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Radioimmunoassay of Plasma 17a–Hydroxyprogesterone

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A radioimmunoassay for the measurement of 17α–hydroxyprogesterone (17α–OHP) in plasma was investigated utilizing antiserum produced by the introduction of 17α–hydroxyproges-terone-3-oxim BSA. Accuracy was such that within-assay variance was 16.2% and between-assay variance 18.3%.

The 17α–OHP mean plasma levels were 930±201 pg/ml (n=8) for normal adult males and 402±186 pg/ml (n=11) in the follicular phase and 1,190±662 pg/ml (n=12) in the luteal phase of females with a normal menstrual cycle.

The 17α–OHP levels in some tissues and tissue fluid also were measured, being 18.06±12.91 ng/ml (n=6) in the normal trophoblastic tissue, 1.7±0.5 ng/ml (n=17) in the trophoblastic tissue of a hydatidiform mole and 855.5±507.4 ng/ml (n=5) in lutein cyst fluid.

INTRODNCTION

We investigated a radioimmunoassay for the measurement of 17α–hydroxyprogesterone (17α–OHP) in plasma utilizing antiserum resulting from the introduction of 17α–hydroxyprogesterone. Levels of 17α–OHP in some tissues and plasma also were measured. The results are reported herein.

MATERIALS AND METHODS

1) Specificity of the Antiserum

The specificity of the antiserum used was tested by cross reaction studies with various

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Table 1. Cross reactions of various steroids with antiserum Produced by 17α-OHP-BSA

steroid compounds	% cross reaction	steroid compounds	% cross reaction
17α-hydroxy progesterone	100	corticosterone	0.04
progesterone	7.85	cortisol	0.04
20α -hydroxy progesterone	3.23	aldosterone	0.04
11-deoxy cortisol	0.88	testosterone	0.04
11-deoxy corticosterone	0.66	dehydroepiandro-sterone	0.04
pregnenolone	0.52	estrone	0.04
androst-4-ene-3,17, dione	0.25	estradiol-17β	0.04
5-β-pregnanediol	0.08	estriol	0.04

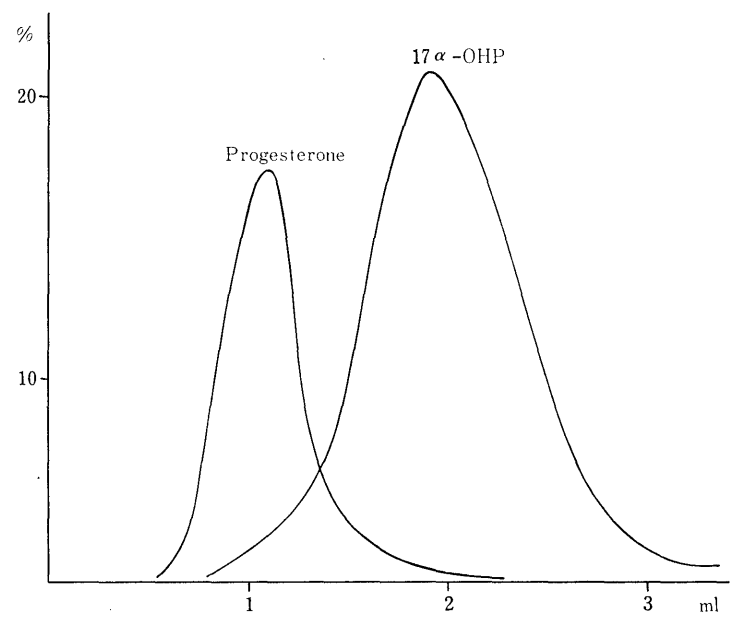

Fig. 1 Chromatographic pattern of progesterone and 17α-OHP in Sephadex LH-20 microcolumn

steroids (Table 1). The cross reaction of 17α-OHP was taken as 100%. Cross reaction of progrsterone and 20α-OHP-progesterone was 7.85% and 3.23%, respectively, but that of all other steroids in the test was less than 1%.

2) Plasma Extraction, Separation and Purification

1×10^3 dpm volume of ^{3}H-17α-OHP was added to plasma to produce an internal standard for recovery estimations. Extraction of 17α-OHP was carried out with 4ml of ether, following which the etheral extract was transferred to other tubes and evaporated to dryness at 37-40°C. The dried residue was dissolved in a benzenemethanol (95 : 5 solvent) and then chromatographic separation of 17α-OHP was accomplished by Sephadex LH-20 microcolumn chromatography (Fig. 1).

3) Standard Curve

Titration of the antiserum revealed that a dilution of $1 : 4 \times 10^4$ was most suitable for assay. Namely, when the antiserum was diluted 40,000 times, the calibration curve became an almost straight line in the range of 0-1,000 pg of 17α-OHP (Fig. 2). In this range, the 17α-OHP percent bound to antiserum displayed a standard deviation of ±0.1-±0.7 and a coefficient of variation of 0.2-3.7%.

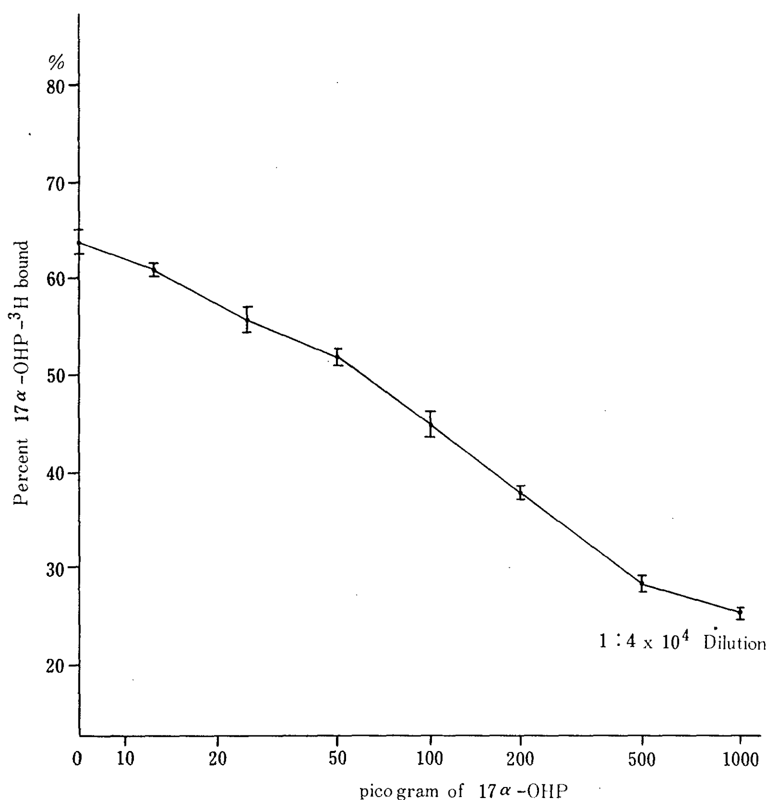

Fig 2 Standard curve with antiserum produced by 17α-OHP-BSA and diluted $1/4 \times 10^4$

4) Measurement Procedure

The measurement procedure is as shown in Figure 3. The concentration of 17α-OHP in plasma was calculated by the following formula:

$$\frac{E}{R} \times (M-m) \times \frac{100}{Re\%} \times \frac{1}{\text{Plasma sample(ml)}} \quad \text{where}$$

E=affluent from column chromatography
R=volume of effluent used in RIA
M=17α-OHP value estimated by
standard curve
m=value of water sample for testing blank of system
Re%=recovery percentage

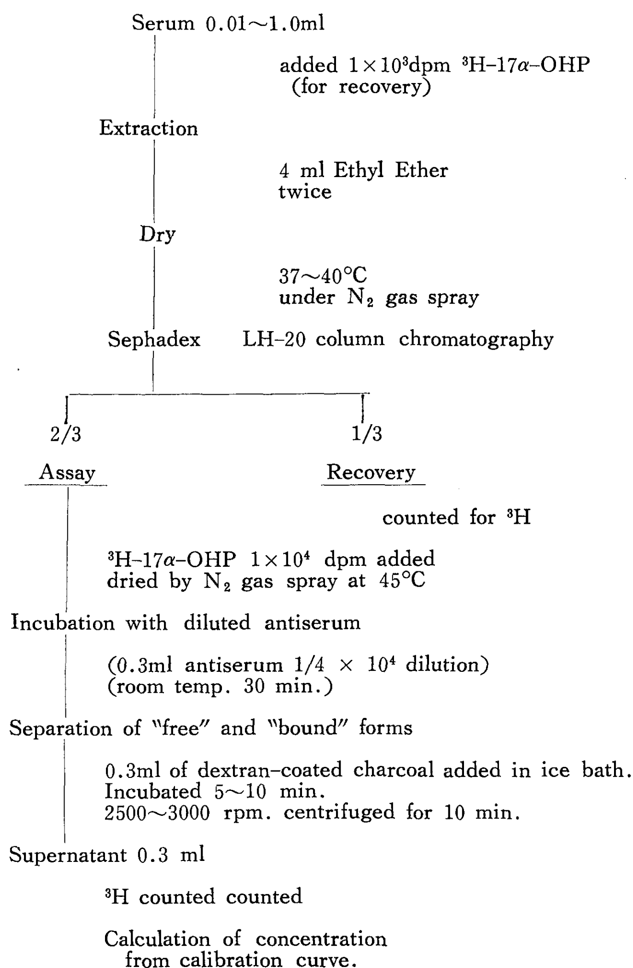

Fig. 3 Measurement procedure

5) Accuracy and Precision

The accuracy of the assay is shown in Table 2. The coefficient of variation varied between 8.3% and 17.3%. Within-assay and between-assay variances were 16.2% (n=17) and 18.3% (n=9), respectively.

Table 2. Accuracy of measurement
(Recovery of 17 -OHP added to 1 ml of distilled water)

	17α -OHP added (pg)	17α -OHP measured (mean) (pg)	standard deviation (±)	coefficient of variation (%)
	25	27.4	2.7	9.9
Distilled	50	53.2	9.2	17.3
water	100	96.2	8.0	8.3
1ml	200	191.0	20.0	10.5
	500	345.2	25.5	7.0

RESULTS

The plasma 17α-OHP levels determined by the assay were 930±201 pg/ml (n=8) in normal adult males, and 402±186 pg/ml (n=11) in the follicular phase and 1,190±662 pg/ml (n=12) in the luteal phase of females with a normal menstrual cycle.

Plasma levels of LH, estradiol, progesterone and 17α-OHP during the menstrual cycle of the same female were examined, the results of this being shown in Fig. 4. In a normal pregnancy, the plasma 17α-OHP level was observed to be 2.03±0.65 ng/ml (n=5) at the 7th to 17th weeks.

We next examined tissue levels of 17α-OHP. Normal trophoblastic tissue and the tissue of a hydatidiform mole were homogenized and the levels of 17α-OHP in the supernatants determined. The levels were 18.06±12.91 ng/ml (n=6) and 1.71±0.5 ng/ml, respectively. Lutein cyst fluid obtained during surgery involving a destructive mole and chorioepithelioma also was examined and the 17α-OHP level was found to be 855.5±507.4 ng/ml (n=5).

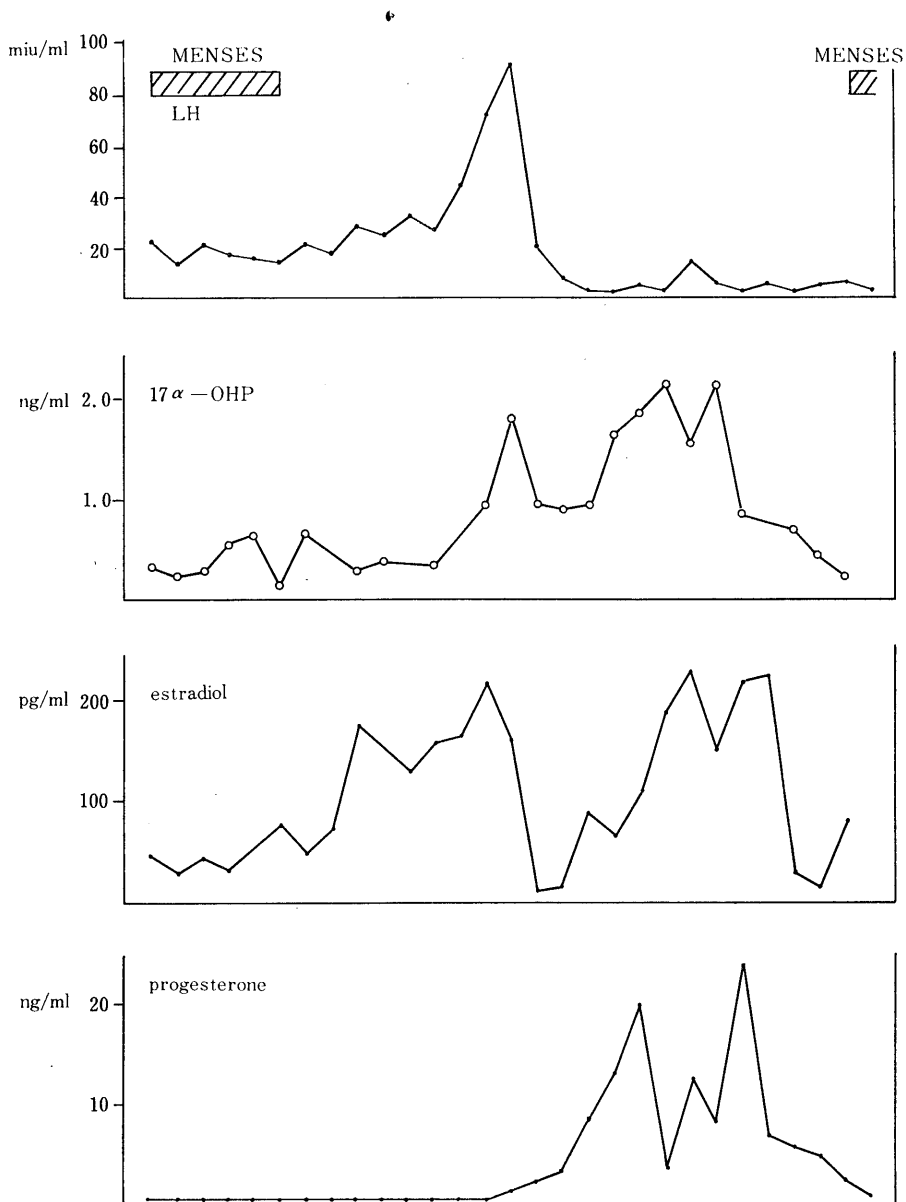

Fig. 4 The plasma levels of LH, 17α-OHP, estradiol and progesterone simultaneously measured in one person during normal menstrual cycle.

DISCUSSION

The antiserum used in the assay displayed cross reactions of 7.85% and 3.23%, respectively, with progesterone and 20α-hydroxyprogesterone, but its cross reactions with all other steroids tested were less than 1%. The specificity of our serum, when compared

with the antiserum in ABRAHAM's RIA[1] and the cortisol binding globulin of competitive protein binding assay (CPBA)[3][4], proved to be far better.

The plasma 17α-OHP level we obtained with normal males was 930$\pm$201 pg/ml (n =8), a value very similar to the 950$\pm$310 pg/ml (n=18) (CPBA) obtained by STROTT[5] and the 870$\pm$530 pg/ml (n=13) (RIA) obtained by Abraham[1]. However, it was rather lower than the value of 1,400$\pm$100 pg/ml (n=10) (RIA) obtained by YOUSSENFNEJADIAN[6]. Values obtained in the follicular and luteral phase of normally menstruating females coincided with those obtained by other investigators [1][4][6].

Normal trophoblastic tissue showed a far higher level of 17α-OHP (18.06$\pm$12.91 ng/ml) than hydatidiform mole tissue (1.71$\pm$0.50 ng/ml). In addition, there have been reports that 17α-hydroxysteroid dehydrogenase is not found in the trophoblastic tissue of either destructive mole or hydatidiform mole[5]. Based on these facts, it can be assumed that there is a considerably lower rate of 17α-OHP biosynthesis in molar tissue.

A great deal of 17α-OHP (855$\pm$507.4 ng/ml) was found to be contained in lutein cyst fluid obtained through surgery involving a destructive mole or chorioepithelioma. MIURA[2], one of the authors, has previously reported that the decrease in plasma levels of hCG, estradiol and progesterone was found to be delayed in a patient with lutein cyst. It is our opinion that in the case of such patients, a large amount of the hormones contained in the lutein cyst is continually released into plasma, and this may be the cause of the delayed decrease. Therefore, the measurement of plasma 17α-OHP levels as that of other steroid hormones could provide useful clues to the condition of a lutein cyst.

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