

The Plasma Estradiol as an Index of Fetoplacental Function

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ABSTRACT A radioligand assay has been developed for the measurement of unconjugated 17β -estradiol in as little as 0.01 ml of pregnancy plasma employing rabbit uterine cytosol as specific binder and activated charcoal as nonspecific adsorbant. Large numbers of samples could be processed simultaneously at relatively little expense and results were obtainable within 3 hr. The procedure was sensitive to 1 ng/ml. No diurnal or positional variations were found. Values from 250 unselected normal patients showed a constantly rising mean plasma E_2 from 18 to 35 wk gestation from 4.5 to 14 ng/ml. From 35 to 40 wk, mean E_2 rose only to 15 ng/ml and the range of values increased substantially. When 22 normal pregnant subjects were followed serially, rising levels of plasma E_2 were found with no significant fall ever seen. By contrast, patients exhibiting fetal distress generally had falling or reduced E_2 levels. However, 3 cases of Rh isoimmunization had elevated or normal E_2 concentrations even after clearcut evidence of fetal demise. A decrease of 45% in E_2 concentration was associated with intraamniotic instillation of hypertonic saline prior to delivery supporting the view that placental conversion of maternal adrenal precursors is responsible for about half of the E_2 production in pregnancy. The postpartum clearance of endogenous E_2 was measured and found to fit a two compartment model with mean half-time of 22 min and 7 hr. Follicular phase levels of E_2 were attained by 35 hr postpartum. The concentration of unconjugated E_2 in pregnancy plasma correlated as well with the state of the placenta as other placental hormone measurements and holds promise of being a rapid, inexpensive, and reliable method of following patients with high-risk pregnancies in a variety of clinical settings.

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INTRODUCTION

Since the discovery of Spielman, Goldberger, and Frank in 1933 (1) that, upon the death of the fetus, urinary bioassayable estrogens decrease markedly, it has been clear that this phenomenon might have clinical applications in assessing fetoplacental function.

Subsequently, with the development of steroid measurement methods, estimation of the urinary estriol (E_3)¹ has been used extensively for assisting the obstetrician in the diagnosis of fetal distress. Aside from technological problems which have been gradually overcome, urinary E_3 assay has intrinsic disadvantages. The need to collect urine results in errors if there are incomplete 24 hr collections. Because of dependence on hepatic conjugation and renal clearance wide daily variations in urinary E_3 excretion have been described (2-6), increasing the complexity of clinical interpretation.

Measurement of the plasma E_2 (6, 7) has not attained widespread acceptance, perhaps because of technical difficulties, but results may be obtained within a single day. A search for other means of evaluating placental function has been carried out recently. Plasma levels of various hormones (8, 9), blood components (10), and enzymes (11, 12) have been suggested as criteria of assessing placental function and some of these techniques such as blood human chorionic somatomammotropin (placental lactogen) termed (HCS) (9) are promising.

Development in our laboratories of a radioligand assay method for the measurement of unconjugated 17β -estradiol (E_2) (13-15) and estrone (E_1) (16) in non-

¹ *Abbreviations used in this paper:* E_1 , Estrone—Estra-1,3,5(10)-triene-17-one,3-hydroxy; E_2 , 17β -Estradiol—Estra-1,3,5(10)-triene-3,17 β -diol; E_3 , Estriol—Estra-1,3,5(10)-triene-3,16 α ,17 β -triol; HCG, human chorionic gonadotropin; HCS, human chorionic somatomammotropin (placental lactogen).

pregnant individuals led us to explore the possibility of measuring E_2 in pregnancy plasma and testing its effectiveness as a predictor of fetal distress.

We now report an extremely simple, inexpensive, and rapid technique for the radioligand assay of unconjugated plasma E_2 in pregnancy. A single individual can reliably measure the concentration of E_2 in large numbers of plasmas in 3 hr using minute amounts of blood.

Over 250 sequential and individual estrogen determinations have revealed the pattern of change during normal pregnancy and have demonstrated the alterations in 16 cases of fetal distress. Changes in the plasma E_2 resulting from induction of abortion using hypertonic saline infusion have been characterized. The pattern of decline of endogenous E_2 from the time of parturition has been shown.

On the basis of these data it is suggested that measurement of E_2 during pregnancy by the radioligand assay procedure may provide considerable physiological information and may be of significant clinical applicability, particularly in the early diagnosis of placental insufficiency.

METHODS

6,7- 3H -Estradiol, 42.0 Ci/mmol, obtained from New England Nuclear Corporation (Boston, Mass.) was used without further purification for about 1 month and discarded. Spectroquality ethylene glycol and activated charcoal (Norite A) were obtained from Matheson, Coleman, and Bell (Cincinnati, Ohio); dextran D grade was obtained from Mann Research Laboratories Inc., New York. Biosolve-BBS® was obtained from Beckman Instruments, Fullerton, Calif. All other chemicals were reagent grade.

The subjects were all volunteers who gave informed written consent. For the postpartum studies, the subjects were housed in the Harbor General Hospital Clinical Research Center.

Assay Procedure. The specific estrogen binder was prepared from immature rabbit uteri as previously described (13-15). Fresh or freshly frozen uteri from immature rabbits were obtained from a commercial slaughterhouse. Uterine estrogen binder has been prepared successfully from rat, calf, horse, and human uterus (15) as well as rabbit. Uteri were homogenized in 3 volumes of a buffer consisting of 0.01 M Tris-HCl pH 8.0, 0.001 M EDTA, 0.25 M sucrose, at 4°C in the Waring Blendor using four 30-sec pulses and cooling intervals of 2 min. The homogenate was centrifuged at 1000 *g* for 15 min and the supernatant centrifuged at 105,000 *g* for 90 min at 4°C. This supernatant (cytosol), stored in liquid N_2 in small portions, was stable as a potent estrogen binder for months.

The E_2 assay was carried out in 10 × 75 mm disposable glass centrifuge tubes. Radioactive E_2 and standard E_2 solutions, were made up in assay buffer consisting of 8 volumes 0.01 M Tris-HCl pH 8.0, 0.001 M EDTA, and 2 volumes of ethylene glycol. To a final vol of 0.5 ml were added either was attained. Furthermore, dried ether extracts of 0.2 ml of 0.02 ml of plasma and 10,000 cpm of 3H -labeled E_2 . The final addition was 0.02 ml of uterine cytosol. Each assay consisted of a standard curve in triplicate and unknowns in

TABLE I
The Ratio of Association Constants of Estrone and Estriol Compared to 17 β -Estradiol for Specific Binding Sites in Rabbit Uterine Cytosol

	Ratio of association constants	
	16 hr 0°C	2 hr 23°C
Estrone	0.41	0.08
Estriol	0.10	0.05

Unradioactive estrone and estriol in increasing concentrations were allowed to compete with 3H -labeled estradiol for binding sites in uterine cytosol at 0°C for 16 hr and at 23°C for 2 hr. From the competition curves, employing previously described equations (19), the ratio of association constants (RAC) were calculated.

duplicate. The tubes were stood at room temperature (23°C) for 2 hr.

The protein-bound and free 3H -labeled E_2 were separated by adding 0.5 ml of a suspension containing 0.5% activated charcoal and 0.05% dextran in 0.01 M Tris-HCl pH 8.0. After incubation for 15 min in an ice bath, samples were centrifuged for 10 min at 4°C and the supernatants decanted into counting vials containing 10 ml of scintillation solution consisting of 160 ml Biosolve, 42 ml Liquiflor and toluene to 1 liter total volume. The samples were counted to a total of 5000 counts at an efficiency of 35%.

Validation of the assay system. As previously described (14, 15) standards ranged from 10 to 200 pg with 20 pg always > 2 sd different from 0. A typical standard curve has been presented (14). For unknowns run at 0.01 ml, then, the sensitivity of the assay was 2 ng/ml. We found that the use of 0.020 ml of plasma did in no way alter the assay so that under those circumstances a sensitivity of 1 ng/ml was attained. Furthermore, dried ether extracts of 0.2 ml of plasma or less also gave identical plasma concentration values at a sensitivity of 0.1 ng/ml. Blanks using nonpregnancy plasma gave values indistinguishable from 0.

The precision of the assay system has been previously evaluated (14) with an index of precision λ of the standard curve of 0.06.

A number of studies have been carried out to validate the specificity of the estrogen assay system. Specificity of the uterine receptor for estrogens has been demonstrated (13, 17). Comparability of estrogen measurements with those of Baird and Guevara, using a double isotope derivative procedure (18), has supported the validity of radioligand assay.

In the case of pregnancy plasma, conditions were required which could minimize the contribution of the E_1 , E_3 , and estrogen conjugates present. Accordingly, conditions were employed in which the ratio of association constants (19) of E_1 and estriol relative to E_2 were low (Table I). In contrast to overnight incubation at 0°C, after incubation at 23°C for 2 hr, E_1 and E_3 had affinities only 8 and 5% that of E_2 for the uterine receptor. It has been our experience that estrogen conjugates do not bind to the uterine cytosol receptor. However, to demonstrate the lack of influence of any component of pregnancy plasma on the plasma E_2 determination, we have carried out a comparison of the above procedure with replicates assayed as previously described (13-15). Other estrogens and estrogen conjugates were separated from the E_2 fraction by ether extraction and

TABLE II

Comparison of Plasma Estradiol Concentrations in Pregnancy Measured Directly and after Processing by Extraction and Column Chromatography

Direct	Processed	Direct	Processed
ng/ml	ng/ml	ng/ml	ng/ml
2.0	2.6	11.6	13.8
15.0	14.9	6.6	4.6
20.0	21.0	8.8	5.8
5.6	4.6	7.4	9.0
1.1	0.6	14.0	16.9
14.0	13.2	18.8	19.5
13.0	12.2	5.4	4.3
6.8	6.3	18.0	19.9
10.8	10.8	11.0	11.0
13.3	14.9	14.4	16.4
3.7	2.3	2.0	1.4
1.5	1.0	10.8	12.1
8.8	7.1	4.1	6.2
6.6	5.1	9.4	11.6

Samples of pregnancy plasma were assayed by the simple and the more complex radioligand assay procedures described in the text and the values obtained compared. The mean E_2 was 9.45 ng/ml on direct assay and 9.65 ng/ml for extracted and chromatographed plasma.

column chromatography, and recovery was estimated by an internal standard. As shown in Table II, the mean E_2 was 9.45 ng/ml on direct and 9.65 ng/ml on processed plasma, an insignificant difference. The mean sd of duplicates run by the different methods was 0.8 ng/ml. Duplicate plasmas run by the "direct" method only, on different days, also gave a constant sd of 0.8 ng/ml. The probability was, therefore, less than 5% that another portion assayed by the same method would vary by more than 1.6 ng/ml.

Further evidence for the validity of the method has been obtained by comparison with values obtained by others during gestation. Very good agreement with the data of Svendsen and Sorensen (20), Smith (21), and Munson, Mueller, and Yannone (22), all of whom used quite different techniques, has been obtained.

TABLE III

Diurnal Variation of Plasma Estradiol

8 am	4 pm	Midnight
ng/ml	ng/ml	ng/ml
20.0	20.5	18.0
42.0	43.0	38.0
29.5	31.5	30.0
20.5	19.5	17.5
23.0	24.0	22.0
29.5	28.0	29.5
22.0	26.0	23.5
14.0	15.5	14.0
29.0	24.5	29.0
Mean	25.5	25.9

RESULTS

The plasma concentration of E_2 was determined every 8 hr during a 24-hr period in nine normal subjects during their last trimester of pregnancy. As shown in Table III, no significant fluctuations in E_2 levels were found.

The effect of short-term postural changes on blood E_2 were checked in patients from whom blood was drawn after 30 min of stabilization in each given position. There was no difference from one position to another.

Blood was drawn from 250 unselected patients visiting the prenatal clinic. Their plasma E_2 values are shown in Fig. 1. The mean E_2 concentration increased gradually until the 35th wk of pregnancy when it leveled off. The scatter of individual values increased with advancing pregnancy and was larger for values above the mean than for values below it, giving a skewed distribution. As a result, 2 sd of the mean represented by the upper and lower lines, were expressed for each group separately. In the last 8 wk of pregnancy, not a single value was found to fall below 5.5 ng/ml. Twin pregnancies resulted in high E_2 values.

22 normal patients were followed periodically during their gestation. The results, depicted in Fig. 2 indicate that E_2 concentrations rose consistently as pregnancy progressed. It is of particular note that no significant fall with time occurred in any of these patients.

Abnormal pregnancies

In this group of patients are those admitted for imminent fetal demise, intrauterine growth retardation or

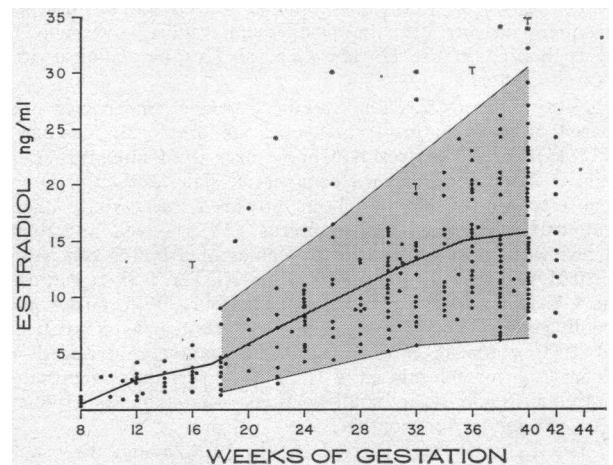


FIGURE 1 Individual and mean plasma estradiol concentrations from 250 pregnant patients. The lines represent the mean and 2 sd above and below the mean. Since the distribution was skewed toward high levels, variances above and below the mean were computed separately. T represents a twin gestation. Points at 42 wk gestation represent patients with uncertain conception dates and not postmaturity.

a condition suspected of leading to fetal wastage. The problem was identified at various stages of gestation and when possible, multiple plasma E_2 determinations were carried out. In Fig. 3 the plasma E_2 concentrations in each individual case are shown. Brief clinical descriptions follow.

Patient 1 was an 18 yr old, primigravida with severe preeclampsia including 4+ proteinuria and a blood pressure of 200/120. Administration of hydralazine resulted in a rapid return of her blood pressure to normal. Her plasma E_2 fell sharply to subnormal levels. 1 day later fetal movement was noted to have ceased and intrauterine fetal demise was confirmed.

Patient 2 was a 33 yr old, gravida 2 para 0, with a history of 10 yr of hypertension. She was admitted for preeclampsia with 3+ proteinuria and a blood pressure of 220/130. Initial and subsequent plasma E_2 concentrations were all subnormal. A further drop was noted in association with fetal death.

Patient 3. See patient Rh3 in next section.

Patients 4, 5, 6, 7, and 10 represent five patients with intrauterine fetal demise of unknown cause. All apparently had a normal gestation and none had any previous abnormal pregnancies. Of the five, patient 7, the only one in labor, had a normal plasma E_2 level. She had noticed sudden cessation of fetal movement 5 days before admission and the placenta on delivery was found to be intact.

Patient 8 was a 24 yr old, gravida 2 para 1, who was thought to have intrauterine growth retardation. Her initial E_2 value was considered to be borderline. She was delivered of a 5 lb. 2 oz., alive, dysmature baby via emergency cesarean section, when fetal movements ceased.

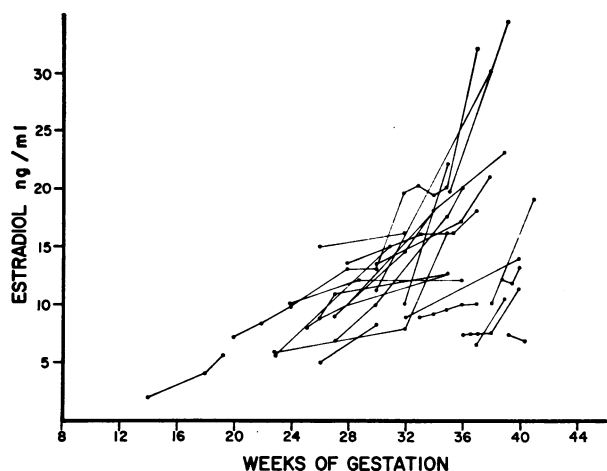


FIGURE 2 Serial plasma estradiol determinations in 22 pregnant patients. The lines connect determinations in an individual.

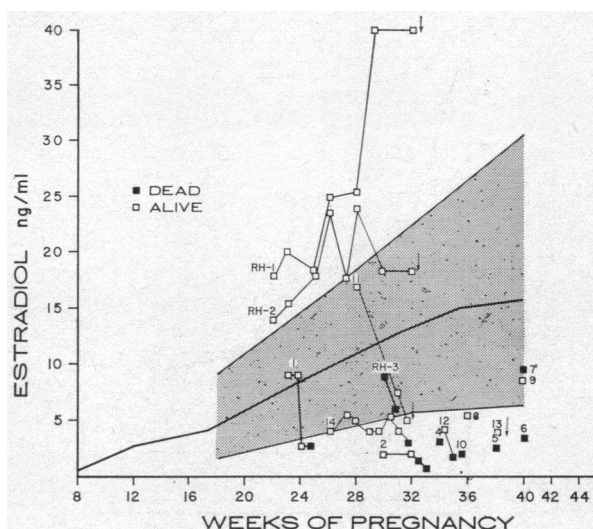


FIGURE 3 The plasma estradiol concentration in 16 abnormal pregnancies. The black squares represent samples obtained after a clinical diagnosis of fetal demise. Lines connect sequential determinations in an individual. Arrows indicate time of delivery.

Patient 9 was a 28 yr old, gravida 3, para 2, admitted in labor with a history of two previous cesarean sections due to cephalopelvic disproportion. A repeat cesarean section resulted in a delivery of a 4 lb. 12 oz., dysmature, alive fetus and the placenta on examination showed multiple infarcts covering 50% of the placental surface. This patient had a normal E_2 concentration.

Patient 11 was a 23 yr old, primigravida who was hospitalized for preeclampsia with 1+ proteinuria and a blood pressure of 180/100. She was maintained on hydralazine and diuretics. Her plasma E_2 concentration fell substantially to below normal values under observation. 1 day after the last determination, fetal heart tones were no longer heard and she was delivered of an alive infant by cesarean section.

Patient 12 was a 24 yr old, primigravida, admitted for preeclampsia with 1+ proteinuria and a blood pressure of 160/100 which was controlled by hydralazine. Fetal heart tones which were present on admission became inaudible 2 days later. Her low initial plasma E_2 fell still further at the time of fetal demise.

Patient 13 was a 30 yr old, gravida 5, para 4 heroin addict who presented at 38 wk gestation with severe malnutrition and a hematocrit of 6%. She was transfused and treated with promazine hydrochloride. Fetal heart tones, which were present on admission, were noted to have disappeared 10 hr later when the patient spontaneously commenced labor. She was delivered of a 3150 g nonmacerated stillborn. The placenta was small (230 g) and contained multiple infarctions. Her plasma E_2 was subnormal.

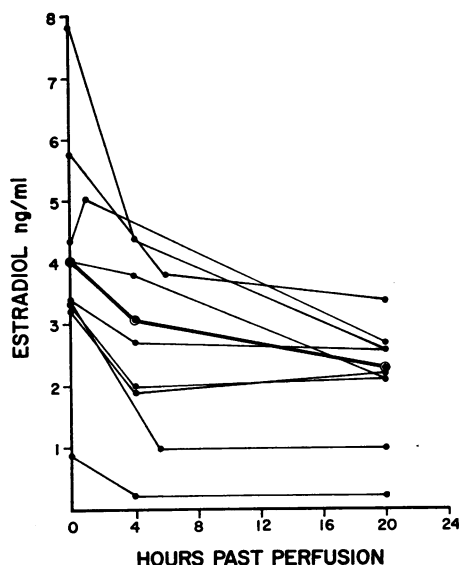


FIGURE 4 Plasma estradiol concentrations during therapeutic abortion, induced by perfusion with hypertonic saline. The thick solid line indicates the mean plasma estradiol concentration.

Patient 14 was a 22 yr old, primigravida, admitted at 26–27 wk of gestation for preeclampsia and fetal growth retardation. She had 3+ proteinuria and a blood pressure of 160/100. The uterus was the size of a 22-wk gestation and fetal heart tones were present. Her blood pressure was controlled with hydralazine over the next 4 wk but no increase in uterine size was noted. Fetal movements and then fetal heart tones ceased and the patient was delivered of an 1100 g macerated fetus by the instillation of hypertonic glucose intraamniotically. The placenta weighed 250 g and had multiple infarcts. The plasma E_2 hovered at the lower limit of normal for a 26 wk gestation and fell before fetal demise.

Patients with Rh isoimmunization disease

Patient Rh1 was a 26 yr old, gravida 3, para 2. Her plasma E_2 was abnormally high for her stage of gestation and continued to be elevated after fetal death.

Patient Rh2 was a 23 yr old, gravida 3, para 2, whose severe Rh isoimmunization disease necessitated intra-uterine fetal transfusion. Fetal death ensued and she went into spontaneous labor 1 day later. Her E_2 levels, initially elevated, fell only to the normal range after fetal demise.

Patient Rh3 was a 22 yr old, gravida 3, para 2, who presented at 30 wk of gestation with evidence of intra-uterine fetal demise. Her initial E_2 was in the normal range and although it fell significantly 1 wk later it remained in the normal range despite unequivocal evidence for fetal demise.

Measurement of the plasma E_2 would have been of assistance in predicting imminent fetal demise in cases 1, 2, 11, 12, 13, and 14. In patients 4, 5, and 6, the clinical presentation was too late for fetal salvage. The value in patient 7, was clearly misleading and in 9, a normal plasma E_2 concentration failed to reveal imminent fetal demise or placental infarction. In the three cases of Rh isoimmunization disease, E_2 levels were elevated or normal even in the face of fetal demise.

Induced abortions

The disappearance rate of E_2 was determined in nine patients at from 14 to 20 wk gestation who underwent therapeutic abortion by the infusion of hypertonic saline intraamniotically for 10 min. Blood was obtained before the procedure and at the times indicated in Fig. 4. The mean initial plasma E_2 was 4.0 ng/ml. By 6 hr, 6 of the patients had attained their maximum fall. At 20 hr, before delivery, the mean value had fallen only to 2.2 ng/ml despite the fact that delivery of a stillborn fetus ensued shortly thereafter. The low values of E_2 occurred in a 22 yr old gravida, 2 para 1 with scleroderma, who had been receiving 20 mg of prednisone throughout

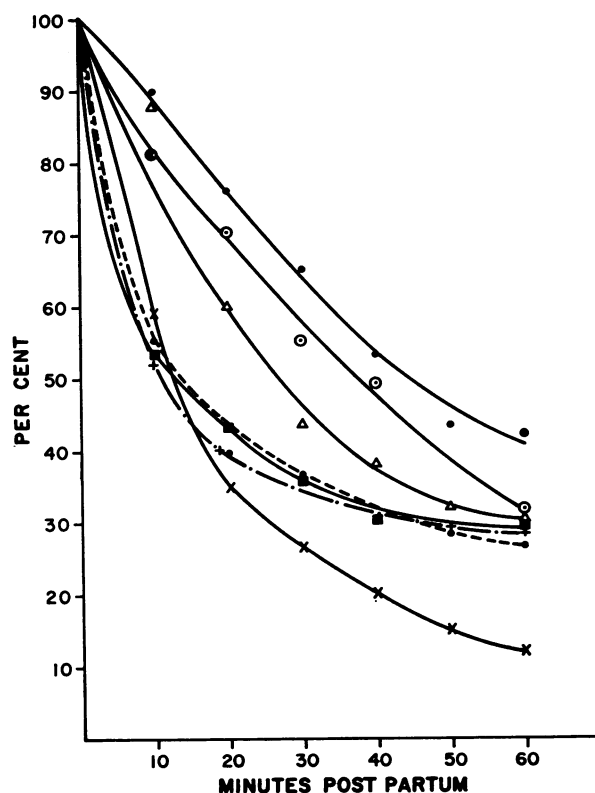


FIGURE 5 Plasma levels of E_2 during the 1st hr postpartum period. Per cent refers to the prepartum concentration of E_2 . Delivery was accomplished in each of these patients in less than 10 min.

pregnancy. Her initial value of 800 pg/ml fell to 200 pg/ml, a value in the nonpregnant range.

Postpartum E_2 clearance

In order to study the disappearance rate of E_2 from the blood, we selected four volunteers who had had an uneventful labor and delivery, and who had not received lactation suppressants. The initial blood sample was drawn upon delivery of the baby's head and subsequently in frequency ranging from 10 min in the first postpartum hr to 6 hr on the 3rd postpartum day. During the first 24 hr, blood was drawn through a heparinized catheter which was removed thereafter and the total amount of blood obtained from each patient did not exceed 75 ml. From three other patients, blood samples were drawn in the 1st postpartum hr only. Sensitivity of the assay system was adapted to the anticipated concentration of E_2 . After 3 hr each sample was analyzed by the method for nonpregnant individuals previously described.

The results for the 1st hr are depicted in Fig. 5. The data are presented as per cent of the initial concentration. The mean initial half-life of the hormone was 22 min. Four of the seven had initial E_2 half-lives of 13 min and the other three 27, 30, and 45 min respectively. Subsequent determinations are presented in Fig. 6. A second exponential decay with half-lives of 6, 6, 6, and 8 hr was apparent. Normal early follicular phase values for E_2 of under 100 pg/ml were reached in each case by 35 hr postpartum.

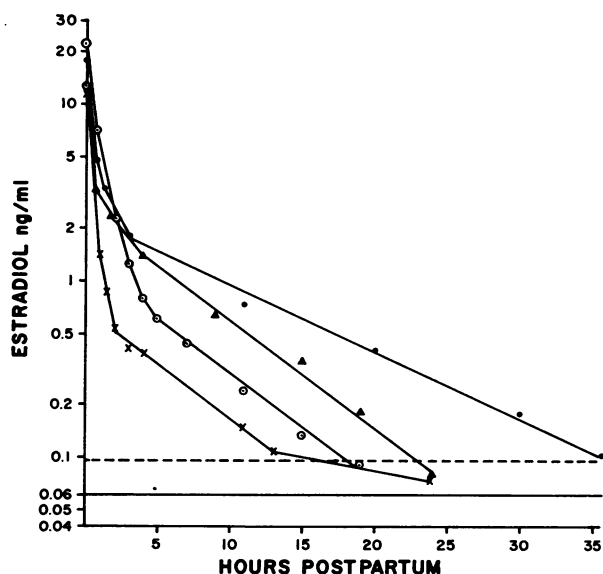


FIGURE 6 Plasma levels of E_2 during the immediate postpartum period. The solid, horizontal line represents the mean early follicular phase plasma concentration of estradiol. The dashed line represents 2 sd above the mean.

DISCUSSION

We have exploited the intracellular estrogen receptor characteristic of target tissues for steroid hormones as specific binding reagent for unconjugated estrogen assay in male and female blood plasma (13–16). During human pregnancy, there are markedly elevated concentrations of unconjugated estrogens present which made it possible for us to simplify the assay procedure by elimination of the extraction and chromatographic steps. This reduced the time required for the assay to 3 hr and increased the number of samples, which could be processed enormously, while reducing costs substantially. We were able to show that neither the numerical value nor the precision of the assay was affected by the simplification, also ensuring that we were not measuring estrogen conjugates. Since we could use up to 0.02 ml of plasma, the lower limit of sensitivity of the assay was 1 ng/ml. In the last trimester of pregnancy the vast bulk of determinations were over 8 ng/ml (Fig. 1) and the coefficient of variation of replicates was < 10% at this concentration or above.

We found that the mean plasma E_2 concentration rose at a fairly constant rate during pregnancy until the 35th wk when the rate of increment decreased. Values at term tended to become more variable ranging from 5.5 to 50 ng/ml (in one instance). These results confirmed previous reports (20–22) using entirely different assay methods; the validity of our procedure was hence indirectly supported. A declining rate of increase in late pregnancy has been shown to be characteristic of the other principal placental hormones progesterone (23, 24) and chorionic somatomammotropin (8, 25).

Of note is the fact that in studies of normal pregnancy, from the 32nd wk no E_2 level below 5.5 ng/ml was found (Fig. 1) and no significant decline occurred during the course of gestation (Fig. 2). By contrast, in each abnormal pregnancy followed serially (Fig. 3), except in Rh isoimmunization disease, falling E_2 levels and abnormally low concentrations were found, suggesting that particularly in cases of placental insufficiency, following the plasma E_2 may be of clinical assistance. Unfortunately we had no diabetic pregnancies.

The high E_2 concentrations persisting after evidence of fetal demise in Rh isoimmunization disease (Fig. 3) are consistent with values obtained for plasma (7), and urinary estriol (25–28), as well as chorionic somatomammotropin (25), when these assays were used to evaluate fetal well being. It is generally accepted that the placenta is hypertrophied in both Rh isoimmunization disease and many diabetic pregnancies, resulting in persistence of normal hormone values despite clear evidence of prior fetal demise. In cases of placental insufficiency however, the plasma E_2 assay provides as good

evidence of the clinical state as any other hormone measurement.

Estimation of the clinical usefulness of any of the hormonal parameters of fetoplacental well being is difficult because the clinical setting in which the assays are performed is of importance. Under conditions of nearly continuous fetal monitoring, clinical evaluation may be more satisfactory than any test with a time delay. However, as previously emphasized (7, 27-29) a fall of serially obtained urinary or plasma E_2 concentrations strongly suggested imminent fetal distress so that significant fetal salvage might be anticipated by appropriate follow-up procedures in general prenatal-care clinics or particularly in high-risk clinics. Our data indicate that measurement of the plasma E_2 can give similar if not superior information. Therefore it is suggested that this rapid, simple, and inexpensive assay may be of considerable help in identifying and following patients suspected of placental insufficiency.

In study of induced abortion at 14-20 wk gestation, the fall in E_2 concentration after fetal death, which presumably represents the fetal contribution, averaged only 40% (Fig. 4). The persistently high plasma concentrations at 20 hr were probably due to a continued supply of precursors by maternal adrenals and the presence of an intact placenta. Persistence of the plasma progesterone (30) and human chorionic gonadotropin (HCG) (8) under similar conditions attests to the viability of the placenta and supports the quantitative contribution of adrenal sources to E_2 secretion at this time of pregnancy. The patient with suppressed adrenals had a fall of E_2 to 200 pg/ml. The source of this concentration of E_2 , a normal luteal phase level, was not clear but presumably ovarian and fetal adrenal sources may contribute.

The half-life of unconjugated E_2 has been reported to be about 20 min based on studies measuring the disappearance rate of intravenous tracers in nonpregnant women (31). We have taken a different approach and measured the disappearance of endogenous hormone from the blood postpartum. Assuming a linear initial disappearance, the mean first half-times of the hormone was found to be 22 min with a range of from 13 to 45 min (Fig. 5). In the four cases, followed for more than 1 hr (Fig. 6), a two compartment model can be shown to characterize the vast bulk of E_2 clearance, with a mean second $t_{1/2}$ of 6-7 hr and a range of from 6 to 8 hr. The E_2 concentration reached follicular phase levels by 14-35 hr, similar to the fall of progesterone (23, 24) at a time when plasma HCG levels remained quite high (32, 33). These data directly support the view that HCG does not stimulate estrogen secretion by the ovary of pregnancy. Previous studies (22), though confirmatory, were not sensitive enough to delineate the

lower portion of the curve. The physiological significance of these two compartments is not known.

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REFERENCES

1. Spielman, F., M. A. Goldberger, and R. T. Frank. 1933. Hormone diagnosis of viability of pregnancy. *J. Amer. Med. Ass.* **101**: 266.
2. Kloppe, A. 1959. The effect of oestriol on plasma water content. *J. Obstet. Gynaecol. Brit. Commonw.* **66**: 822.
3. Dickey, R. P. 1966. Effect of posture on estrogen excretion during pregnancy. *Amer. J. Obstet. Gynecol.* **96**: 127.
4. Timonen, S., E. Hirvonen, and R. Sokkanen. 1965. Urinary volume and excretion of estrogens in late pregnancy. *Acta Endocrinol.* **49**: 393.
5. Eraz, J., and R. Hausknecht. 1969. Diminished urinary estriol due to mandelamine administration during pregnancy. *Amer. J. Obstet. Gynecol.* **104**: 924.
6. Nachtigall, L., M. Bassett, U. Hogsander, S. Slagle, and M. Levitz. 1966. A rapid method for the assay of plasma estriol in pregnancy. *J. Clin. Endocrinol. Metab.* **26**: 941.
7. Ratansopa, V., A. E. Schindler, T. Y. Lee, and W. L. Herrmann. 1967. Measurement of estriol in plasma by gas-liquid chromatography. *Amer. J. Obstet. Gynecol.* **99**: 295.
8. Saxena, B. N., N. K. Emerson, Jr., and H. A. Selenkow. 1969. Serum placental lactogen levels as an index of placental function. *N. Engl. J. Med.* **281**: 225.
9. Naguib, A. S., J. T. Bradbury, and C. P. Goplerud. 1969. Serial hormonal studies in normal and abnormal pregnancy. *Amer. J. Obstet. Gynecol.* **104**: 781.
10. Nicoloff, J. T., H. A. Gross, D. W. Warren, J. H. Mestman, and G. V. Anderson. 1970. Thyroxine binding globulin values as a measure of placental adequacy. *Obstet. Gynecol.* **35**: 191.
11. Diamant, Y. Z., H. Zuckerman, E. Sadovsky, and W. Z. Polishuk. 1970. Leukocyte alkaline phosphatase in cases of bleeding in the first half of pregnancy. *Amer. J. Obstet. Gynecol.* **106**: 872.
12. Southren, A. L. 1970. Plasma diamine oxidase in pregnancy complicated by age and weight factors. *Amer. J. Obstet. Gynecol.* **106**: 607.
13. Korenman, S. G. 1968. Radioligand binding assay of specific estrogens using a soluble uterine macromolecule. *J. Clin. Endocrinol. Metab.* **28**: 127.
14. Korenman, S. G., L. E. Perrin, and T. P. McCallum. 1969. A radioligand binding assay system for estradiol measurement in human plasma. *J. Clin. Endocrinol. Metab.* **29**: 879.
15. Korenman, S. G., D. Tulchinsky, and L. William Eaton. 1970. Radioligand procedures for estrogen assay in normal and pregnancy plasma. Second Karolinska Symposium on Research Methods in Reproductive Endocrinology, *Acta Endocrinol. Suppl.* **147**: 291.

16. Tulchinsky, D., and S. G. Korenman. 1970. A radio-ligand assay for plasma estrone; normal values and variations during the menstrual cycle. *J. Clin. Endocrinol. Metab.* 31: 76.
17. Korenman, S. G. 1969. Comparative binding affinity of estrogens and its relation to estrogenic potency. *Steroids.* 13: 163.
18. Baird, D. T., and A. Guevara. 1969. Concentration of unconjugated estrone and estradiol in peripheral plasma in non-pregnant women throughout the menstrual cycle, castrate, and post-menopausal women and in men. *J. Clin. Endocrinol. Metab.* 29: 149.
19. Korenman, S. G. 1970. The relation between estrogen inhibitory activity and binding to cytosol of rabbit and human uterus. *Endocrinology.* 87: 1119.
20. Svendsen, R., and B. Sorensen. 1964. The concentration of unconjugated oestrone and 17β -oestradiol in plasma during pregnancy. *Acta Endocrinol.* 47: 237.
21. Smith, O. W. 1966. Free and conjugated estrogens in blood and urine before and during parturition in normal human pregnancy. *Acta Endocrinol.* 104: 1.
22. Munson, A. K., J. R. Mueller, and M. E. Yannone. 1970. Free plasma 17β -estradiol in normal pregnancy, labor, and the puerperium. *Amer. J. Obstet. Gynecol.* 108: 340.
23. Llauro, J. L., B. Runnebaum, and J. Zander. 1968. Progesterone in human peripheral blood before, during and after labor. *Amer. J. Obstet. Gynecol.* 101: 861.
24. Yannone, M. E., J. R. Mueller, and R. H. Osborn. 1969. Protein binding of progesterone in the peripheral plasma during pregnancy and labor. *Steroids.* 13: 773.
25. Samaan, N. A., J. T. Bradbury, and C. P. Goplerud. 1969. Serial hormonal studies in normal and abnormal pregnancy. *Amer. J. Obstet. Gynecol.* 104: 781.
26. Scommegna, A., S. C. Chattoraj. 1967. Gas chromatographic estimation of urinary estriol in pregnancy. *Amer. J. Obstet. Gynecol.* 99: 1087.
27. Heys, R. F., J. S. Scott, R. E. Oakey, and S. R. Stitch. 1968. Urinary oestrogen in late pregnancy. Oestriol excretion as a guide to impending fetal death before term. *Lancet.* 1: 328.
28. Magendantz, H. G., D. Klausner, K. J. Ryan, and S. S. C. Yen. 1968. Estriol determinations in the management of high risk pregnancies. *Obstet. Gynecol.* 32: 610.
29. Beischer, N. A., J. B. Brown, and C. Wood. 1968. The value of urinary estriol measurements during pregnancy. *Obstet. Gynecol. Digest.* 10: 37.
30. Short, R. V., G. Wagner, A. R. Fuchs, and F. Fuchs. 1965. Progesterone concentrations in uterine venous blood after intra-amniotic injection of hypertonic saline in midpregnancy. *Amer. J. Obstet. Gynecol.* 91: 132.
31. Sandberg, A. A., and W. R. Slaunwhite, Jr. 1957. Studies on phenolic steroids in human subjects. II. The metabolic fate and hepato-biliary enteric circulation of esterone- C^{14} and estradiol- C^{14} in women. *J. Clin. Invest.* 36: 1266.
32. Yen, S. C., O. Llerena, B. Little, and O. H. Pearson. 1968. Disappearance rates of endogenous luteinizing hormone and chorionic gonadotropin in men. *J. Clin. Endocrinol. Metab.* 28: 1763.
33. Jaffe, R. B., P. A. Lee, and A. R. Midgley. 1969. Serum gonadotropins before, at the inception of, and following human pregnancy. *J. Clin. Endocrinol. Metab.* 29: 1281.