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A RADIOIMMUNOASSAY FOR TOTAL (UNCONJUGATED + CONJUGATED) OESTRONE IN PLASMA FROM PREGNANT WOMEN; THE RESPONSE OF TOTAL OESTRONE IN PLASMA TO AN INTRAVENOUS INJECTION OF DEHYDROEPIANDROSTERONE SULPHATE (DHAS)

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SUMMARY

A non-chromatographic radioimmunoassay for estimation of total oestrone in plasma from pregnant women is described. A purified *Helix pomatia* extract is used for the hydrolysis. The antiserum has a high specificity to oestrone. The technical procedure is simple and rapid. Only small amounts of plasma (0.01–0.05) are needed for the analysis. The method has been applied to the measurement of total oestrone in plasma from pregnant women. During normal pregnancy a gradual increase of total oestrone was noticed from about 75 ng/ml in the 33rd–34th week to slightly more than 100 ng/ml in the 39th–40th week. Great individual variations were observed. Patients giving birth to small-for-date children showed a tendency towards lower values. In response to an intravenous injection of dehydroepiandrosterone sulphate (DHAS) a marked increase of total oestrone in plasma was recorded with peak values 360 min after the injection. A considerable individual variation in the magnitude of the rise was observed. The rise was generally lower in patients with small-for-date babies. The results do not suggest that measurement of total oestrone before or after an injection of DHAS will be useful for assessment of placental function.

INTRODUCTION

In the last ten years radioimmunoassays have been developed for estimations of oestrogens in blood. The continuous elaboration of more specific antibodies has made it possible to simplify the laboratory procedure. Although attempts have been made [1] to develop antibodies for oestrone sulphate a hydrolysis is still necessary for the estimation of total oestrone in plasma. Previous radioimmunoassays e.g. [2] are rather laborious. In 1977 a simplified technique was described for the estimation of total oestrone in serum from non-pregnant humans [3]. One of the aims of the present study was to apply this method for analysis of total oestrone in plasma from pregnant women.

The major part of circulating oestrone found in plasma from pregnant women is conjugated [4, 5] mainly as oestrone sulphate [6]. Total oestrone and oestriol are the most abundant oestrogens in plasma from pregnant females [5, 7]. Data on the plasma concentration of total oestrone or oestrone sulphate during normal and abnormal pregnancy are available [4, 7–9].

In human pregnancy oestrone is formed by the placenta from DHAS [10, 11]. The administration of DHAS in combination with measurement of the oestrogen increase in urine [12] or in plasma [13, 14] has been said to indicate the functional status of the placenta. Few reports [13], however, have dealt with the response of total oestrone in plasma to an injection of DHAS. In the present report total oestrone in plasma was measured during normal and abnormal pregnancy before and after the administration of DHAS to the mother with the intention to study if the data obtained could be used for the assessment of placental function.

EXPERIMENTAL

Materials

[2,4,6,7-^{3}H]-oestrone (S.A. 98.5 Ci/mmol) was obtained from New England Nuclear Corp., Boston, U.S.A. [6,7-^{3}H]-oestrone sulphate (S.A. 470 mCi/mmol) was purchased from The Radiochemical Centre, Amersham, England. Non-radioactive oestrone and oestrone sulphate were supplied by Ikapharm, Ramat-Gan, Israel. Dehydroepiandrosterone sulphate (DHAS) was furnished by F. Hoffman-La Roche & Co. A.G., Basel, Switzerland. The antiserum was supplied by Dr. Gordon Niswender, Colorado State University, Fort Collins, Colorado, U.S.A. This antiserum was diluted to 1:1000 in phosphate buffered saline containing 0.1% gelatin. Amberlite XAD-2, bead size 0.30–0.45 mm (Rohm & Haas Co., Philadelphia, Pennsylvania, U.S.A.) washed with methanol, acetone and glass distilled water [3] was provided by Dr. Kjell Carlström, Sabbatsberg Hospital, Stockholm, Sweden. *Helix pomatia* extract was

obtained from Industri Biologique Francaise, Gennevilliers, France.

Assay method

The content of 1 ampoule *Helix pomatia* extract was diluted to 10 ml with 0.15 M sodium acetate buffer pH 4.2. This solution was mixed with 3 ml of drained Amberlite XAD-2. The mixture was incubated at room temperature under slow rotation for 15 min. After that the Amberlite XAD-2 was removed by filtration. To 10–50 μl plasma 10 μl of 1.5 M sodium acetate pH 4.2 was added, followed by vortex mixing. Four hundred μl of the purified *Helix pomatia* extract were added and after vortex mixing the sample was incubated at 55°C for 30 min. Another 400 μl of the enzyme solution were added, and the sample was again incubated at 55°C for 30 min. The plasma sample was cooled to room temperature and extraction of the steroid was carried out once with 10 vol. of diethyl ether. The ether solution was decanted after freezing of the aqueous layer. The supernatant was evaporated to dryness before the radioimmunological analysis. All steps in this assay system were the same as those described by Edqvist and Johansson[15]. One hundred μl of the antibody solution were added to each tube. Then 100 μl of the tracer solution were added. This solution contained 138 pg of radioactive and 500 pg of cold oestrone. Standard samples made up with pure oestrone (0, 0.25, 0.5, 1.0, 2.5, 5.0, and 10 ng in duplicate) were processed simultaneously for each set of samples assayed.

Evaluation of the method

Specificity. The antibody used is sufficiently specific for a non-chromatographic radioimmunoassay of oestrone. For the crossreaction with other steroids the reader is referred to a previous publication [16].

Precision. The within assay variation calculated in accordance with Abraham[17], was 4.45%. The between assay variation was calculated from a plasma pool containing a known amount of oestrone. The coefficient of variation was 6.15%.

Accuracy. The accuracy of the method was tested by a recovery experiment (Fig. 1). Acceptable accuracy was found within the working range. The recovery of radioactive oestrone sulphate added to plasma prior to hydrolysis and extraction was 96.5%.

Sensitivity. When making up the standard curves, the binding in the tube with no extra oestrone added was arbitrarily set to 100%. The addition of 0.25 ng steroid reduced this binding to 86.3% (S.D. 2.6) as read off the standard curve. This difference is statistically significant ($P < 0.01$). When assaying comparable amounts of saline instead of plasma with the purified enzyme, blank values corresponding to an amount of 22.3 pg (S.D. 5.8) oestrone was measured. These values were read against a more sensitive standard curve [16]. The presented concentrations of total oestrone are not corrected for the enzyme blank nor for procedural losses.

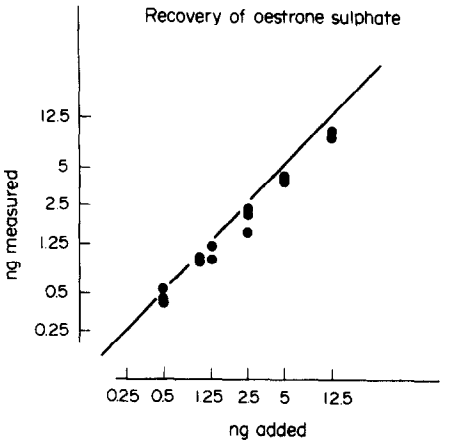

Fig. 1. Recovery of oestrone sulphate expressed as oestrone. Oestrone sulphate was added to plasma. Analysis was performed on 10, 25 and 50 μl of plasma. Correction was made for blank values.

Subjects

Forty-one women with uncomplicated pregnancies and 11 with pathological pregnancies volunteered for this study. All women with normal pregnancies gave birth to one healthy infant with birth weight exceeding 2800 g. Two women felt a slight pain and 3 noticed a taste of nut at the injection of DHAS. Otherwise no side effects were observed.

DHAS-test

Fifty mg of DHAS were injected i.v. at 8 o'clock a.m. Blood was withdrawn before and at 15, 30, 60, 120, 180, 360, and 600 min after the DHAS injection. In some women blood was collected also after 24 h. All tests were performed from the 33rd to the 40th week of pregnancy. Blood was collected into heparinized tubes. Plasma was separated by centrifugation and stored at below −15°C until assayed.

RESULTS

The pre-injection plasma levels of total oestrone appear in Table 1. In women with normal pregnancies the mean values tended to rise with advancing gestational age, but large variations were noticed. Patients with pre-eclampsia and diabetes had values well within ± 2 S.D. of the mean values of women with uncomplicated pregnancies. One pre-eclamptic patient, who gave birth to a child who was small for gestational age [18], had a value in the lower range. The other pre-eclamptic patients delivered children of normal weights and with Agpar scores of at least 8. Also the diabetic women gave birth to healthy infants of normal weight. Lower values were found in pregnant women with intrauterine growth retardation (IUGR).

The response of total oestrone in plasma after an injection of DHAS is shown in Fig. 2 and Table 2. The results from the groups of different gestational ages were pooled, since an analysis of variance

Table 1. Mean and variation of total oestrone in plasma during late pregnancy; The results are given in ng/ml

Week of pregnancy	Uncomplicated pregnancy			Pre-eclampsia			IUGR			Diabetes		
	Number of women	Mean	S.D.	Number of women	Mean	Range	Number of women	Mean	Range	Number of women	Mean	Range
33–34	10	77.6	47.4				3	18.0	5.0–36.0			
35–36	9	87.9	46.3	2	89.0	48.0–130.0				1	250.0	
37–38	11	110.5	60.6	1	160.0					1	76.0	
39–40	11	102.6	96.8	1	365.0					2	70.3	58.5–76.0

Table 2. Mean and variation of total oestrone following an intravenous injection of 50 mg DHAS in uncomplicated pregnancy; The results are given in ng/ml

Minutes after DHAS injection	15	30	60	120	180	360	600
Number of samples	40	40	40	40	38	38	13
Mean rise	8.2	18.9	28.2	59.9	76.4	90.0	52.2
Standard deviation	11.3	23.8	17.7	40.9	49.5	89.1	38.2

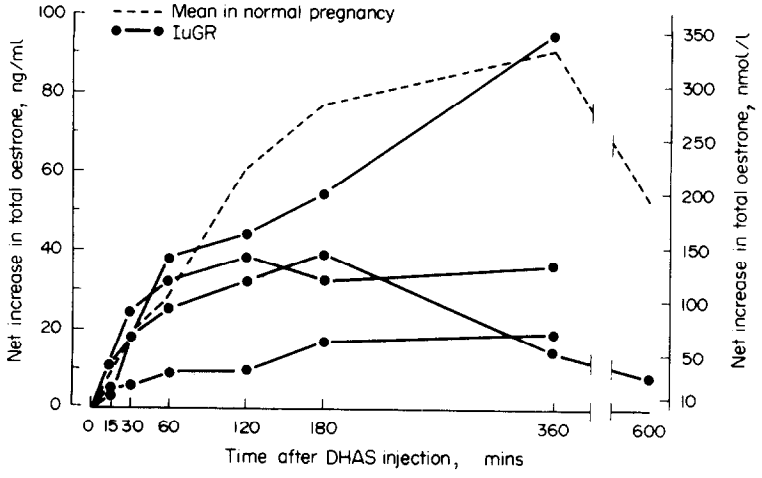

Fig. 2. The response of total oestrone in plasma to an i.v. injection of 50 mg DHAS in women with normal and abnormal pregnancies.

between these groups did not show any significant differences. For women with uncomplicated pregnancies an increase of total oestrone appeared at 15 min. The peak value was reached 360 min after the injection. At 600 min a decrease was noticed. Twenty-four h after the DHAS injection the plasma oestrone concentration was slightly elevated compared to the pre-injection values. As shown in Table 2 there was a great variability in the response of total oestrone levels to the DHAS load. Three women with IUGR and one with pre-eclampsia gave birth to children small for their gestational age. In 3 of these women the oestrone increase was lower than the mean rise found in women with normal pregnancies (Fig. 2). In the diabetic women the oestrogenic response showed no evident differences from that of women with uncomplicated pregnancies.

DISCUSSION

Different kinds of methods including colorimetric [8], fluorometric [19–21], and gas chromatographic [7] procedures have been used to measure total oestrone or oestrone sulphate in blood from pregnant women. Also radioimmunoassays have been presented [4]. These methods have been rather complicated and thus less suitable for clinical purposes. In a recent publication [3], a radioimmunoassay for total oestrone in serum from non-pregnant humans was presented and a simplified procedure was described. In this method the high reagent blanks often occurring when using enzymatic hydrolysis were efficiently reduced by treating the enzyme preparation with Amberlite XAD-2 resin before use. In the present report this method was modified to a minor degree and applied to the analysis of total oestrone in plasma from pregnant women. The chromatographic separation of the plasma oestrogens could be omitted due to the high specificity of the antiserum [16]. Amberlite XAD-2 will absorb steroids and other large organic molecules [22]. Although the exact nature of the compounds producing blank effects of *Helix pomatia* extracts is not known, treatment of the *Helix pomatia* extract with Amberlite XAD-2 was found to reduce the blank effect [3]. When assaying plasma from pregnant women the blank effect produced by the purified enzyme can be neglected. If, however, plasma from non-pregnant females is to be analysed, it is our experience that the blank effect could be minimized by processing the standard samples in the same way as the unknown samples.

The pre-injection levels of total oestrone in the present investigation are in the same range as those of other investigators. This holds true for different kinds of methods [4, 7, 8, 20]. Most investigations on total plasma oestrone concentrations during late pregnancy have revealed a slow increase of the oestrone level throughout pregnancy [4, 8]. This is in good agreement with our findings. As also found by others [4, 7, 8] we noted a large spread within the group of uncomplicated pregnancies. Roy *et al.*[9] and Fischer-Rasmussen[7] found that low and subnormal levels of total oestrone were more commonly met in patients with pre-eclampsia. This tendency was more marked in cases of stillbirth, neonatal asphyxia or low birth weight. Although our observations are few, they are in accordance with those of Roy *et al.*[9] and Fischer-Rasmussen[7]. The great variability in total oestrone concentration in plasma from one woman to another makes it unlikely that this hormone will provide a discriminating test of placental function. Lauritzen *et al.*[13] have described the response of total oestrone after an i.v. injection of DHAS. In 10 pregnant women from the 38th to the 40th week of pregnancy they found a slow increase with peak values between 60–240 min after the injection. The magnitude of the rise was 200–400% of the pre-injection value. These findings are in good agreement with ours. The present method cannot separate the different kinds of oestrone conjugates. Therefore,

it is not possible to state whether the increase of plasma oestrone after giving DHAS is mainly oestrone sulphate or other conjugates of oestrone. The rise of unconjugated plasma oestrone after administration of DHAS is of a much less magnitude [13]. Although the oestrone increase was less in pregnancies resulting in small-for-date babies than the mean increase of normal pregnancies (Fig. 2), the considerable variation within the group of uncomplicated pregnancies suggests that the measurement of total oestrone in response to DHAS will not be a useful addition to available tests of placental function. Since the oestrogenic steroids formed by the placenta are secreted into the maternal circulation as unconjugated hormones [10] estimation of unconjugated steroids might give a more accurate reflection of the placental function. A study on this matter is under preparation.

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