HORMONE AND ENZYME ASSAYS IN PREGNANCY

IV. *The human chorionic somatomammotrophin, placental cystine-aminopeptidase, progesterone and the urinary oestrogens in pregnancies complicated with essential hypertension, mild or severe pre-eclampsia*

By

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ABSTRACT

A comparative study of human chorionic somatomammotrophin (HCS), placental cystine-aminopeptidase (P-CAP), progesterone and total urinary oestrogens (Oe) in pregnancies complicated with essential hypertension, mild or severe pre-eclampsia was performed.

No significant reduction in the 4 parameters could be demonstrated in cases complicated with essential hypertension.

In cases complicated with mild pre-eclampsia, however, a significant reduction was found in the HCS ($P < 0.001$) and P-CAP ($0.01 < P < 0.02$) but not in the urinary oestrogen values ($0.1 < P < 0.2$). A tendency to increased progesterone values could be demonstrated. Furthermore, in those cases of mild pre-eclampsia associated with a birth weight below the 10 percentile, the HCS and P-CAP assays proved to be more sensitive than the urinary oestrogen assay. No influence upon the progesterone levels was observed in these cases.

On the other hand, in pregnancies complicated with severe pre-eclampsia the HCS, P-CAP and urinary oestrogen values were significantly reduced ($P < 0.001$) but not the progesterone values ($P > 0.5$). Again, when severe

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pre-eclampsia was associated with low birth weight, HCS and P-CAP were the most reliable tests.
Based upon the HCS, P-CAP and the urinary oestrogen readings placental and foeto-placental scores were introduced. The placental score provided valuable information concerning the viability of the infants but no additional information was obtained by the foeto-placental score. The results indicated that simultaneous measurements of HCS and P-CAP as a placental function test and total urinary oestrogens as a foeto-placental test contribute reliable information in pregnancies complicated with pre-eclampsia. The measurement of progesterone could not be recommended as a test for the placental function.

In previous studies the human chorionic somatomammotrophin (HCS), placental cystine-aminopeptidase (P-CAP) and progesterone patterns during normal pregnancy were investigated (Christensen 1974a; Christensen et al. 1974). In addition, the P-CAP and total urinary oestrogens were studied in pregnancies complicated with essential hypertension, mild or severe pre-eclampsia. In cases complicated with essential hypertension neither the enzyme activity nor the urinary output of oestrogens were significantly reduced. Both in mild and severe pre-eclampsia the P-CAP and the urinary oestrogens were significantly reduced, but the reduction in the P-CAP values was more marked than the reduction in the oestrogen values. When P-CAP and the urinary oestrogens were measured simultaneously the possibility of predicting the viability of the foetus was increased. Even in pregnancies complicated with severe pre-eclampsia, however, some normal P-CAP and oestrogen values were found.

It has recently been reported that HCS is a sensitive test for the placental function (Genazzani et al. 1972; Spellacy et al. 1971; Lindberg & Nilsson 1973; Gitsch et al. 1973). There has been some doubt about the value of progesterone as an index for the placental function due to the large range in the normal pregnancy values (Johansson 1969a; Christensen et al. 1974). The aim of the present study was therefore to ascertain the value of HCS and progesterone in pregnancies complicated with pre-eclampsia and essential hypertension, and further to determine whether simultaneous serial measurements of HCS, P-CAP, progesterone and total urinary oestrogens would be of further support in the clinical evaluation concerning the viability of the foetus.

MATERIAL AND METHODS

Definitions

**Essential hypertension.** – Blood pressure (BP) ≥ 140/90 mmHg without proteinuria or oedema.

**Mild pre-eclampsia.** – BP rise during pregnancy to 140/90 – 160/110 mmHg and proteinuria < 2 %.

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Severe pre-eclampsia. – BP ≥ 160/110 mmHg and proteinuria ≥ 2 %.

Foeto-placental score. – The P-CAP, HCS and urinary oestrogen output is set to 100 per cent. In an actual case the average percentage deviation from the normal median and mean is the foeto-placental score. The following formula was used:

\[
\text{Foeto-placental score} = \left( \frac{\text{P-CAP} \times 100}{\text{normal median}} + \frac{\text{HCS} \times 100}{\text{normal median}} + \frac{\text{urinary oestrogens} \times 100}{\text{normal mean}} \right)^{-1} \]

Placental score. – For the foeto-placental score excluding total urinary oestrogens. The following formula was used:

\[
\text{Placental score} = \left( \frac{\text{P-CAP} \times 100}{\text{normal median}} + \frac{\text{HCS} \times 100}{\text{normal median}} \right)^{-1}
\]

Foetal danger zone. – When plotting the 2 scores in a diagram (Fig. 5) an upper limit of a lower area named “foetal danger zone” can be determined by using the following formula:

\[
\text{Foeto-placental score} = \left( \frac{2.5 \text{ percentile of P-CAP} \times 100}{\text{normal median}} + \frac{2.5 \text{ percentile of HCS} \times 100}{\text{normal median}} + \frac{-2 \text{ sd of total urinary oestrogens} \times 100}{\text{normal mean}} \right)^{-1}
\]

Placental score = \left( \frac{2.5 \text{ percentile of P-CAP} \times 100}{\text{normal median}} + \frac{2.5 \text{ percentile of HCS} \times 100}{\text{normal median}} \right)^{-1}

Foetal observation zone. – The area between the upper limit for the foetal danger zones and the respective median or mean.

Foetal well zone – The area higher than the normal median or mean.

Subjects

In the present series are included 82 pregnant women, 17 cases with essential hypertension, 31 cases with mild pre-eclampsia and 34 cases with severe pre-eclampsia. The observation period varied from day 200 to day 250 in the pregnancy until parturition, and at least the last of the blood and urine samples were collected while the women were hospitalized.

Collection and storage of the blood and urine samples

The urine was collected for 24 h and stored in a refrigerator until it could be assayed. During the same 24 h period blood samples were drawn into heparinized test tubes, centrifuged and the plasma stored at -20°C.

Assay procedures

HCS was determined (in duplicate) by a rapid radioimmunoassay method (Phadebas® HCS Test, Pharmacia). The method described by Pharmacia was followed in detail (Lindberg et al. 1972). P-CAP (in duplicate) was measured using 1-cystine-di-β-
naphtylamide as substrate (Babuna & Yenen 1966). The method is described in a previous report (Christensen 1974a).

Duplicate measurements of progesterone in plasma were carried out by the rapid protein binding technique described by Johansson (1969b) with minor modification (Fylling 1970).

Total urinary oestrogens were determined as Kober chromogen following acid hydrolysis and the extraction-partition procedure described in the Instruction Manual for the Paton-Brown Partition Extractor (Brown et al. 1968).

Statistics

Conventional statistical methods were applied. The following statistical handbooks were used: Documenta Geigy (1962) and Therkelsen (1968). The calculations were performed on a Compucorp 141 calculator.

RESULTS

Essential hypertension

In this group the mean placental weight was 650 g (range 450–850 g) and the mean infant weight was 3450 g (range 1950–4150 g). There were no foetal

Fig. 1.
The P-CAP, HCS, urinary oestrogen (Oe) and progesterone values in 17 cases of essential hypertension plotted into the normal P-CAP, HCS and progesterone pattern (median and 2.5 percentile) and the normal urinary oestrogen pattern (mean and –2 sd).

* : Birthweight higher than the 10 percentile.

* : Birthweight lower than the 10 percentile (see text).
deaths and one single infant had a birth weight under the 10 percentile according to the classification of Engstrom & Sterky (1966).

In Fig. 1 the values of the 4 assays one to 5 days before parturition are illustrated. No reduced values were found in any of the 4 assays.

*Mild pre-eclampsia*

The mean placental weight in this group was 605 g (range 350–1000 g) and the mean infant weight was 2850 g (range 1750–4050 g). There were no foetal deaths.

As shown in Fig. 2 a significant reduction in both P-CAP ($0.01 < P < 0.02$) and HCS ($P < 0.001$) was found in this group, but no significant reduction in the oestrogen values ($0.1 < P < 0.2$) could be demonstrated. The progesterone assay showed a tendency to increased values as the results of 18 assays showed values higher than the median compared with 13 values lower than the median.

The infants in 18 cases with mild pre-eclampsia showed a birth weight under the 10 percentile (Fig. 2). Seven-teen of these patients had low P-CAP values ($P < 0.001$), and 16 had low HCS values ($P < 0.001$). In 15 cases low oestrogen values were observed ($0.001 < P < 0.01$), but in only 10 cases low
progesterone values could be demonstrated \((P > 0.5)\). Only one, respectively 2 cases were found with “false positive” P-CAP and HCS values (values higher than the median). “False positive” progesterone results were found in 8 cases, and “false positive” oestrogen values (values higher than the mean) were found in 5 cases. In all but one case at least 2 out of 3 assays (P-CAP, HCS and oestrogens) showed values lower than the median or mean. Hence, simultaneous measurements of these 3 assays predicted that the foetus was influenced by the pre-eclamptic state with a high accuracy.

**Severe pre-eclampsia**

In this group the mean infant weight was 2250 g (range 1400–3720 g) and the mean placental weight was 475 g (range 250–850 g). There were 4 foetal deaths.

In Fig. 3 the values of the 4 assays one to 4 days before parturition are illustrated. A significant decrease in P-CAP, HCS and oestrogen values \((P < 0.001)\) was found, but no significant reduction in the progesterone values \((P > 0.5)\).

![Chart showing severe pre-eclampsia data](chart.png)

**Fig. 3.**
Severe pre-eclampsia (30 cases). Further explanation Fig. 1.
Table 1.
Gestational age, infant weight, placental weight and the P-CAP, HCS, progesterone and urinary oestrogen values in 4 cases of intra-uterine foetal death.

<table>
<thead>
<tr>
<th>Days of pregnancy</th>
<th>Infant weight (g)</th>
<th>Placental weight (g)</th>
<th>P-CAP Δ O. D.</th>
<th>HCS μg/ml</th>
<th>Progesterone ng/ml</th>
<th>Urinary oestrogens mg/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>237</td>
<td>1530</td>
<td>300</td>
<td>0.080</td>
<td>1.9</td>
<td>55.2</td>
<td>3.2</td>
</tr>
<tr>
<td>227</td>
<td>1400</td>
<td>250</td>
<td>0.100</td>
<td>2.3</td>
<td>19.0</td>
<td>3.1</td>
</tr>
<tr>
<td>252</td>
<td>2000</td>
<td>350</td>
<td>0.118</td>
<td>5.8</td>
<td>60.0</td>
<td>19.1</td>
</tr>
<tr>
<td>274</td>
<td>2040</td>
<td>285</td>
<td>0.145</td>
<td>4.5</td>
<td>112.4</td>
<td>11.6</td>
</tr>
</tbody>
</table>

In 25 cases of severe pre-eclampsia the infant had a birth weight under the 10 percentile (Fig. 3). Twenty-three of these patients showed low P-CAP values and in 24 patients low HCS values could be demonstrated \( P < 0.001 \). In 18 cases low oestrogen values were found \( P < 0.001 \), but in only 13 cases low progesterone values could be demonstrated \( P > 0.5 \). This group showed 2 "false positive" values of P-CAP and HCS, but not in the same 2 cases. "False positive" oestrogen values were observed in 7 cases, and "false posi-

Fig. 4.
The HCS, P-CAP, total urinary oestrogens and progesterone in 4 patients (1 to 4) with severe pre-eclampsia. All 4 infants had a birthweight lower than the 10 percentile (see text).
tive" progesterone values in 12 cases. In all but one case at least 2 out of 3 assays (P-CAP, HCS, oestrogens) showed values lower than the median or mean.

Table 1 shows the gestational age, infant weight, placental weight and the hormone and enzyme values one to two days before the intra-uterine foetal death. The P-CAP turned out to be the most accurate assay. The progesterone and oestrogen assay showed values in the normal range.

In Fig. 4 the HCS, P-CAP, progesterone and oestrogen values are illustrated in another 4 cases of severe pre-eclampsia. Blood and urine samples were collected up to at least 2 days before parturition and in all the cases the birth weights were under the 10 percentile. Both the HCS, the progesterone and the oestrogens showed a greater variation from one assay to another than the P-CAP. Again it was possible to demonstrate that simultaneous measurements of HCS, P-CAP and oestrogens give good support in the clinical evaluation concerning the viability of the foetus. The progesterone assay, however, did not supply any more information than the HCS and P-CAP assays.

**Placental and foeto-placental scores**

In Fig. 5 the placental and foeto-placental scores are illustrated.

a) **Mild pre-eclampsia.** – The women whose infants had a birth weight higher than the 10 percentile showed a score in the “Foetal well zone” in 8 cases, in the “Foetal observation zone” in 3 cases and in the “Foetal danger zone” in 2 cases both in the placental and the foeto-placental score diagrams. None of the infants with a birth weight under the 10 percentile, however, had scores in the “Foetal well zone” on the diagram. A score in the “Foetal observation zone” of the foeto-placental score diagram was found in 8 cases compared with 7 cases in the placental score diagram. Nine cases had a score in the “Foetal danger zone” of the foeto-placental score diagram, compared with 10 cases in the placental score diagram. Hence, the foeto-placental score did not supply any more information than the placental score.

b) **Severe pre-eclampsia.** – The placental and the foeto-placental scores in cases of severe pre-eclampsia are presented in Fig. 5. Of the women whose infants had a birth weight higher than the 10 percentile, 2 showed a score in the “Foetal well zone”, one in the “Foetal observation zone” and 2 in the “Foetal danger zone” both in the placental and foeto-placental score diagram. However, in cases with a birth weight lower than the 10 percentile no score was found in the “Foetal well zone” but 10, respectively 9 scores in the “Foetal observation zone” of the foeto-placental and the placental score diagram. Fifteen, respectively 16 cases had a score in the “Foetal danger zone” of the foeto-placental or placental score diagram. Hence, once again the foeto-placental score did not supply any more information than the placental score.
The placental and foeto-placental scores in pregnancies complicated with mild (C and D) and severe (A and B) pre-eclampsia.

- = infant weight lower than the 10 percentile.
\(\triangledown\) = infant weight higher than the 10 percentile.
1: foetal well zone. 2: foetal observation zone. 3: foetal danger zone.

**DISCUSSION**

The present work demonstrates that essential hypertension seldom influences the foeto-placental function or the foetal growth. *Ward et al.* (1973) found decreased HCS values in pregnancies complicated with essential hypertension but this could not be confirmed in the present investigation. Difference in the cases studied might be an explanation.
The P-CAP and HCS proved to be the most sensitive and accurate methods for detecting a placental dysfunction in cases of pre-eclampsia. This is in good accordance with the findings in a previous work (Christensen 1974b) which indicated that pre-eclampsia primarily affected the placental function. On the other hand, we have previously reported that the oestrogen excretion was significantly reduced in cases of mild pre-eclampsia (Christensen 1974b), but this could not be confirmed in the present work. This could partly be due to the spread in the oestrogen values from one assay to another which has been reported by several investigators (for review see Diczfalussy & Mancuso 1969). This is certainly the problem in the progesterone assay too, where day to day variations up to 100 ng/ml have been registered in normal pregnancies (Christensen et al. 1974). As the reduction was greater in the P-CAP and HCS than in the oestrogen values in cases of pre-eclampsia it might be concluded that pre-eclampsia primarily affects the placental function and that the reduced infant weight probably is secondary to the reduced placental function.

Our HCS results are in good accordance with the findings of the majority of previous investigators (Josphovich et al. 1970; Spellacy et al. 1971; Genazzani et al. 1972; Gitsch et al. 1973; Lindberg & Nilsson 1973) who found low HCS values in pregnancies complicated with pre-eclampsia. Letchworth & Chard (1972) found lower values in mild than in severe pre-eclampsia but this could not be stated in the present work. This might be due to a difference in the duration of the disease in the 2 groups compared.

The progesterone assay showed a relatively high incidence of “false positive” results in cases of retarded foetal growth where the other 3 parameters were more in accordance with the outcome of the pregnancy. Hence, the progesterone assay cannot be recommended as a test for the placental function. Johansson (1969a) reported the progesterone values in 5 cases of foetal death due to severe toxaemia, diabetes and unknown reasons. Very low values were found in 2 cases of severe toxaemia but the other 3 cases showed low or normal values. This impression of a limited value of the progesterone assay was further verified in the present work. A tendency to increased progesterone values in cases of mild pre-eclampsia was observed in the present work without any clear tendency of increased placental or infant weight. Further investigation is required to determine whether this tendency is coincidental or whether it indicates an increased formation or reduced metabolism of the hormone.

The use of simultaneous measurements of different hormones and enzymes in the evaluation of complicated pregnancies has recently been reported. Keller et al. (1971) recommended simultaneous measurements of HCS and urinary oestrogens in pregnancies complicated with hypertension, mild and severe pre-eclampsia. As all cases were reported in one group, no further comparison with the present work is possible. Watson et al. (1973) found that the estimation of oestriol better reflected the condition of the foeto-placental unit than the
assay of P-CAP and HCS in 12 cases of foeto-placental insufficiency. Again it is impossible to make a comparison as several diseases may cause a foeto-placental insufficiency.

A reduction in P-CAP, HCS and oestrogen values did not occur simultaneously in the different cases although there was a good correlation especially between the HCS and P-CAP. In order to get a combined evaluation of the assays the foeto-placental and the placental scores were introduced. Even though the foeto-placental score did not supply any more information than the placental score, the determination of both HCS, P-CAP and the oestrogens simultaneously is advisable. In a previous work (Christensen 1974b) the determination of P-CAP as a placental test and of the urinary oestrogens as a foeto-placental test gave valuable information in cases with a low placental coefficient. Relatively high oestrogen and low P-CAP values indicated an interrelation between the infant weight and the placental function.

The observation in the present work that none of the women with an infant of low birth weight showed placental scores in the “Foetal well zone”, indicates a correlation between the score and the foetal well being. The combination of a normal birth weight and a score in the “Foetal danger zone” can be explained by a late onset of the placental dysfunction.

The concept of “placental dysfunction” or “placental insufficiency” is commonly used to explain otherwise unexplained perinatal deaths occurring in pregnancies complicated with such conditions as pre-eclampsia, diabetes or postmaturity. Further, foetal deaths in utero for unknown reasons are often considered to be caused by “placental insufficiency” (Lewis 1964). More exact definitions are desirable, and we suggest low values of the placental assays to be a prerequisite for the use of the terms “placental insufficiency” or “placental dysfunction”.

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REFERENCES

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