HORMONE AND ENZYME ASSAYS IN PREGNANCY

III. The placental cystine-aminopeptidase and the urinary oestrogens in pregnancies complicated with essential hypertension, mild or severe pre-eclampsia

By
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ABSTRACT

Simultaneous measurements of placental cystine-aminopeptidase ("plasma oxytocinase") (P-CAP) and total urinary oestrogens were performed in pregnancies complicated with essential hypertension, mild or severe pre-eclampsia.

Essential hypertension (n = 31): Neither the enzyme activity nor the urinary output of oestrogens were significantly reduced in these cases.

Mild pre-eclampsia (n = 59). In this group of patients a significant reduction of the 2 parameters was observed (P-CAP: \( P < 0.001 \); total oestrogens: \( 0.02 < P < 0.05 \)). Expressed in per cent of the normal mean the P-CAP and total urinary oestrogens were 71.0 and 83.2 per cent, respectively.

Severe pre-eclampsia (n = 41): A more marked reduction both in P-CAP (\( P < 0.001 \)) and total urinary oestrogens (\( P < 0.001 \)) was observed in this group. The percentage reduction from the normal mean of P-CAP and total urinary oestrogens was 46.0 and 35.2 per cent, respectively.

In the whole material (n = 131), including both essential hypertension and pre-eclampsia, secondarily retarded foetal growth was found in 39 pregnancies. The percentage reduction from the normal mean of both P-CAP and total primary oestrogens in this group was 46.0 and 53.2 per cent, respectively.

In 12 cases with a low placental coefficient (placental weight: infant weight) the P-CAP was 41.7 per cent and the total urinary oestrogen excretion 74.6 per cent of the normal mean. When evaluating such cases, simultaneous measurements of P-CAP as a placental function test and the urinary output of oestrogens as a foeto-placental function test, might be beneficial.

In each of 4 cases of intra-uterine foetal death the P-CAP values were below 2.5 percentile of the normal pattern. The total urinary oestrogen levels, however, showed a more diffuse pattern and in 1 case the levels were within the normal range.

The total urinary oestrogen excretion has been widely accepted as the criteria in the clinical evaluation of the placental function. The total oestrogen excretion, however, is considered to be a parameter of the whole foeto-placental unit as the precursors mainly arrive from the foetus (for review see Diczfalusy & Mancuso 1969).

The placental cystine-aminopeptidase (P-CAP) increases gradually during pregnancy and reaches a maximum near term (for review see Christensen 1974). The placental origin of the enzyme is well established (Rydén 1966), and it has been suggested that measurement of the enzyme activity in maternal plasma may reflect the functional state of the placenta (Babuna & Yenen 1966; Tovey 1969; Rydén 1972). However, the value of the assay as a test for the placental function is still under dispute (Page 1946; Riad 1962; Babuna & Yenen 1966; Melander 1965; Rydén 1966, 1972). Chapman et al. (1971) estimated the plasma P-CAP in normal and diabetic pregnancies and compared these results with those found by measuring total urinary oestrogens. They found significantly lower P-CAP values in diabetic pregnancies compared with those found in normal pregnancies. No such difference could be demonstrated using the urinary oestrogen assay. Curzen & Varma (1973) have recently reported data on P-CAP and urinary oestrogen output in high risk pregnancies. They found that serial assays of urinary oestrogens enabled the “light for dates” infant to be predicted in 70 per cent of the cases. P-CAP determinations were of no value in predicting “foetal distress”.

In an unpublished study during the years 1969 to 1971 including essential hypertension, mild or severe pre-eclampsia, P-CAP was measured. The results indicated that the enzyme activity was not influenced by essential hypertension. On the other hand, both in mild and severe pre-eclampsia the P-CAP was reduced as compared to the values obtained in normal pregnancies. However, even in pregnancies complicated with severe pre-eclampsia, some of the values were within the normal range. Hence, the aim of the present investigation was to carry out simultaneous measurements of plasma P-CAP and total urinary oestrogens in pregnancies complicated with essential hypertension, mild or severe pre-eclampsia.
MATERIAL AND METHODS

Definitions

Essential hypertension. – Blood pressure (BP) of \( \geq 140/90 \) mmHg without proteinuria or oedema.

Mild pre-eclampsia. – BP rise during the pregnancy to between 140/90 mmHg and 160/110 mmHg and proteinuria < 20/00.

Severe pre-eclampsia. – BP \( \geq 160/110 \) mmHg and proteinuria \( \geq 20/00 \). BP was recorded at least twice with a minimum interval of 6 h.

Secondarily retarded foetal growth. – Infants delivered after pregnancies complicated with essential hypertension, mild or severe pre-eclampsia with a birth weight below the 10 percentile according to the classification of Engström & Sterky (1966).

Subjects

In the present series are included 31 cases with essential hypertension, 59 cases with mild pre-eclampsia and 41 cases with severe pre-eclampsia. The work was carried out during the years 1971 to 1973. At least the collection of the last blood and urine sample was performed with the patients hospitalized. Drugs, such as diuretics, antihypertensive and psychopharmaceutical agents were given to the patients when necessary. The patients received the regular hospital diet.

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 samples stored at \(-20^\circ\)C until they could be analyzed. The last blood sample was drawn not later than 7 days prior to parturition.

Enzyme and total urinary oestrogen measurements

P-CAP was measured using l-cystine-di-\( \beta \)-naphtylamide as substrate (Babuna & Yenen 1966). The method is described in a previous report (Christensen 1974).

Total urinary oestrogen excretion was 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).

Statistical methods

Conventional statistical methods were applied. The following statistical handbooks were used: Scientific Tables, Documenta Geigy (1962), Medicinsk Statistik, Therkelsen (1968). The statistical calculations were performed on a Wang 500 calculator and a Compucorp 141 calculator.

RESULTS

P-CAP and total urinary oestrogens in pregnancies complicated with essential hypertension

The mean placental weight in this group was 610 g (range 420–800 g), and the mean infant weight was 3250 g (range 2200–4350 g). All infants were born alive.
The P-CAP in 31 cases of maternal essential hypertension. The result of the last assay before parturition is illustrated.

Neither the P-CAP nor the oestrogen values were significantly reduced. As shown in Fig. 1 and Table 1, however, 18 of the 31 P-CAP readings showed values under the median of the normal activity pattern compared with 13 of the 31 oestrogen readings showing values under the mean.

In Fig. 4 the results of the P-CAP and oestrogens are expressed in per cent

Table 1.
The distribution of the patients with essential hypertension, mild and severe pre-eclampsia related to the normal excretion pattern of total urinary oestrogens.

<table>
<thead>
<tr>
<th></th>
<th>No. of cases essential hypertension</th>
<th>No. of cases mild pre-eclampsia</th>
<th>No. of cases severe pre-eclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; mean</td>
<td>18</td>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td>&lt; mean &gt; -2 (sd)</td>
<td>10</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>&lt; -2 (sd)</td>
<td>3</td>
<td>15</td>
<td>19</td>
</tr>
</tbody>
</table>

0.02 < P < 0.05 P < 0.001
of normal mean. The P-CAP was more reduced (82.1 per cent) than the oestrogens (91.5 per cent). The correlation between the P-CAP and the crude placental weight was +0.66.

The P-CAP and total urinary oestrogens in pregnancies complicated with mild pre-eclampsia

The mean placental weight in this group was 600 g (range 300–1050 g) and the infant weight varied between 1750 and 4050 g (mean 3050 g). One infant died in utero.

The P-CAP values are plotted in Fig. 2, and the result of the oestrogen readings are shown in Table 1. The reduction in the P-CAP activity ($P<0.001$) was greater than the reduction in the oestrogen excretion ($0.02<P<0.05$) (Table 1).

In Fig. 4 the results of the P-CAP and the oestrogens are expressed as per cent of the normal mean. Again the reduction in the P-CAP (71.0 per cent) was greater than the reduction in the oestrogens (83.2 per cent). In the 18 cases

![Graph](image-url)
Fig. 3.
The P-CAP in 41 cases of severe pre-eclampsia. The result of the last assay before parturition is illustrated \((P < 0.001)\).

Fig. 4.
The placental cystine-aminopeptidase (P-CAP) and the total urinary oestrogens calculated as per cent of normal mean.
where the results of the last P-CAP assay showed values under the 2.5 percentile, the mean placental weight was 450 g. The correlation between the crude placental weight and the P-CAP was +0.67.

The P-CAP and total urinary oestrogens in pregnancies complicated with severe pre-eclampsia

In this group the mean placental weight was 430 g (range 280–1000 g), and the infant weight varied between 1350 and 3750 g (mean 2170 g). Three infants died in utero. As shown in Fig. 3 and Table 1 both the P-CAP and the oestrogen values were significantly reduced \((P < 0.001)\). However, in this group also the reduction in the P-CAP activity was more marked than the reduction in the oestrogen excretion. This is illustrated in Fig. 4, where the results of the P-CAP and the oestrogens are expressed in per cent of the normal mean. When compared with the 2 former groups the reduction was more marked in both the P-CAP values (56.0 per cent) and the oestrogen values (65.1 per cent).

In 23 out of 41 cases, where the result of the last P-CAP assay before parturition showed values under the 2.5 percentile, the mean placental weight was 410 g. The correlation between the crude placental weight and the P-CAP was +0.69.

The P-CAP and the total urinary oestrogens in cases of secondarily retarded foetal growth

In 39 of 131 cases the infant weight was below the 10 percentile for the respective gestational age. The maternal complications in this group were essential hypertension in 4 cases mild pre-eclampsia in 11 cases and severe

![Graph](attachment:image.png)

Fig. 5.
The P-CAP and the total urinary oestrogens in 12 cases with low placental coefficient (0.090–0.110).
pre-eclampsia in 24 cases. The mean placental weight was 430 g (range 280–600 g). As shown in Fig. 4 there was a marked percentage reduction of normal mean both in the P-CAP (46.0 per cent) and the oestrogens (53.2 per cent). This reduction was even more marked than in cases complicated with severe pre-eclampsia.

*The P-CAP activity and the total urinary oestrogens in cases with a low placental coefficient*

In Fig. 5 are illustrated 12 cases where the placental coefficient (placental weight: infant weight) varied between 0.090 and 0.110. In these cases the P-CAP was 41.7 per cent and the oestrogen excretion 74.6 per cent of the normal mean indicating a disproportion between the infant weight and the placental function.

*The P-CAP activity and the total urinary oestrogens in cases of intra-uterine foetal death*

In 4 out of 131 pregnancies the infant died in utero. In all of these cases the P-CAP activity was below the 2.5 percentile at least during the last 7 days before delivery. The oestrogen excretion showed more variable values, and in one of the cases the oestrogen excretion was in the normal range. A decrease in the oestrogen excretion a short time before the foetal death was seen in 2 cases, both illustrated in Fig. 6. In both cases the mother was in a severe pre-eclamptic state.

![Fig. 6.](image)

The P-CAP (—.—.) and the total urinary oestrogens (.....) in 2 cases (1 and 2) of intra-uterine foetal death. + = Day of foetal death.
DISCUSSION

The menstrual history has been used in estimating the gestational age, although this method is never fully reliable. The menstrual history, however, was carefully examined in all women to minimize errors.

The diagnosis, hypertension, was always based upon repeated readings. However, the blood pressure varies with many factors, and there is probably no clear-cut limit between normotensive and hypertensive pregnant women. The first blood pressure was usually recorded before any drugs were administered, and in preliminary studies it was found that the drugs that were administered to the hypertensive women did not influence the enzyme activity or the hormone excretion.

In the present study essential hypertension caused no significant reduction in either the P-CAP or the oestrogen values. Hence, it can be concluded that the influence of hypertension on the placental and the foeto-placental metabolism is minor. In a recent report Bjøro (1972) showed that the oestrogen metabolism was little influenced by essential hypertension, and his result could be confirmed in the present work. In addition, McGillivray (1967) found that a rise in the blood pressure to 140/90 mmHg or more, occurring alone without the development of proteinuria, may have no ill effects, either on the mother or on the foetus, and may be physiological. An essential hypertension existing for years, however, may affect the renal function causing a reduced clearance of the oestrogens.

Pre-eclampsia represents a potential danger to the foetus. The present work indicates that the affect is mainly on the metabolism of the placenta since the reduction in the P-CAP values is more pronounced than in the urinary production of oestrogens in both mild and severe pre-eclampsia. Furthermore, the results suggest that the degree of the disease can be determined by these assays since the decrease in both values was greater in severe than in mild pre-eclampsia. When comparing the results of serial assays from single cases it appears that the oestrogen values were more variable than the P-CAP values, the latter apparently more related to the placental function. However, since P-CAP has a longer half-life than oestrogens (Christensen 1974) an indication of sudden changes in the metabolism of the placenta reflected in the P-CAP values will be delayed.

No comparable investigation of essential hypertension in pregnancy and pre-eclampsia can be found in the literature. Page (1946) and Riad (1962) came to no conclusive results concerning the relationship between the P-CAP activity and pre-eclampsia, probably due to different methods used for estimating the enzyme activity. Josephides & Turkington (1967) found the P-CAP values in pregnancies complicated with pre-eclampsia to be very similar to those found in normal pregnancies. Their conclusions, however, were based upon rather few cases. Babuna & Yenen (1966) reported decreasing and or
low values in 6 of 8 cases of toxaemia, and Rydén (1972) found very low values in 3 out of 6 patients with severe pre-eclampsia, which is in good accordance with the results in the present study. Curzen & Varma (1973) found that the P-CAP assay was of no use in predicting foetal distress, the Apgar score at birth or the “light for dates” infants. However, since “foetal distress” may be caused by several factors and as the maternal complications were not related to the results of the P-CAP readings, it is difficult to compare their results with the present ones.

In pre-eclampsia the danger to the foetus may be related to the duration of the disease and the time of onset of the symptoms. In the present work low P-CAP values were mostly found in those cases where the pre-eclampsia occurred before about 252 days of pregnancy. This may indicate that an early onset or a long duration of the pre-eclampsia affect the placental function more than a late onset and/or a short duration of the symptoms. The decrease in the urinary oestrogen output in the same cases, however, seemed to appear later than the decrease in the P-CAP values.

Both the P-CAP and the oestrogen values were below the lower normal limit in cases of severe pre-eclampsia where the foetus died in utero. The decrease in the P-CAP, however, was again observed before a decrease in the urinary output of oestrogens. Again this supports the view that P-CAP is a placental function test and pre-eclampsia is a disease that primarily affects the placental function.

Secondary retardation of foetal growth was associated with low placental weight, low P-CAP and oestrogen values indicating that low birth weight was secondary to a reduced placental function. A low placental coefficient presents a potential danger to the foetus, and in such cases simultaneous measurements of P-CAP as a placental test and oestrogen as a foeto-placental test gave valuable information in the present study. According to our data relatively high oestrogen and low P-CAP values might indicate a disproportion between the infant weight and the placental function.

Rydén (1972) reported occasionally very high P-CAP values in cases of severe pre-eclampsia. He attributed this finding mainly to aminopeptidases from other organs, such as liver and myometrium, interfering with the assay of P-CAP. However, it has been shown in a previous report that aminopeptidases of tissue origin do not show any increase in severe pre-eclampsia as compared with normal pregnancy (Christensen 1974). Very high P-CAP values were found in the present work too, but mostly combined with a high placental weight. It seems more likely that a combination of high P-CAP values and low or normal placental weight is due to a massive flow of P-CAP arising from a severe and sudden damage to the placenta. On the other hand, it is reasonable to assume that the functional capacity of the placenta is not always proportional to the crude placental weight.
On the basis of the present work it is possible to conclude that the P-CAP assay is valuable in determining the placental functional capacity, and as a placental test it provides more accurate information than the total urinary oestrogen assay which is an assay for the whole foeto-placental unit. However, a combination of both assays might be of importance in evaluating the viability of the foetus.

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REFERENCES


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