Plasma estrone, estradiol, estriol, progesterone, and 17-hydroxyprogesterone in human pregnancy

I. Normal pregnancy

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The plasma concentrations of progesterone, 17-hydroxyprogesterone, and unconjugated estrone, estradiol, and estriol, were measured by radioligand assays in 0.21 ml plasma aliquots obtained during the second half of normal human pregnancy.

The mean plasma concentrations of unconjugated estrone, estradiol, and estriol increased toward term whereas the mean plasma 17-hydroxyprogesterone concentrations increased significantly only after the thirty-third week of gestation. Mean plasma progesterone concentrations were at least ninefold higher than any of the unconjugated estrogens measured in this study.

Estradiol was quantitatively the most important unconjugated estrogen throughout the second half of pregnancy. The ratios of plasma progesterone:estradiol (unconjugated), progesterone:estriol (unconjugated), and estradiol (unconjugated):estriol (unconjugated) remained constant throughout the second half of gestation.

There appears to be no preferential increase in the plasma concentrations of any one of these hormones as measured.

Information concerning the relationship between estrogens and progesterone during late human pregnancy is based primarily on data derived from urinary studies.1 No direct information is available on the progesterone estrogen relationship in plasma and the information on the interrelationship between the various unconjugated estrogens in plasma is limited to a small number of patients and is devoid of any statistical analysis.2-5 Yet, an understanding of these interrelationships may be of considerable clinical importance. Progesterone is largely of placental origin while estradiol is derived from both fetal and maternal sources and estriol is mainly of fetal origin.6 Comparisons of these plasma hormone levels may provide an additional indicator of the integrity of the fetoplacental unit and may be a diagnostic adjunct in the management of disorders of late pregnancy. Comparing estrogens and pro-
gestogens in plasma may also be important in clarifying their possible effect on uterine motility prior to or during labor.

The purpose of this study is to describe normal relationships between the various plasma unconjugated estrogens and progesterone during the second half of human pregnancy. Subsequent papers will deal with the clinical and physiologic applications of these determinations.

Materials and methods

The subjects of the study were apparently healthy pregnant patients who came to the Harbor General Hospital obstetric clinic. One milliliter plasma samples were obtained throughout pregnancy for determination of progesterone, 17-hydroxyprogesterone and unconjugated estrone (E₁), unconjugated estradiol (E₂), and unconjugated estriol (E₃). Plasma progesterone, 17-hydroxyprogesterone, and unconjugated E₂ were measured by radioimmunoassay while unconjugated E₁ and E₃ were measured by radioligand assay with cytosol as a specific binder. In brief, the methods for measurement of these hormones consisted of extraction of 0.1 to 0.2 ml. plasma with ether after addition of radioactive tracer to monitor recovery, purification of the steroids by chromatography and measurement of mass by their capacity to compete with labeled steroid on a limited number of binding sites. These methods have coefficients of variation of less than 10 per cent at plasma levels above 1 ng. per milliliter. Plasma estradiol was measured directly from 0.01 ml. of unextracted plasma by our rapid radioligand assay with uterine cytosol as specific binding reagent. The coefficient of variation obtained by this method is less than 10 per cent at plasma levels above 5 ng. per milliliter.

Results

The mean plasma values calculated from 308 individual progesterone and 126 17-hydroxyprogesterone, 140 unconjugated E₁, 310 unconjugated E₂, and 201 unconjugated E₃ plasma determinations. The bars represent the standard error of the means.

![Graph showing mean plasma values calculated from 308 individual progesterone (P), 126 17-hydroxyprogesterone (17-OHP), 140 unconjugated E₁, 310 unconjugated E₂, and 210 unconjugated E₃ plasma determinations. The bars represent the standard error of the means.](image-url)
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Fig. 2. P/E₂ (unconjugated) ratios of 125 normal patients at from 20 to 42 weeks' pregnancy. Each dot represents an individual P/E₂ (unconjugated) ratio while the middle dashed line indicates the regression line. Because of the skewed distribution, the 95 per cent confidence interval represented by the shaded area was calculated separately for values above and below the mean.

The relationship between progesterone and unconjugated E₂ during the second half of human pregnancy is shown in Fig. 2. The progesterone/E₂ (unconjugated) ratio is constant from the twentieth through the forty-second week of gestation with correlation coefficient “r” of = 0.003. The regression line, represented by the middle dashed line, has an equation $y = 0.034x +16.3$, indicating negligible slope and mean plasma progesterone concentration 16.3 fold higher than that of unconjugated E₂. The shaded area representing the 95 per cent confidence interval has a skewed distribution with the wider scatter of values observed above the mean.

The normal progesterone/E₂ (unconjugated) distribution in the second half of pregnancy is presented again in Fig. 3. Here, however, the actual progesterone and unconjugated E₂ values were given for each patient while the week of gestation was eliminated, since it was shown (Fig. 2) to have no effect on the progesterone/E₂ ratio. The shaded area represents the area of normalcy. The regression line had an equation of $y = 0.0533x +8.7$ and the correlation coefficient “r” was 0.433.

The relationship between the plasma concentrations of progesterone and unconjugated E₂ in the second half of pregnancy is shown in Fig. 4. There is no change in the progesterone/E₂ unconjugated ratios with increasing stage of gestation (correlation coefficient “r” = 0.021). The regression line $y = 0.034x +16.3$ indicates negligible slope and a mean plasma progesterone concentration 16.3 fold higher than that of unconjugated E₂. Again values above the mean are more widely scattered than those below the mean.

The progesterone/E₂ (unconjugated) relationship in the second half of pregnancy is presented again in Fig. 5. Here, however, the actual progesterone and unconjugated E₂ values are shown for each patient, while the week of gestation is eliminated. The shaded area representing the area of normalcy is asymmetric, indicating the skewed distribution of the values. The regression line is y
Fig. 4. P/Es (unconjugated) ratios of 97 normal patients at from 22 to 41 weeks' gestation. The middle dashed line is the regression line and the shaded area represents the P/Es 95 per cent confidence interval calculated as described in Fig. 2.

\[ \text{P} = 0.028x + 4.39 \]  and the correlation coefficient “r” = 0.557.

The unconjugated E2/Es relationship in the second half of pregnancy is shown in Fig. 6. There is no significant change in the unconjugated E2/Es ratio with increased stage of gestation (correlation coefficient “r” = -0.04). The regression line \( y = 0.009x + 2.52 \) indicates an insignificant slope, and mean unconjugated E2 plasma concentration which is 2.5 times higher than that of unconjugated Es.

The unconjugated E2/Es relationship in the second half of pregnancy is presented again in Fig. 7. Here, the actual unconjugated E2 and E2 values are shown for each patient, while the week of gestation is eliminated. The shaded area represents the area of normalcy. The regression line is \( y = 0.242x + 3.82 \) with correlation coefficient “r” of 0.51.

Comment

Comparing hormones which are primarily of placental origin (progesterone) with those which arise partially from maternal (Es) or largely of fetal sources (E2) may provide a more thorough assessment of the components of the maternal fetoplacental unit than is possible by only looking at single hormone levels. It may perhaps permit differentiation of whether abnormal hormonal production is due to placental or fetal abnormalities. It may perhaps also identify certain hormone concentrations as abnormal for an individual particular fetoplacental unit in circumstances where the usual comparison to a range of normal values would have failed to recognize the abnormality. For instance, a low normal E2 concentration may have different meanings if associated with a small placenta making low normal levels of progesterone or if associated with a large placenta making large amounts of progesterone. Obviously, abnormal levels of hormones may not necessarily be associated with an abnormal ratio. On the other hand, an abnormal ratio may be found even if the individual values fall within the normal limit. Both indices are therefore complementary. Utilization of this principle for the follow-up of patients with abnormalities of gestation will be the subject of subsequent communications.

In pregnancy it may be desirable to measure the concentrations of the unconjugated rather than the conjugated estrogens. The unconjugated estrogens lack substantial diurnal variations, they are produced and secreted primarily by the placenta and are more biologically active. Because of low plasma levels, their measurement requires use of the more sensitive methods such as the
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Fig. 6. The E₂ (unconjugated)/E₃ (unconjugated) ratios of 94 normal patients at from 21 to 42 weeks' gestation. The dashed line represents the regression line and the shaded area indicates 95 per cent confidence interval.

Radioligand technique and radioimmunoassays.

The plasma levels of progesterone, 17-hydroxyprogesterone, and unconjugated E₂ and E₃ obtained during pregnancy by these methods have previously been reported and are in good agreement with other studies. The plasma levels of unconjugated E₂ reported here are also in the range reported for pregnancy.

These results confirm the fact that progesterone is the main steroidal hormone in human pregnancy with mean plasma concentrations of at least ninefold higher than any of the three classical unconjugated estrogens. E₂ is the main unconjugated estrogen in human pregnancy with plasma concentration higher than either unconjugated E₃ or unconjugated E₁. Of interest is the observation that 17-hydroxyprogesterone, which is considered to reflect corpus luteum activity during pregnancy shows significant increase of its plasma levels after the thirty-third week of gestation. This observation will be subject to further investigation.

Urinary E₃ has been reported to increase 1,000 fold during pregnancy, whereas urinary E₁ and E₂ have been estimated to increase 100 fold. Our plasma unconjugated E₁ and E₂ levels at term are also about 100-fold higher than luteal phase levels previously reported and therefore agree with the urinary data. No estimate of the increase in plasma unconjugated E₃ during pregnancy can be made until luteal phase levels of unconjugated E₃ are well established. It is also conceivable that a 1,000-fold increase in plasma unconjugated E₃ will be found considering the minimal concentration of unconjugated E₃ reported for the non-pregnancy state.

Calculation of ratios and comparison of plasma hormone concentrations require that measurements of the various hormones be made on the same sample. Meaningful ratios cannot be calculated using normal value as a "base line." Using this approach we found constant progesterone/E₂ (unconjugated) progesterone/E₃ (unconjugated) and E₂/E₃ (unconjugated) ratios during the second half of normal pregnancy, indicating no preferential increase in the plasma concentration of any one of these hormones as mea-
sured. This conclusion seems to be in discrepancy with the data of Klopper and Billewicz who found that the ratio of urinary pregnanediol/urinary estriol decreases during the second half of gestation. However, urinary excretory products such as pregnanediol and conjugated estriol should not be expected to reflect precisely the levels of progesterone and unconjugated estriol in plasma. The increased clearance rate of conjugated estriol observed toward term will increase urinary estriol excretion, whereas it is unlikely to affect plasma unconjugated estriol, which is cleared by the liver. Likewise, in the pregnant patient pregnanediol is derived mostly but not solely from progesterone. Moreover, as pointed out by Hytten and Leitch, the pattern of plasma progesterone is quite different from the pattern of pregnanediol excretion during pregnancy, suggesting a changing clearance rate of pregnanediol or change in the metabolism of circulating progesterone. Therefore, it is conceivable that Klopper and Billewicz urinary data as well as our data may both be correct. They should not be expected either to support or to contradict each other but rather to reflect different aspects of normal hormonal relationships in human pregnancy.

REFERENCES
