UNCONJUGATED OESTETROL IN PLASMA IN RESPONSE TO AN INTRAVENOUS LOAD OF DEHYDROEPIANDROSTERONE SULPHATE (DHAS) IN UNCOMPLICATED AND COMPLICATED HUMAN PREGNANCY

By

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ABSTRACT

A non-chromatographic radioimmunoassay for estimation of unconjugated oestetrol in plasma from pregnant women is described. The antiserum has a high specificity to oestetrol. The technical procedure is simple and rapid. Only small amounts of plasma (0.2–0.4 ml) are needed for the analysis. The method has been applied to the measurement of oestetrol in plasma from pregnant women before and after an intravenous injection of 50 mg DHAS. In women with uncomplicated pregnancies a rise of plasma oestetrol was found 60 min after the injection. From 120 to 360 min there was a plateau level, at 600 min a decrease from this level was observed. No changes in the oestetrol response were found with advancing gestational age from the 33rd to the 40th week of pregnancy. A great spread in the individual responses were recorded. Patients with pre-eclampsia and intrauterine growth retardation had a tendency to a lower increase and patients with diabetes a tendency to a higher increase of plasma oestetrol after the DHAS administration. From the data obtained it is concluded that the increase of plasma oestetrol after an intravenous injection of DHAS in most cases is secondary to the increase of plasma oestradiol. The results suggest that measurement of unconjugated oestetrol in plasma after an intravenous load of DHAS is no safe way to assess foetal wellbeing. In women with intrauterine growth retardation (IUGR) the simultaneous measurement of plasma oestradiol and oestetrol after an injection of DHAS indicates a possibility to distinguish placental from foetal causes of this syndrome.
The 3 “classical” oestrogens, oestrone, oestradiol and oestriol are finally formed by the placenta during late human pregnancy (Diczfalusy 1964). Adrenal dehydroepiandrosterone sulphate (DHAS) is the main precursor for the placental synthesis of these oestrogens (Diczfalusy 1964; Siiteri & MacDonald 1966). The maternal contribution of precursor substance is substantial for the placental production of oestrone and oestradiol. Oestriol, on the other hand, is synthesized mainly from foetal precursors (Diczfalusy 1964; Siiteri & MacDonald 1966).

Oestetrol (15α-hydroxyoestriol), a steroid isolated from urine of pregnant women and newborn infants by Zucconi et al. (1967). is supposed to be a “foetal oestrogen”, since it is produced by the foetal liver (Zucconi et al. 1967; Adlercreutz & Laukkainen 1970). The 15α-hydroxylase activity is quite extensive in the liver of a term foetus (Hagen 1970). With advancing age this activity declines (Hagen 1970), although some 15α-hydroxylation of oestrogens may still be present in adults (Jirku & Levitz 1969).

DHAS is an important precursor for the formation of oestetrol (Younglai & Solomon 1968). Two main pathways are proposed for the formation of oestetrol from DHAS. The phenolic way passes via oestradiol, of which oestetrol is the major metabolite in the foetus (Schwers et al. 1967). The neutral way will probably involve 15α-hydroxylation of DHAS and then aromatization to 15α-hydroxyoestradiol (Younglai & Solomon 1968). Although oestetrol can be derived from oestriol, this pathway seems to be of minor importance (Schwers et al. 1967; Younglai & Solomon 1968).

About 10 years ago Lauritzen (1967) introduced a test where DHAS was administered to women in late pregnancy. The rate of conversion to urinary oestrogens was estimated and subsequently Lauritzen (1969) claimed that the test could be useful for detection of placental insufficiency. In later reports (Strecker & Lauritzen 1974; Korda et al. 1975; Tulchinsky et al. 1976) the increase of plasma oestrogens in response to DHAS was used to assess placental or foeto-placental function.

In the present study a radioimmunoassay of plasma oestetrol was used to estimate the rise of the oestetrol levels after an intravenous injection of DHAS to women in late pregnancy. The plasma oestriadiol or oestrone response to injections of DHAS may be used to assess placental function. The aim of the present communication was to investigate if the rise of plasma oestetrol in response to a DHAS load could be of clinical usefulness in the management of high risk pregnancies.

**Material and Methods**

**Subjects**

Sixty-three pregnant women volunteered for this study. Most women are the same as the “subjects” of a recent report (Axelsson et al. 1978). They can be divided into the following groups:
Uncomplicated pregnancy

All 41 women in this group gave birth to 1 healthy infant of normal birthweight (birthweight within ±2 SD of the mean for the actual gestational age according to Engström & Sterky (1966)). All children were born in the 37th to the 42nd week of pregnancy. Since an analysis of variance performed on the oestetrol values recorded in women with uncomplicated pregnancies did not reveal any differences in the oestetrol response with advancing gestational age, the results from women in different weeks of pregnancy were pooled.

Pre-eclampsia

Of the 5 patients studied, 2 had a history of hypertension already before the pregnancy. Three patients delivered infants small for gestational age (birthweight below −2 SD) or with Apgar scores of 6 or less at 1 min.

Intrauterine growth retardation (IUGR)

Nine women with this syndrome were investigated. They all delivered infants with birthweights more than −2 SD below the normal mean for the respective gestational week.

Diabetes mellitus

Of the 5 women with diabetes, 3 belonged to White group D. 1 to group C and 1 to group B (Marble et al. 1971). The infants were all healthy and of normal birthweights.

Low basal excretion of urinary oestrogens

Six women with low urinary excretion of oestrogens (Persson & Hofstedt 1969) are presented separately. Two of these women (M. L. and S. M. M.) are also included in the group of pre-eclampsia and one (M. A.) in the IUGR group.

DHAS-test

Fifty mg of DHAS (supplied by F. Hoffman-La Roche) was injected intravenously. Blood was withdrawn immediately before and at 30, 60, 120, 180, 360 and 600 min after the DHAS injection. In some women blood was collected also after 24 h.

Blood was collected into heparinized tubes. Plasma was separated by centrifugation and stored at −15°C until assayed.

Assay method

The plasma concentration of unconjugated oestetrol was estimated by a radioimmunoassay1). Radioactive and non-radioactive oestetrol was obtained via AB Kemila-Preparat, Stockholm, Sweden. The specificity of the antiserum is high. The cross-reaction with oestriol was <0.4% (Kundu & Grant 1976). The sensitivity of the assay was 25 pg as read off the standard curve. When assaying plasma volumes of 0.2–1.0 ml from women post-partum (5–6 days after delivery) no oestetrol could be detected. Hence the plasma blank was considered as negligible. The accuracy was tested by

1) The antiserum was a gift from Dr. N. Kundu. Department of Obstetrics and Gynecology, School of Medicine, The University of South Dakota, Yankton, South Dakota, USA.
Table 1.
Mean and variation of unconjugated oestetrol in plasma during late pregnancy. The values are given in ng/ml. n denotes number of women.

<table>
<thead>
<tr>
<th>Week of pregnancy</th>
<th>Uncomplicated pregnancy</th>
<th>Pre-eclampsia</th>
<th>IUGR</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>sd</td>
<td>n</td>
</tr>
<tr>
<td>33–34</td>
<td>9</td>
<td>0.19</td>
<td>0.06</td>
<td>2</td>
</tr>
<tr>
<td>35–36</td>
<td>7</td>
<td>0.28</td>
<td>0.14</td>
<td>1</td>
</tr>
<tr>
<td>37–38</td>
<td>14</td>
<td>0.49</td>
<td>0.21</td>
<td>1</td>
</tr>
<tr>
<td>39–40</td>
<td>11</td>
<td>0.61</td>
<td>0.32</td>
<td>1</td>
</tr>
</tbody>
</table>
assaying 0.2 ml of plasma from post-partum women containing known amounts (0.25–1.0 ng/ml) of non-radioactive oestetrol. The mean recovery was 76% (± 4.9). The precision was calculated from duplicate determinations. The intra-assay variation was 8.2% (n = 73). The inter-assay variation was 18.1% (n = 21). All samples obtained from a certain woman were analysed in the same assay. The analyses were performed on small amounts of plasma (0.2–0.4 ml). All steps in the assay were the same as those described by Edqvist & Johansson (1972).

RESULTS

The pre-injection plasma levels of unconjugated oestetrol appear in Table 1. In women with uncomplicated pregnancies the rise of the mean values with advancing gestational age was obvious. Large variations were noticed. Of the 5 patients with pre-eclampsia, 3 gave birth to children small for gestational age. Two of these women had low values of plasma oestetrol. the 3rd woman showed a high value. The 2 women with pre-eclampsia who delivered infants of normal weights had oestetrol values well within ± 2 s.d of the normal mean. The mean values recorded in women with IUGR did not differ significantly from those observed in women with uncomplicated pregnancies. In the group of diabetic women 2 out of 5 had high values of plasma oestetrol.

The response of unconjugated plasma oestetrol to an injection of DHAS is shown in Table 2. Figs. 1 and 2. For women with uncomplicated pregnancies a noticeable increase appeared 60 min after the injection. From 120 to 360 min there was a plateau level with the maximum rise of 0.27 ng/ml recorded at 180 min. A decrease from the plateau level was noticed at 600 min. The net increase of 0.27 ng/ml corresponds to a 1.7-fold increase of the base-line value. Twenty-four hours after the DHAS injection the mean plasma oestetrol value was still slightly elevated compared to the pre-injection value. The large standard deviations (Table 2. Fig. 2) show that there was a great variation in the individual responses.

In women with pre-eclampsia (Fig. 1) the mean rise was less than that observed in normal pregnancy. A peak value (0.21 ng/ml) was observed 360 min after the injection. Also in women with IUGR (Fig. 1) lower responses were recorded. The maximum value (0.22 ng/ml). however, was obtained at 180 min. In the 5 women with diabetes mellitus (Fig. 1) the mean rise of plasma oestetrol was higher than that observed in women with undisturbed pregnancies. The differences between the pathological groups and the normal group were, however, not statistically significant (Student's t-test).

The test results in 6 individual subjects (Table 3). all of whom had sub-normal excretion of urinary oestrogens. are shown in Fig. 2. Patient E. H. delivered a baby with a severe malformation of the heart. The child died 3 days after birth. The liver was found to be in a congestive condition at
The mean increase of unconjugated oestetrol in plasma after an intravenous injection of 50 mg DHAS to women with uncomplicated and complicated pregnancies.

The mean increase and spread (± 1 sd) of unconjugated oestetrol after an intravenous injection of 50 mg DHAS to women with uncomplicated pregnancies. Individual responses in 6 women with low urinary excretion of oestrogens.
Table 2.
Mean and variation of unconjugated oestetrol in plasma following an intravenous injection of 50 mg DHAS in uncomplicated pregnancy. The values are given in ng/ml.

<table>
<thead>
<tr>
<th>min after DHAS injection</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>360</th>
<th>600</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>41</td>
<td>41</td>
<td>39</td>
<td>39</td>
<td>34</td>
<td>18</td>
</tr>
<tr>
<td>Mean rise</td>
<td>0.05</td>
<td>0.15</td>
<td>0.23</td>
<td>0.27</td>
<td>0.25</td>
<td>0.11</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.8</td>
<td>0.13</td>
<td>0.18</td>
<td>0.21</td>
<td>0.17</td>
<td>0.16</td>
</tr>
</tbody>
</table>

autopsy. The DHAS-test revealed a pronounced rise in the oestetrol response (Fig. 2). M. H. gave birth to an anencephalic child. Although the test was performed already in the 30th week of pregnancy, the response resembles that seen in women with uncomplicated pregnancies (Fig. 2). Patient L. B. L. was hospitalised due to threatening premature delivery and treated with glucocorticoides to induce foetal lung maturity. However, her pregnancy went on uneventfully and later she delivered a healthy term infant. The oestetrol increase recorded in L. B. L. was in the upper normal range (Fig. 2). Patients M. L. and S. M. M. had their pregnancies complicated with pre-eclampsia. M. A. belonged to the group of IUGR. The oestetrol response in these 3 women was very weak (Fig. 2).

Table 3.
Data on 6 pregnant patients with subnormal excretion of urinary oestrogens.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gestation at DHAS-test (weeks)</th>
<th>Gestation at delivery (weeks)</th>
<th>Birthweight (g)</th>
<th>Placental weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. H.</td>
<td>38</td>
<td>39</td>
<td>3000</td>
<td>625</td>
</tr>
<tr>
<td>L. B. L.</td>
<td>35</td>
<td>40</td>
<td>2810</td>
<td>400</td>
</tr>
<tr>
<td>M. L.</td>
<td>34</td>
<td>34</td>
<td>840</td>
<td>135</td>
</tr>
<tr>
<td>S. M. M.</td>
<td>33</td>
<td>33</td>
<td>890</td>
<td>550</td>
</tr>
<tr>
<td>M. A.</td>
<td>33</td>
<td>34</td>
<td>1720</td>
<td>330</td>
</tr>
<tr>
<td>M. H.</td>
<td>30</td>
<td>35</td>
<td>not weighed</td>
<td>350</td>
</tr>
</tbody>
</table>

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A few radioimmunoassays of plasma oestetrol have been described in the last years (Fishman & Guzik 1972; Kundu & Grant 1976; Den et al. 1977). Of the antisera produced, that obtained by Kundu & Grant (1976) has the lowest cross-reaction with oestriol. This antiserum was used in the present investigation and due to its high specificity, there was no need for a separation of the plasma oestrogens prior to the final analysis. The recovery of the present method was somewhat lower than that observed by others (Kundu & Grant 1976; Den et al. 1977). It is our experience that the recovery could be higher if 2 extractions were performed. The omission, however, of the second extraction step will save time and if an assay method is to be applied for clinical purposes, it must be fast and simple.

The plasma levels of unconjugated oestetrol estimated by the present method are in the same range as those found in most previous reports (Tulchinsky et al. 1975; Kundu & Grant 1976; Notation & Tagatz 1977). The measurement of plasma oestetrol does not seem to be superior to the measurement of plasma oestriol for the assessment of foetal wellbeing (Tulchinsky et al. 1975; Notation & Tagatz 1977). The present investigation was not undertaken to study the usefulness of plasma oestetrol per se as a test of foetal wellbeing. The data obtained, however (Table 1) do not suggest that plasma oestetrol would be a useful aid in the management of high risk pregnancies.

Tulchinsky et al. (1976) estimated the increment of unconjugated oestetrol after giving DHAS to women in late pregnancy. As found in the present investigation a significant rise of plasma oestetrol was not seen until 60 min after the injection (Table 2. Fig. 1).

Tulchinsky et al. (1976) found maximal levels of plasma oestetrol 4 h after the DHAS injection. This is in good agreement with the present data (Fig. 1). Also the estimated magnitude of the rise in plasma oestetrol resembles that found by Tulchinsky et al. (1976).

The fact that oestetrol rose more slowly than oestradiol (Axelsson et al. 1978) after the DHAS administration suggests that the main part of the oestetrol is formed from oestradiol.

The oestetrol response found in women with pre-eclampsia and IUGR reminds of that observed for oestradiol (Axelsson et al. 1978), although the oestradiol response recorded in these 2 groups of pregnant women is more different from that of normal pregnancy than is the oestetrol response. From previous investigations (Strecker & Lauritzen 1974; Axelsson et al. 1978) it is evident that the oestradiol response to DHAS in pregnant women with diabetes is almost indistinguishable from that of normal pregnancy. The tendency to an increased response of plasma oestetrol noticed in the present work could be explained by an enhanced 15α-hydroxylation capacity of the foetal liver in diabetic pregnancies.
There is generally a good parallelism between the oestradiol and oestetrol response in individual subjects; that is, a marked or low increase of oestradiol is accompanied by a similar response of oestetrol. In a previous publication (Axelsson et al. 1978) the response of plasma oestradiol to DHAS is described in 4 subjects with low urinary excretion of oestrogens. In the present report the response of plasma oestetrol was estimated in these 4 (E. H., L. B. L., M. L. and S. M. M.) and 2 other (M. H. and M. A.) individuals with low urinary excretion of oestrogens (Table 3). A positive correlation between the oestra-
diol and oestetrol responses to DHAS was evident in 5 of the 6 subjects. A positive correlation between maximal post-injection levels of oestradiol and oestetrol was also found by Tulchinsky et al. (1976). Patient M. A. had an increase of plasma oestradiol in the upper normal range but an extremely low increase of plasma oestetrol (Fig. 2). Tulchinsky et al. (1976) have reported on 1 subject where a normal rise of oestradiol was accompanied by a subnormal rise of oestetrol. From this finding they suggested that "chronic foetal distress" not associated with placental insufficiency seldom occurs. The low increase of plasma oestetrol in subject M. A. could be suggestive of an impaired function of the foetal metabolism and thereby indicating a foetal and not a placental cause of the IUGR in this patient. Such a piece of information might be of value when to decide on the optimal time for delivery in patients with IUGR.

The low urinary oestrogen levels found in patient E. H. were probably caused by a shortage of foetal precursors since the foetus was severely diseased. The great increase of plasma oestetrol after the DHAS load found in patient E. H. (Fig. 2) suggests that the oestetrol response is of no help in detecting severely diseased foetuses; an observation confirmed by the quite normal oestetrol increase observed in patient M. H. (Fig. 2) who carried an anencephalic foetus. Hagen (1970) could demonstrate that human anencephalics were able to 15α-
hydroxylate oestrogens. Accepting the view that 15α-hydroxylation of oestro-
gens during pregnancy is due mainly to foetal metabolism (Schut et al. 1978) the data obtained from the DHAS-test in patient M. H. constitute a further piece of evidence for the capacity of 15α-hydroxylation by the anencephalic foetus.

As previously stated (Axelsson et al. 1978) the increase of plasma oestradiol after an injection of DHAS might be used to rule out or verify suspicion of placental insufficiency in pregnant women with low basal production of oestrogens.

That a low increase of plasma oestetrol in combination with a normal or high rise of oestradiol should be indicative of a severely diseased foetus in the presence of a normal placenta seems to be contradicted by the findings of the present study. In patients with IUGR, however, this response pattern might indicate that the cause of the IUGR is primarily foetal and not placental.
The estimation of plasma oestetrol after an intravenous injection of DHAS to women in late pregnancy seems to be of limited value in the management of high risk pregnancies although the final answer needs further investigations.

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