

URINARY OESTRIOL AS AN INDEX OF PLACENTAL FUNCTION*

by

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Introduction

Since placental insufficiency has established itself as a definite entity in modern obstetric practice, it was natural for obstetricians all over the world to evolve methods which could be used to assess it. Various techniques have been employed from time to time but the problem largely remains unsettled. Urinary oestrogen assay has been employed increasingly in recent times for this purpose. Zondek (1957, 1959), Taylor *et al.* (1958, 1961 and 1962) Frandsen and Stakemann (1960), ten Berge (1959, 1960), Keller *et al.* (1959), Lenters (1958), Greene *et al.* (1961, 1962, 1963, Banerjee (1962), Coyle *et al.* (1963), Wray and Russel (1963).

The authors set out to determine the reliability of assessing placental function by urinary oestriol assay. Placenta is known to produce large quantities of oestrogens during preg-

nancy and the pattern of oestrogen fractions in the placenta is characterised by the predominance of oestriol. Diczfalusy, (1953); Mitchell and Davies, (1954); Diczfalusy and Lindkvist (1956). About 90% of the oestrogen excreted in the urine during later stages of gestation is in the form of sodium oestriol glucosiduronate (Cohen *et al.* 1935, Doisy, 1931). Thus oestriol is considered as the specific oestrogenic hormone of pregnancy.

Cassmer (1959) showed that with the placenta and foetus left in situ, there is an abrupt fall in maternal urinary oestriol excretion when foetal circulation is interrupted. Perfusion of the foetal side of placenta, even after removal of the foetus, maintains maternal urinary oestriol at its normal levels. It would appear from these studies that the maternal urinary oestriol excretion is dependent upon a properly functioning foeto-placental unit.

Method

Various chemical methods have been employed for the estimation of urinary oestrogens. The authors were interested in estimating urinary oestriol by a method which is expected to be used as an aid to clinical

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Received for publication on 8-6-66.

obstetrics. It should be easy to perform, less time consuming and less expensive; the equipment necessary should be within the reach of a clinical laboratory attached to an obstetric service. Besides, it should be reliable and the results reproducible. In this study oestriol was estimated by the method of Eberlein, Bogiovanni and Francis (1958) with certain modifications suggested by Greene *et al.* (1963) and also minor modifications introduced by the authors. From our experience it is concluded that the method fulfils the conditions laid down above and a trained technician can tackle 6 to 8 samples in 4 to 6 hours. The recovery rate from distilled water and also from hydrolysed male urine was $94 \pm 6\%$.

The estimations were carried out from accurately collected 24 hours urine specimens and all the laboratory work was done by the first author himself.

Material

Oestriol assays were carried out in 136 patients involving 590 estimations. The cases were as under:

1. Normal pregnancy ..	58
2. Toxaemia of pregnancy & essential hypertension ..	25
3. Post-date pregnancy ..	10
4. Cases of unexplained foetal loss	6
5. Twin pregnancy ..	10
6. Non-toxaemic antepartum haemorrhage ..	5
7. Doubtful foetal viability	3
8. Suspected placental insufficiency	2
9. Threatened abortion ..	14
10. Miscellaneous ..	3

Results

I. Normal pregnancy

269 estimations were carried out in 58 cases of normal pregnancy at various periods of gestation. These women did not show any abnormality at any stage. The pattern of oestriol excretion in normal pregnancy as determined is shown in Fig. I.

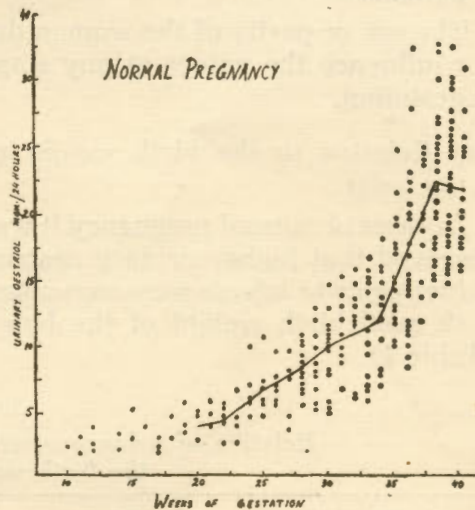


Fig. 1

Scatter graph of urinary oestriol values in normal pregnancy. The solid line represents the mean values.

The mean curve is generally a rising one from the beginning of pregnancy to term, the rise being rather steep after the 23rd week. It shows a maximum value at 38th week after which there is a plateau and no significant fall is observed prior to labour. There is a wide scatter of results which becomes more marked in the third trimester. The daily variation in excretion in the same patient is as much as 5-25%.

In spite of the wide scatter of results and the significant daily variation, the lower limits of normal preg-

nancy values have been determined in the present series. During 35th to 40th week no value fell below 8.5 mg. per 24 hours and only on two occasions was it below 10 mg. per 24 hours. Most of the values were over 15 mg. per 24 hours.

(a) *Relation to age and parity of the patient*

The age or parity of the woman did not influence the values at any stage of gestation.

(b) *Relation to the birth weight of the baby*

In cases of normal pregnancy it was observed that higher urinary oestriol values prior to labour were associated with good birth weight of the baby. (Table I).

values reported by certain Western workers.

II. *Toxaemia of pregnancy and essential hypertension*

Twenty-five cases belonged to this category and their distribution was as under:

(a) *Pre-eclamptic toxaemia*

The oestriol values in all cases were below the mean values observed in cases of normal pregnancy. (Fig. 2). The increase in oestriol excretion with advancing gestation was found to be lower as compared to normal pregnant women.

Two patients developed toxaemia at 37th and 38th week under antenatal care and remarkably enough their oestriol values showed a fall by

TABLE I

Relation of urinary oestriol value prior to labour with the birth weight of the baby

Weight of the baby at birth	Below 5 lbs.	5 to 6 lbs.	6-7 lbs.	Over 7 lbs.
Mean oestriol mg. per 24 hrs.	16.38 (4 cases)	20.32 (17 cases)	23.82 (10 cases)	29.89 (7 cases)

TABLE II

	No. of cases	No. of estimations
(a) Pre-eclamptic toxaemia	20	75
(b) Antepartum eclampsia	2	8
(c) Essential hypertension with pregnancy	2	6
(d) Essential hypertension with superadded toxaemia of pregnancy	1	8

It is worth noting that since most of the patients studied by us gave birth to babies weighing 5 to 7 lbs. the oestriol values obtained in this series of normal pregnant women are somewhat lower in comparison to the 34% and 40% prior to the clinical onset of toxaemia. Such a finding has been reported earlier by Keller *et al.* (1959) in one of their cases.

In 14 cases where the babies survived, all the oestriol values were

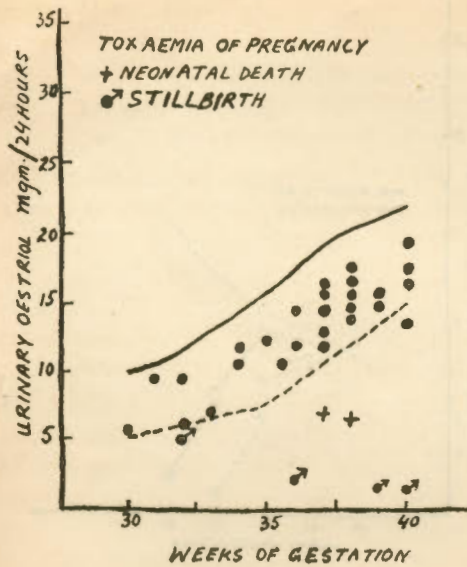


Fig. 2

Scatter graph of urinary oestriol values in cases of toxæmia of pregnancy (overlapping values omitted for clarity) Dotted line represents the lower limit of normal range and solid line the normal mean.

within the normal range.

Four patients had stillbirth after 36th week of gestation and in all of them the oestriol values prior to confinement were below 5 mg. per 24 hours. In 3 of these cases falling values were observed prior to labour. Two patients delivered small babies (4 lbs. and 4 lbs. 8 ozs) after 37th week of gestation and both babies died in the immediate neonatal period. Their oestriol values prior to confinement were 6.2 mg. per 24 hours and 7.1 mg. per 24 hours.

(b) Ante-partum eclampsia

One case was admitted at 34 weeks of gestation with eclampsia. The fits were soon controlled and the pregnancy was allowed to continue under close supervision. At 37th week she

delivered spontaneously a living child weighing 2 lbs. 14 ozs. who died after 2 days. The placenta was quite small for the period of gestation. The oestriol assays were done twice weekly and all the values were below the lower limits observed in normal pregnancy.

The second case was admitted with eclampsia at 38th week and she delivered after 2 days. The baby was 6 lbs. 10 ozs. in weight and died after 12 hours. Oestriol assays were done daily and the values were 7 mg. per 24 hours and 9.5 mg. per 24 hours.

(c) Essential hypertension with pregnancy

Two cases of essential hypertension with pregnancy showed oestriol values in the low normal range and in both cases the babies survived.

(d) Essential hypertension with superadded toxæmia of pregnancy

This patient, a known case of essential hypertension, came under our care at 22 weeks of pregnancy when she already had superadded toxæmia. The oestriol values till 34th week were in the low normal range. At 35th week it showed a fall by 45% and the value was 5 mg. per 24 hours. Three days later she gave birth to a fresh still-born baby after a relatively short labour.

III. Post-date pregnancy (10 cases, 60 determination)

Urinary oestriol assays were carried out in 10 cases of post-date pregnancy; 3 cases delivered at 41 weeks, 4 cases at 42 weeks, one case each at 43, 44 and 46 weeks, the last one was an anencephalic baby. The oestriol values started falling after 40

weeks but the fall was significant only after 42 weeks. In 9 cases, the oestriol value did not fall below 16 mg. per 24 hours, which is well above the lower limit observed in case of normal pregnancy, and all their babies survived. One patient who had a stillbirth at 42 weeks showed oestriol values of 5.83 mg. per 24 hours at 41 weeks and 4.38 mg. per 24 hours at 42 weeks.

IV. Cases of unexplained foetal loss (6 cases, 13 determinations)

(a) Stillbirths

There were 2 cases where the pregnancy was progressing normally but resulted in stillbirth at 39 and 40 weeks. Both these cases showed falling oestriol levels much below the lower limit observed in normal pregnancy. (Fig. 3).

(b) Neo-natal deaths

There were 4 patients where the pregnancy and labour were normal but the babies died within 48 hours of delivery without any obvious cause. Unfortunately, post-mortem examination could not be carried out on these babies. The babies were premature according to weight (less than 5 lbs.) but were mature according to the period of gestation. It is interesting to note that the urinary oestriol values of these patients prior to confinement were below the lower limit determined for normal pregnancy. (Fig. 3).

This is consistent with the possibility of placental insufficiency existing in these patients where premature babies were born after 37th week of gestation and they had early neonatal death.

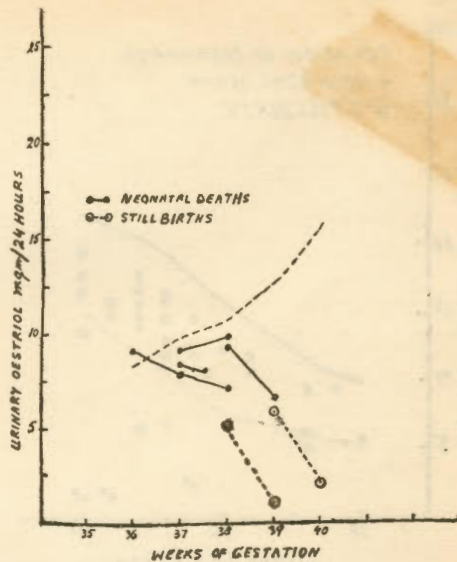


Fig. 3

Urinary oestriol values in cases of unexplained foetal loss. Dotted line represents the lower limit of normal range.

V. Twin pregnancy (10 cases, 52 determination).

Urinary oestriol assays were carried out in 10 cases of twin pregnancy, of which 3 cases were associated with toxæmia of pregnancy. Oestriol values were found to be higher in cases of twin pregnancy as compared to single pregnancy, the range in last 5 weeks of gestation being 14-41 mg. per 24 hours. The values in cases of twins with toxæmia were lower than those in twins without toxæmia, but even these were higher than normal single pregnancy values.

All the babies survived.

VI. Non-toxæmic antepartum hæmorrhage (5 cases, 16 determination)

Five such cases were studied in this

series. Extraplacental causes of bleeding were excluded. In one case placenta praevia was diagnosed at caesarean section and in the other 4 the aetiological factor could not be ascertained. The oestriol values were essentially normal and all the babies survived.

VII. *Doubtful foetal viability*

(3 cases, 7 determinations)

Three such cases were studied, two were at 30 weeks and one was at 28 weeks of gestation. These patients came as emergencies and their foetal heart sounds were doubtful. In two cases the oestriol values were below 1 mg. per 24 hours and they later delivered still-born macerated babies. In one case the oestriol values were within normal limits and at a later date the foetal heart sounds were located and she delivered a living baby at term.

VIII. *Suspected placental insufficiency* (2 cases, 11 determination)

Two cases were referred to us at 31 and 34 weeks of gestation as cases of placental insufficiency diagnosed clinically. In both cases the fundal height was less for the period of gestation and the obstetrician had an impression that the foetus was not growing with the advancing gestation. They were put on serial urinary oestriol assays which showed rising values within the normal range. Both of them delivered normally and the babies weighed 6 lbs. 2 ozs. and 9 lbs. In one case the pregnancy went to 47 weeks according to her dates, but the baby did not show any evidence of post-maturity. Presumably her dates were wrong.

IX. *Cases of threatened abortion* (14 cases, 49 determination)

Oestriol assays were carried out on 14 cases of threatened abortion put on conservative therapy. The pregnancy ranged from 7 to 24 weeks. In 7 cases rising oestriol values within the normal range were observed and their pregnancy continued. In the other 7 falling values were obtained and in some oestriol was undetectable by the present method. These patients eventually aborted.

X. *Miscellaneous*

(a) One case of threatened miscarriage carried to term. She was followed with serial urinary oestriol assay from 26th week of gestation. The oestriol values were essentially normal and she delivered a healthy baby at term.

(b) One case of diabetic pregnancy showed oestriol values within normal range. The baby survived.

(c) One case of anencephalic pregnancy showed normal oestriol values.

Discussion

The urinary oestriol values at different periods of gestation in cases of normal and abnormal pregnancy have been presented. It is difficult to compare the values obtained by different workers as their assay methods, their criteria for normal pregnancy and their experimental designs have been different, yet an attempt has been made to compare their results with those obtained in the present series (Table III).

The values obtained by the authors, using the method of Eberlein *et al.* (1958) with some modifications, are higher than those reported by Greene

TABLE III
 Urinary oestriol values in normal pregnancy
 (mean, mg. per 24 hours)

Authors	Method used	Period of gestation in weeks			
		20	30	36	40
1. Lenters (1958) ..	Flourimetric	—	14-35	—	19.40
2. Keller (1959) ..	Brown's (1956)	5	15	25	32
3. Greene et al (1961)	Flourimetric	—	1-9	—	12-22
4. Zondek (1960) ..	-do-	—	1-9	—	12-22
5. Frandsen & Stake- (1960)	Their own method	5	10	—	24
6. Hobkirk & Nilsen (1962)	Not stated	6.5	10	12.5	19.8
7. Greene et al (1963)	Modified Eberlein et al (1958)	2.5 to 3.5	3.7 to 10.5	7.0 to 22	11.5 to 26
8. Wray and Russel (1963)	Modified Brown's method (1957)	5	10	20	25
9. Present Authors ..	Modified Eberlein et al (1959)	4.37 ± 0.94	10.02 ± 3.78	17.41 ± 5.75	22.1 ± 4.16

et al. (1963) using the same method. This might be due to the higher recovery of $94 \pm 6\%$ obtained by the authors compared to the average recovery of $54 \pm 10\%$ reported by Greene *et al.* Nevertheless the pattern of oestriol excretion in normal pregnancy as reported by different authors is essentially the same. The normal range is wide and the day to day variation in the same patient may be as much as 5-25%. Great caution has to be exercised in evaluating the results and more than one determination is necessary before a low value is taken to imply that the foetus is in danger. There has been a significant relation in the oestriol value prior to labour and the birth weight of the baby. The same findings have been reported by Hobkirk and Nilsen (1962), Greene *et al.* (1963), Coyle and Brown (1963), while others have not corroborated

this finding (Klopper *et al.* 1963). Such correlation may be of assistance in deciding upon the optimum time for intervention in various complications of pregnancy.

In 98 patients where the oestriol values in last five weeks of gestation were over 8.5 mg. per 24 hours, all the babies survived except one who died in the immediate neonatal period. The value in this case was 10 mg. per 24 hour at 38th weeks.

In 14 cases where the value fell below 5 mg. per 24 hours, all of them had stillbirth and in three cases where the value was between 5 mg. per 24 hours and 8.5 mg. per 24 hours immediate neonatal death occurred without any obvious reason.

Here it needs to be cautioned that owing to the wide normal range and the significant daily variation, a single low value is not of much importance except as an indication to repeat the

assay. From this limited study it appears that any value which is falling, persistently low or shows a persistent drop from a previous high value should be viewed as suggestive of placental insufficiency and if otherwise feasible, early termination of the pregnancy may be desirable.

It is worth nothing that urinary oestriol assay may prove of value in cases of threatened abortion where one has to decide whether to adopt a conservative policy or to complete the process.

It is concluded that urinary oestriol assay would be of benefit in cases where placental insufficiency is suspected or anticipated. This laboratory aid along with the clinical criteria may solve the dilemma which the obstetrician has often to face in such conditions. Such investigations would presuppose, however, that the method of oestriol assay could be further simplified in order to make it widely acceptable.

Summary

1. Urinary oestriol values for normal pregnancy have been determined using the modified Eberlein *et al.* (1958) method. From the authors' experience the method is reliable and can be carried out in the laboratory attached to an obstetric unit.

2. The normal range of oestriol excretion is wide and the day to day variation is as much as 25%.

3. In patients where the oestriol values were above 8.5 mg. per 24 hours all the babies except one survived. Values below 5 mg. per 24 hours were associated with stillbirth and values between 5 mg. per 24 hours and 8.5 mg. per 24 hours sug-

gest foetal jeopardy.

4. Patients in whom placental insufficiency is suspected or anticipated may be kept on serial urinary oestriol assay and the best time for intervention decided. This would result in improved perinatal mortality.

Acknowledgements

I owe my gratitude to Dr. M. K. P. Amma, Lecturer in Biochemistry in our Institute, for her constant help and guidance in the laboratory. My thanks are due to the authorities of Institute of Postgraduate Medical Education and Research, Chandigarh for all the facilities afforded in carrying out this work.

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