterine tissues, placental cytosol produced a much larger stimulatory effect on prostaglandin F2α and 6-oxoprostaglandin F1α biosynthesis than did the cytosols derived from amnion, chorion laeve and decidua vera. It also is noteworthy that, except for placenta, none of the other tissue cytosols affected prostaglandin E2 synthesis. The capacity of each cytosol to stimulate prostaglandin biosynthesis also was found to be completely abolished by indomethacin. These results therefore point to the existence of a highly specialized mechanism for modulating prostaglandin F2α and prostacyclin production in human intra-uterine tissue.

We thank Mrs. Lydia Morris for expert editorial assistance. This work was supported in part by U.S. Public Health Service Grant no. 5-P50-HD11149.
As part of a programme to investigate the possible role of
human endogenous inhibitors in the regulation of prostaglandin
biosynthesis during pregnancy and parturition, we studied the
effect of human amniotic fluid and pregnancy plasma on the
biosynthesis of various prostaglandins.

Experiments were performed with arachidonic acid and
bovine seminal-vesicle prostaglandin synthase (Miles Labora-
tories, Elkhart, IN, U.S.A.). The standard assay mixture
contained, in a final volume of 1.0 ml, 50 mM-phosphate buffer,
ph 7.4, reduced glutathione (1.3 mM), with and without test
fluid, 4 mg of prostaglandin synthase and sodium arachidonate
(61 µM). After 20 min incubation with gentle shaking at 37°C,
the reaction was stopped by adding 0.5 ml of 0.4 M-Citric acid,
and the prostaglandins were extracted with 7.0 ml of ethyl
acetate. After thorough mixing and centrifugation for 5 min at
600 g the ethyl acetate layer was removed, evaporated to
dryness under N2, and the residue dissolved in 1.0 ml of
phosphate buffer, pH 7.4. Prostaglandin E2 and 6-oxopros-
glandin F1α were measured by specific radioimmunoassays
(Mitchell & Flint, 1978; Mitchell, 1978) that have been fully
described and validated. All experiments were performed with
appropriate controls.

The concentration-dependent degrees of inhibition of pros-
taglandin biosynthesis by human amniotic fluid, plasmas of
pregnant and non-pregnant women and indomethacin are presented
in Table 1. It is evident that amniotic fluid, in common

Table 1. Comparative effects of human amniotic fluid and
pregnancy plasma on the inhibition of biosynthesis of prosta-
glandin E2 and 6-oxoprostaglandin F1α.

<table>
<thead>
<tr>
<th>Source of human fluid</th>
<th>Concentration (%)</th>
<th>Inhibition of prostaglandin biosynthesis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amniotic fluid</td>
<td>3</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>69</td>
</tr>
<tr>
<td>Pregnant female</td>
<td>0.3</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>90</td>
</tr>
<tr>
<td>Non-pregnant female</td>
<td>0.25</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>84</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.28*</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>1.40*</td>
<td>93</td>
</tr>
</tbody>
</table>

* Concentration expressed as µM.

with plasma of pregnant women, inhibited prostaglandin
biosynthesis. The inhibitory effects of amniotic fluid were more
pronounced against the synthesis of prostaglandin E2 than that of
6-oxoprostaglandin F1α. Plasmas from pregnant and non-
pregnant women inhibited prostaglandin synthesis strikingly.
This effect of plasma was abolished by heating at 100°C for
5 min and was associated with a protein.

In preliminary experiments designed to investigate the nature
of the proteins with endogenous inhibitor activity, the plasma of
pregnant women was fractionated by continuous sucrose-
density-gradient ultracentrifugation (Beckman model L265,
rotor SW60; 200000 g for 19 h at 4°C). The results of this
analysis indicated that the endogenous inhibitor activity of
pregnancy plasma was mainly associated with protein fractions
sedimenting in the region of 4S and 5S of the gradient. Further
purification of these proteins is required to establish the presence
of a pregnancy-associated endogenous inhibitor of pros-
taglandin synthase in humans.

These results demonstrate the presence of endogenous
inhibitor activity in amniotic fluid and pregnancy plasma. Since
albumin is the major protein found in the amniotic fluid and
because albumin has been shown previously to inhibit prosta-
glandin E2 biosynthesis, the question remains: by what
mechanism does albumin inhibit prostaglandin biosynthesis?
Since albumin does not affect fatty acid cyclo-oxygenase (Collier
et al., 1982), we speculate that albumin could inhibit
prostaglandin production either (i) by binding to the fatty acid
substrate or (ii) by enhancing uptake of arachidonic acid into
glycerophospholphid and triacylglycerols. The latter prop-
osition is supported further by our recent finding (S. A. Saeed &
M. D. Mitchell, unpublished work) that a cytotoxic factor in
human amnion enhances incorporation of arachidonate into
lipids during incubation with a microsomal fraction from human
amnion.

We thank Mrs. Lydia Morris for expert editorial assistance. This
work was supported in part by U.S. Public Health Service Grant no.
5-P50-HD11149.

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Interactions of human lipoproteins with bovine seminal-vesicle prostaglandin synthase

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Human low-density and high-density lipoproteins have been
added recently to a new class of plasma inhibitors of
prostaglandin synthesis (Saeed & Mitchell, 1982). In the present
communication we describe the findings of additional investiga-
tions of the effects of human lipoproteins, including very-
low-density lipoprotein, on prostaglandin biosynthesis.

The lipoproteins were isolated from human plasma of healthy
men by zonal ultracentrifugation by methods described pre-
viously (Havel et al., 1955; Goldstein & Brown, 1974). Briefly,
very-low-density lipoprotein (density < 1.006 g/ml), low density
lipoprotein (density = 1.019-1.063 g/ml) and high-density