

Urinary Estriol Determinations in Normal and Pathological Pregnancies*

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Estriol excretion was studied in 216 normal and 61 pathologic pregnancies. The 95 % fiducial limits of the normal excretion of estriol, within which 95% out of 100 future determinations in normal pregnancies are expected to fall, were established. The estriol curve in normal pregnancy in this study agrees well in its general shape with those presented by previous investigators who used different chemical methods of determination.

The estriol values in pathologic pregnancies with preeclampsia, intrauterine fetal death and antepartum hemorrhage have been analyzed.

The clinical significance of estriol determinations during pregnancy was discussed.

During the normal pregnancy, the urinary excretion of estrogens increases enormously in comparison with the nonpregnant state, but the striking features of this increase is the great preponderance of estriol, compared with estrone and estradiol. The critical role of the fetus in the increased estrogen production was first shown by Cassmer (1959) who found that after inducing fetal death in utero, the maternal pregnanediol excretion fell slightly but the estrogen excretion showed a marked decrease. It is now known that while both the placenta and fetus work together in the metabolism of steroid hormones in human pregnancy, the fetus plays a specific and essential

role in the synthesis of estriol (Diczfalusy, 1969; Diczfalussy and Mancuso, 1969).

Estriol assay is mainly of value to assess the function of the placenta and the growth and well-being of the fetus. Since the publication of the basic principles of estrogen assays in the form of Kober chromogen (Brown, 1955), a great number of modified methods have been described for the clinical routine estimation of estriol (Frandsen and Stakemann, 1960; Greene et al., 1961; Taylor et al., 1961; Beling, 1963; Brown and Coyle, 1963). Most of the methods are, however, either laborious and time consuming, or of insufficient specificity. Urinary estriol assays are of great value to the clinician in monitoring the condition of the "intrauterine patient": however the value of such assays is considerably diminished if the results of such assays are not available to the clinician within a few

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hours and if the patients can not be followed by serial (preferably daily) estriol assay.

In this communication a method has been employed, which yields reliable estimates of estriol in pregnancy urine specimens containing at least 2.0 ug per 24 hours. The method which has been evolved from that described by Beling (1967) is so simple and rapid that the average technician can complete the analysis of 24 urine specimens within 5 hours. A prerequisite for the evaluation of estriol excretion values found in pathologic conditions is a thorough knowledge of the normal excretion values and the variations in the excretion encountered in normal pregnancy. The purpose of the present study was to establish normal values for the excretion of estriol during pregnancy and to illustrate the clinical usefulness of the method in a few cases of pathological pregnancy.

MATERIALS AND METHOD

Materials

Urinary estriol excretion was studied in Korean women during the second and third trimester of their pregnancies at the Department of Obstetrics and Gynecology, Yonsei University College of Medicine between 1976 and 1977. In order to obtain the variance of the excretion of estriol during normal pregnancy, 216 normal subjects have been evaluated. The clinical material of pregnancy consists of 31 preeclampsia cases, 16 intrauterine fetal deaths and 14 antepartum hemorrhages.

All solvents were analytical grade and were redistilled before use. Standard estriol was a gift from the National Institute of Health, U.S.A. Ethyl ether (Mallinckrodt Co.) was peroxide free. Carbonate buffer of pH 10.4 was prepared according to Brown (1955) by adding 5 N sodium hydroxide (150 ml) to 1 M sodium bicarbonate solution (1000 ml). The hydro-

quinone sulphuric acid reagent was prepared by adding concentrated sulphuric acid (790 ml) to distilled water (210 ml). The final volume was made up to 1000 ml and 20 g of hydroquinone (Sigma Co.) was added.

Method

Acid hydrolysis: An aliquot of 4 ml of filtered urine from a carefully mixed 24-hour specimen is pipetted into a glass-stoppered tube (150×22 mm). Following the addition of 0.6 ml of concentrated hydrochloric acid the sample is incubated at 110°C for 1 hour.

Extraction: After cooling in ice water, 5 ml of distilled water is added to the hydrolyzed urine and estrogens are extracted with 10 ml of ethyl ether by shaking. Following the separation of the organic and aqueous phases, the ether is transferred to another clean tube by means of a syringe pipette. The urine specimens are extracted with another aliquot of 10 ml of ether, and the ether is transferred to the clean tube. For the purification of ether extracts, 6 ml of carbonate buffer (pH=10.4) is then added to a clean tube with ether extract and is thoroughly shaken for a minute. After the complete separation of the two layers, the lower layer (aqueous phase) is removed by suction and discarded. The organic phase is shaken with 5 ml of distilled water for a minute. After 10 minutes standing at room temperature the lower layer (aqueous phase) is removed by suction and discarded. In order to separate estriol from estrone and estradiol, 1.5 ml of NaOH (2 N) is then added to the ether extract and is thoroughly shaken for a minute. After the complete separation of the two layers, the upper phase (organic phase) is discarded. Six ml of sodium bicarbonate buffer and 15 ml of ether are added to the aqueous phase and shaken for a minute. Following the separation of the organic and aqueous phase, the ether is

transferred to another clean tube by means of a syringe pipette. The ether is carefully evaporated to dryness under a stream of nitrogen gas in a water bath at 40°C.

Color development and colorimetry: The color reaction was performed as described by Nocke (1961). To each tube, including urine samples, one water blank and 5 concentrations of estriol standard, 2.1 ml of hydroquinone sulphuric acid reagent is added and tubes are heated in a boiling water bath for exactly 20 minutes. In order to ensure proper mixing, the samples must be shaken a couple of times during the first 5 minutes of boiling. Following careful cooling, 1.1 ml of distilled water is added to each tube by gentle shaking and the tubes are heated for a second time in a boiling water bath for 14 minutes. Following cooling in ice water the optical density is measured in a spectrophotometer (Spectronic 88, Baush and Lomb) at wavelengths 464, 510 and 556 mμ, and the corrected optical density is calculated according to the formula of Allen (1950); as modified by Brown (1955). Whenever a new batch of freshly prepared hydroquinone sulphuric acid reagent was used, it was checked by preparing a new calibration curve.

RESULTS

The estriol values of 302 determinations in 216 normal pregnancies are shown in Table 1. Pregnancies, which showed no clinical abnormalities and resulted in the delivery of healthy infants, were included. The values are expressed as mg estriol per 24 hour urine specimen. The length of pregnancy is expressed as weeks after the last menstrual period.

When the frequency distribution of the individual excretion values was assessed it was found that the logarithms of the individual values rather than the values themselves were

normally distributed. However, in order to facilitate the rapid assessment of individual excretion values, the 95% fiducial limits of the normal excretion of estriol are shown on an

Table 1. Estriol values in 216 normal pregnancies

Weeks of pregnancy	No. of determinations	Mean estriol mg/24 hour	95% fiducial limits
25	10	6.7	3.4~10
26	11	7.3	3.8~10.8
27	21	8.0	4.0~12.0
28	16	8.6	4.4~12.8
29	13	9.4	4.8~13.2
30	19	10.2	5.2~14.4
31	20	10.9	5.6~16.2
32	17	11.4	5.8~17.0
33	27	12.1	6.0~18.2
34	16	12.3	6.8~19.0
35	21	15.2	7.2~21.4
36	19	15.8	7.8~22.6
37	22	16.8	8.2~25.4
38	29	18.1	9.0~27.2
39	17	20.0	9.8~30.2
40	24	22.4	10.4~34.4

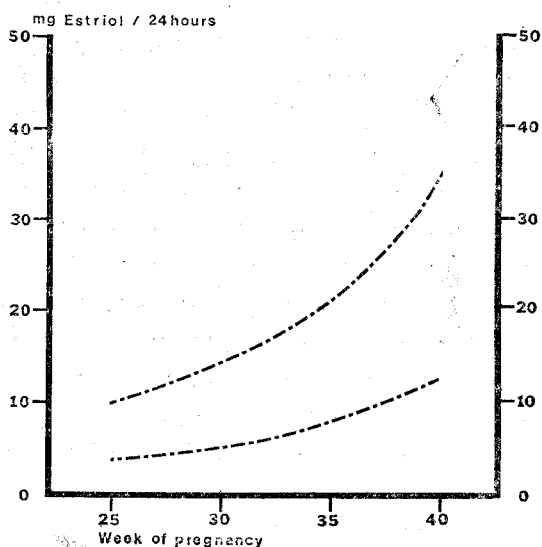


Fig. 1. 95% fiducial limits (dotted lines) of estriol excretion based on 302 determinations in 216 normal pregnancies.

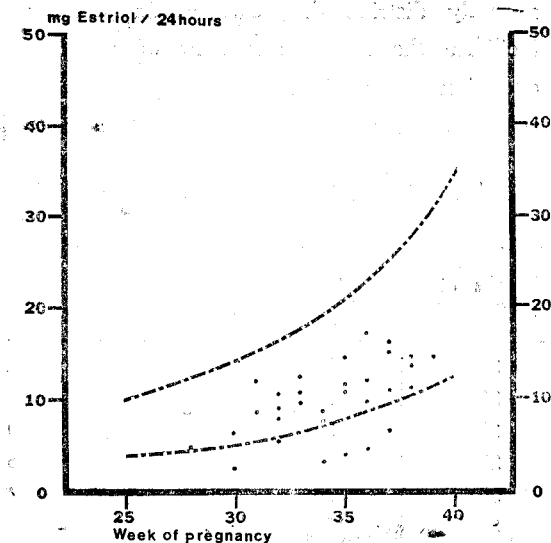


Fig. 2. The values of estriol determinations in 31 pregnancies complicated by preeclampsia. Dotted lines: 95% fiducial limits of the estriol excretion in normal pregnancy.

arithmatic scale in Figure 1. These limits, within which 95 out of 100 future determinations in normal pregnancies are expected to fall, are shown in Figure 1.

The estriol values in cases with preeclampsia are plotted in Figure 2. In general the estriol excretion was slightly depressed. Only 2 out of 31 showed higher values than mean values. Six out of the 31 patients exhibited lower values of estriol than the 95% fiducial limits. After abortion, it was shown that the estriol excretion in two cases was associated with an intrauterine fetal death (arrows indicated).

The estriol values in 14 patients having episodes of minor antepartum bleeding are plotted in Figure 3. No estriol value lower than the 95% fiducial limits was obtained.

The estriol excretion was studied in 16 patients diagnosed as intrauterine fetal death (Figure 4). All patients showed low estriol values below the lower limit of the normal curve suggesting that low estriol value is related to an intrauterine fetal death.

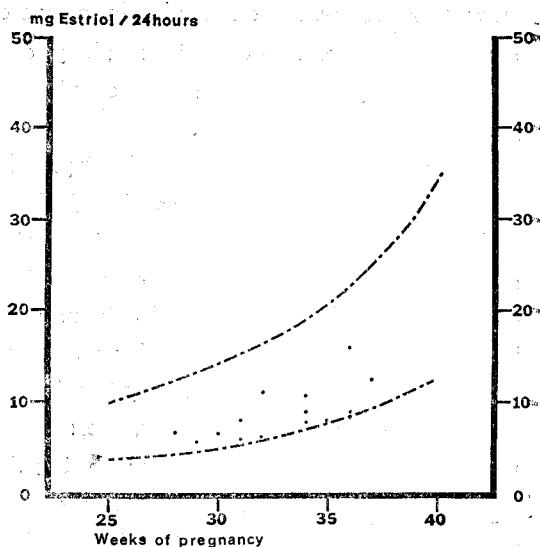


Fig. 3. Estriol determination in 14 cases of pregnancies with antepartum hemorrhage. Dotted lines: 95% fiducial limits of the estriol excretion in normal pregnancy.

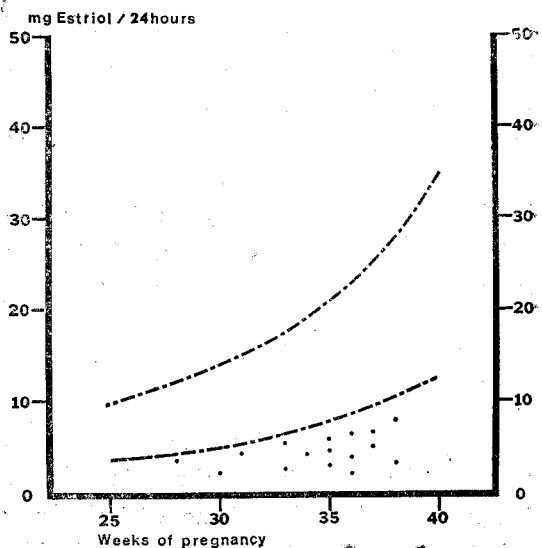


Fig. 4. The values of estriol determinations in 16 pregnancies complicated by intrauterine fetal death. Dotted lines: 95% fiducial limits of the estriol excretion in normal pregnancy.

DISCUSSION

The estriol curve in normal pregnancy presented in this investigation agrees well in its general shape with those presented by previous

investigators (Brown, 1956; Greene et al., 1961 and Beling, 1963) who used different chemical methods of determination.

It appears from Figure 1 that there is a considerable variation in the amount of estriol excreted during normal pregnancy. When urinary estriol excretion studies in normal pregnancies are performed, one must keep in mind other factors which possibly influence the range of values obtained: (a) the reliability of using a menstrual history estimate of gestational age, (b) the effect of work and posture of pregnant women, (c) technic of urine collection and (d) interference of certain drugs. It is difficult to get an indication of true postconceptional age. Problems are involved in the use of a menstrual history estimate of gestational age. Though in the present series all the women were sure of the date of the last menstrual period, the estimated gestational age can not be claimed to be reliable. Estriol excretion during pregnancy is found to increase by bed rest (Dickay et al., 1966; Courey et al., 1970; Wyss, 1970). The effect of posture was observed in women near term without complications of pregnancy as well as in women in high risk pregnancy (Courey et al., 1970). There is good reason to believe that rest in bed ensures that the blood flow to the myometrium and placenta is maintained at a maximum, since during exercise there is probably at least a temporary diversion of blood flow away from the uterus (Morris et al., 1956). Morris and Smalley (1967), however, found no difference between the estriol output in two groups of women; one of which was at moderate rest, the other was at total rest. The estriol excretion was in the present series determined from urine specimens collected during a normal amount of activity. The difficulty of collecting complete 24 hour urine specimens has been recognized. In our obstetric

unit the collection was controlled by well trained nurses. In order to confirm the completeness of 24 hour collections, creatine was measured in urine specimens. Urine samples with a value less than 800 mg creatine per 24 hour were omitted. In view of the known wide variation in the rate of creatine excretion in pregnant women the estriol was expressed in absolute values in mg/24 hour instead of expressing by reference to any level of creatine excretion. Various drugs given to the mother may influence the estriol output. The medical staff was informed of the interference of certain drugs on the estriol excretion. It is therefore unlikely that there was any drug effect to invalidate results. Beling (1963) suggested that the spreading of the value is mainly due to variation between the patients, and that the estriol level is directly correlated with the fetal weight. However, Kloppe and Billewicz (1963) and Morris and Smalley (1967) could not establish a relationship in normal pregnancy between fetal weight and estriol excretion. Wide ranges of estriol values may also be due to a day to day variation in the estriol excretion. In 14 normal pregnancies this variation was studied and in 2 cases the day to day variation was in the range of 40~50%, 4 cases had a 20~40% variation and in 8 cases the values differed less than 20% from day to day (unpublished observation).

Fetal mortality becomes high in cases of preeclampsia where the onset of symptoms occurs before the 32nd week. For a pregnant patient to excrete normal amounts of estrogen, the following steps appear to be necessary: (a) the fetus must synthesize precursors in adequate amounts; (b) the placenta must convert these precursors to estrogen at a normal rate; (c) estrogen must gain access to maternal blood and be cleared by kidney at a normal rate. None of patients with preeclampsia in the

present series presented obvious signs of renal failure, and it may be presumed that the estriol excretion might express the function of the feto-placental unit. Most authors, like Wurtele (1962) and Furuhjelm (1962) found no great change from the normal urinary estriol excretion in mild preeclampsia. Kloppe (1965) calculated the estriol excretion in various categories of toxemia. The reduction in mild toxemia was slight, averaging 85% of normal, while in severe toxemia the average was 51% of normal. In the present study, estriol values in cases of preeclampsia were slightly depressed, but some patients had a normal estriol level. It is possible that the estriol excretion may remain normal in preeclampsia of short duration when the fetal growth and development are almost completed. No certain prediction can, however, be made as to the outcome of pregnancy in cases of preeclampsia with normal levels of estriol in our study since no serial determinations were performed on patients.

In cases of antepartum hemorrhage, Beischer et al. (1967) found that estriol levels were of value in differentiating cases in which pregnancy was progressing normally from those in which the fetus was in jeopardy. Beischer et al. (1970), underlined the fact that normal urinary estriol values provide no assurance against imminent placental abruption.

It was found by many authors that intrauterine fetal death is associated with a decreased estriol excretion and that therefore estriol determinations are of diagnostic value in threatened abortions (Beling, 1963; Frandsen and Stakemann, 1963; Kloppe, 1969). In the present investigation, pathologically low estriol excretion below the lower fiducial limit of the normal curve suggests very strongly an intrauterine fetal death (Figure 4).

The present investigation was carried out in

order to collect some experience in the field of clinical application of estriol determination. The number of patients, however, is small and serial daily determinations in pathological pregnancies to realize the potential of estriol determination were not performed. All workers are agreed that the single level of estriol might not be a useful indicator to diagnosis and management. Therefore, a meaningful evaluation of estriol determinations in the management of patients with pathologic pregnancy can not be reached. A prospective study of feto-placental function in a large number of patients would be required to determine how often low levels of estriol excretion precede placental insufficiency.

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