# A RAPID AND INEXPENSIVE METHOD FOR THE ASSAY OF TOTAL URINARY ESTROGENS DURING PREGNANCY

## Part I

by

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Assessment of foetal condition in clinical practice is still a problem to obstetricians. Attempts were made to find out some criteria which could warn well ahead the impending foetal danger. The different methods of assessment of foetal condition and their merits and demerits have been discussed by Klopper (1969). The combined role of foetus and placenta in the excretion of estriol by the mother and different aspects of estrogen metabolism during pregnancy has also been discussed by Diczfalusy and Mancuso (1969). An evaluation of the role of measurement of hormone excretion in pregnancy was done by Macnaughton (1967). It is generally believed that serial estimation of estriol in the urine of the mother coupled with ultrasonic measurement of foetal head give best information about the foetal well being at the present moment. In absence of ultrasonic equipment estriol excretion values in the urine can be considered as a valuable guide.

Estriol assays in pregnant subjects may be prepared by the shorter methods than in the non-pregnant subject, because of larger amounts of estriol (1000 times or more at term) excreted during pregnancy. Again, since estriol comprises about 90-95 per cent of the total estrogen in pregnancy urine, total estrogen estimation is an alternate to estriol estimation and this procedure will also eliminate chromatographic procedures.

*Principle of the Test*

A milliliter aliquot of a 24 hour urine specimen from pregnant women is used for assay. The urine is hydrolysed with oxalic acid and the liberated estrogens (Estriol comprising 95 per cent of the total estrogen) are extracted with dichloromethane. An aliquot of the solvent extracted material is subjected to colour formation in the presence of 76 per cent sulphuric acid with 2 per cent resublimed hydroquinone followed by colour extraction with 2(N) trichloroacetic acid initially, and finally with pure distilled chloroform. The developed colour is then compared with known standard in B & L Spectronic 20, at three different wave lengths, making use of Allen correction to minimize the background for non-specific chromogens.

*Hydrolysis*: 1 ml. of urine is hydrolys-

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ed with 1 gm of oxalic acid and 200 mg. sodium chloride in a boiling water bath for 1 hour. It is then cooled to room temperature in an inclined position, when oxalic acid crystals are reformed on one side of the tube.

*Extraction*: 10 ml. of dichloromethane s added to the tube and extraction is completed by vigorously shaking the tube for 30 seconds. The tubes should be fitted with tight rubber caps. The rubber cap should be slowly released after the extraction is completed. The tube is centrifuged for five minutes at 2000 r.p.m. for better resolution of the two phases. Th urinary phase is discarded from the organic phase (the top urinary layer could be pipetted off). 5 ml. of the solvent extract is transferred to a tube containin 4 mg. of resublimed hydroquinone. The solvent is evaporated at 40°C by passing compressed air (nitrogen will be preferable).

*Colour development*: To each dried tube 1.5 ml colour reagent (76 per cent sulphuric acid and 2 per cent hydroquinone) is added and mixed. It is again boiled for twenty minutes mixing twice during the bath and 0.5 ml. of distilled water is added to the tube and replaced in the boiling bath after mixing. The tube is removed after 10 minutes and placed in a chilled ice bath.

*Colour extraction*: 1.2 ml. of 2(N) aqueous trichloro-acetic acid is added to each tube and is shaken 15 seconds and again transferred to the chilled ice bath for 2 mins. Next 5 ml. chloroform is added to each tube and the tubes are shaker gently for 15 seconds. The tubes are centrifuged for five minutes at 2000 r.p.m. The upper layer (aqueous phase) is discarded. The lower layer containing the chloroform extracted colour is transferred to the colorimetry tube and absorbance read at 504, 534 and 564 mμ. The corrected absorbance is calculated as follows:

Corrected absorbance =

Absorbance at 534 mμ minus half of the sum of absorbance at 504 and 564 mμ.

Calculation for 24 hour specimen:

$$\frac{\text{Corrected absorbance of unknown}}{\text{Corrected absorbance of standard}} = \frac{\text{Concentration of unknown}}{\text{Concentration of standard in } \mu \text{ gm/tube}}$$

$$\frac{\mu \text{ gm/tube} \times 2 \times \text{total volume}}{1000} \qquad \text{mg/total volume}$$

Recovery for added known

| | | | |
|---|---|---|---|
| added | 5 μ gm | average recovery | 50 percent |
| „ | 10 μ gm | -do- | 65 „ |
| „ | 20 μ gm | -do- | 66.5 „ |

Reproducibility

| | Mean | S.D. |
|---|---|---|
| 5 μg | 5.27 | ± 0.18 |
| 10 μg | 10.11 | ± 0.52 |
| 15 μg | 15.18 | ± 0.43 |
| 20 μg | 19.9 | ± 0.58 |
| 25 μg | 24.75 | ± 0.66 |

*Standard curve*: As shown in Fig. 1 the standard curve is linear between 5 and 25 microgram. If the load of work is too much the standard curve for a particular batch of reagent could also be used for calculation instead of running different strength of the standard everytime.

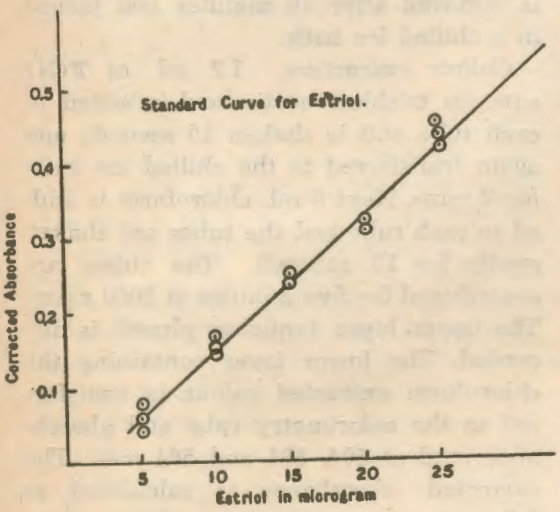


Fig. 1

The basis of chemical assay for estrogen utilises the reagent of Kober. The initial technique was modified in subsequent years by Brown (1955), which offer the determination of estrogens in the urine of men, non-pregnant and pregnant women. Brown's method is good, specially when estrogen excretion is very low. The method is elaborate and the main steps are: acid hydrolysis, ether extraction, methylation of the phenyl group and separation of estrogen methyl ester by chromatography on alumina before the Kober reaction is carried out. The later modification and short method of Brown (1963) or Klopper and Wilson (1962) did not eliminate the step of chromatography, which was necessary for removal of the interfering chromogens. The modification for reducing the non-specific absorption and thus enhancing the sensitivity is that of Ittrich (1958) and later modified by Dale *et al* (1965). Bradshaw (1961) developed the method by diluting the acid solution with trichloracetic acid after which the pink colou was extracted with chloroform. Correc ed readings were three times higher tha those derived from the measurements directly after development of Kober colour. Hydroquinone was recommended as the reducing agents instead of phenol by Butt (1967).

The clinical work calls for a method which should offer an opportunity to warn impending foetal distress, and should be rapid and reliable. The present method serves to fulfil the cardinal role in determining the maternal urinary estrogens. The method utilizes certain modifications for hydrolysis extraction and removal of interfering chromogens after Bradshaw. This method is suitable for assay of total estrogen from pregnancy urine and is not applicable for non-pregnant conditions. Without sacrificing the required specificity the method has been made short simple and inexpensive and is applicable for clinical evaluation of pregnant cases and can be undertaken by a moderately equipped laboratory attache to the hospital.

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