COMPARISON OF TWO SIMPLE METHODS OF ESTROGEN ESTIMATION*

by

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The measurement of urinary estrogen excretion during pregnancy has been recognised as a reliable method of assessing placental function (Banerjee, 1962; Frandsen, 1963). However, its usefulness as a routine test in antenatal clinics is limited by the fact that most methods for determination of estrogens, currently in use, are too time-consuming, complex, require expensive apparatus, not readily available, and call for 24 hour urine collection (Brown, 1955; Wotiz *et al* 1964).

Simplified colorimetric procedures are now available for measuring urinary estriol which constitutes approximately 90% of estrogen excreted during pregnancy. It is the purpose of this investigation to present data and observations comparing the use of two such colorimetric methods, i.e. those of Montague (1964) and Laumas (1966) for the analysis of urinary estrogens in the urine of pregnant Indian women. These methods are not too expensive or time consuming. They can readily be performed in any well-equipped laboratory.

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Received for publication on 1-4-68.

Further, an attempt has been also made to study the estrogen excretion on 2 hour morning samples of urine and compare the values obtained with those on 24 hour urine collections by expressing them in terms of creatinine excretion. This was done to investigate the possibility of evolving a suitable procedure for estrogen estimation that could be used in routine clinical practice.

Material and Methods

Solvents used for analysis were of Analar quality. They were distilled before use. Peroxide free diethyl ether was used.

Thirty-nine pregnant women belonging to the low socio-economic group were admitted to the hospital at 36 weeks of pregnancy and a twenty-four hour as well as a single morning two hour samples of urine were collected and stored without any preservative at 5°C until analysis, which was done within one week of collection.

Estrogen levels in all the samples were determined by the method of Montague (1964). In sixteen of the 24-hour urine samples, estrogen was also determined by the method described by Laumas (1966). Creatinine in urine was determined by the method of Folin (1914).

Montafue's method for the determination of estrogens

The details of this method have been published. In brief, the urine samples were acid hydrolysed and the estrogens were extracted with ether. The ether extract was evaporated and a colour reaction was carried out according to the method of Ittrich (1959). Instead of using internal standards for calculating the values of estrogens for all the estimations, a standard calibration curve was prepared, using standard estriol,* and used for calculating the amount of estrogen in urine sample. However, from several experiments, it was found that the extent of recovery of added estriol was 90-100%.

The original method described by Laumas was followed but for the separation of the estriol fraction. Thin layer chromatography using a solvent system of ethyl acetate benzene (1:1) was used instead of column chromatography for isolating the estriol. The recovery of added estrogens by this method was 70-80%.

In both the methods, the values of estrogens were expressed per gm. creatinine.

Results

There was a highly significant correlation between the values obtained by the two methods, the correlation coefficient being 0.9497 ($P < 0.001$) (Fig. 1). Also, there was a highly significant correlation between the values obtained on the single morning urine sample and the 24 hour sample when the values were expressed per gram creatinine, the correlation coefficient being 0.9497 ($P < 0.001$) (Fig. 2).

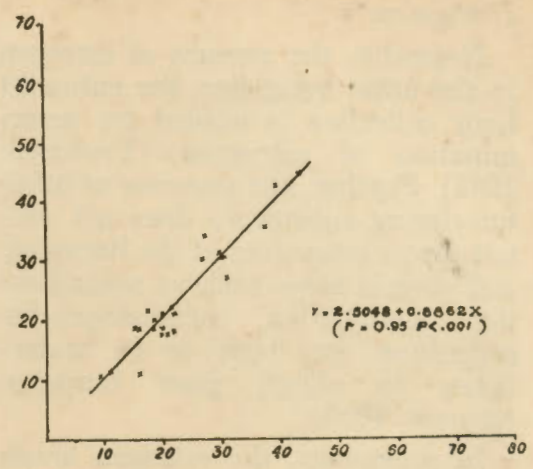

Fig. 1
Comparison of Laumas's with Montague's technique for estrogen estimation.
Laumas method: Estrogens in mg/gm. creatinine.
Estrogen mg/gm creatinine Montague's method.

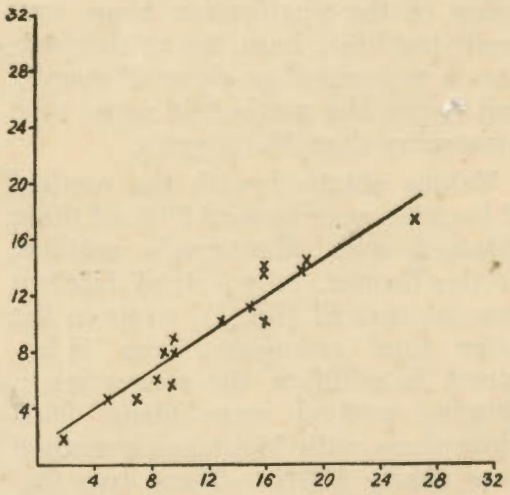

Fig. 2
A comparison of 24 hour and 2 hour estrogen excretion in urine.
24 hours samples: estrogens mg/gm creatinine.
2 hour sample: estrogen mg/gm creatinine.

*"*Standard Estriol was obtained as gift sample from Sigma Co.*"

Discussion

Normally, the amount of estrogen in the urine being low, the entire 24 hour collection is needed for determination of estrogens (Frandsen, 1965). Further, the presence of other interfering substances does not permit direct estimation of the hormone, and several steps such as saponification, methylation, chromatographic separation, etc. have to be undertaken to obtain pure hormone (Brown, 1959).

In pregnancy, the estrogen levels in the urine are high and 95% of the estrogen exists as estriol. Hence, many of the purification steps can be omitted. In Montague's method, total estrogens are determined colorimetrically after intial hydrolysis to liberate the estrogens from their glucuronide conjugates. The simplicity of this procedure permits 15-20 estimations to be made in a single working day. Laumas' method also does not call for many of the purification steps routinely used but, here, the estriol fraction is separated by chromatography and hence the method is more time consuming than Montague's.

Values obtained with the method of Laumas were around 80% of those obtained with Montague's method. In the former, only estriol fraction was determined (fig. 1), while in the latter total estrogens were determined. In addition, the recoveries in Laumas method were about 80% while those with Montague's method were nearly 100%. These two factors, may, therefore account for the higher values obtained with Montague's method.

The observation made here that the estrogen values obtained in random urine samples and 24 hour samples were essentially similar when expressed in terms of creatinine excretion, suggests that a random urine sample provides as much information as a 24 hour urine sample. This is consistent with the observations made by Dickey *et al* (1966), who found a close correlation between creatinine and estrogen levels in urine. The practical implications of this observation are obvious.

Weekly measurement of placental function becomes necessary under such situations as, diabetes, hypertension, habitual abortion, toxaemia of pregnancy, etc. By using Montague's method on a single morning urine sample, determination of urinary estrogen can become an easy, simple, and routine investigation for antenatal cases, especially where placental insufficiency is suspected. This may also be useful in assessing the foetal prognosis and indicating the line of treatment in hospitalised patients.

The observations described in this paper have been utilized in these laboratories to study the pattern of estrogen excretion in Indian women and the results will appear in another publication.

Summary

1. Urinary estrogens were estimated by the methods described by Montague and Laumas.

2. Comparative analyses of 24 hour and single morning urine samples were done.

3. It was found that the method of Montague was as accurate as the more time consuming method of

Laumas, when applied to pregnancy urine, and with this method, 15-20 samples of urine in duplicate, could be analysed in a single working day.

4. Values obtained on single morning urine samples are comparable with those on 24 hour collections, when expressed in terms of creatinine excretion.

Acknowledgement

The author is grateful to Dr. C. Gopalan, Director, and Dr. S. G. Srikantia, Deputy Director for their interest and guidance in this study. She acknowledges gratefully the help of Squadron Leader Mrs. V. Ramanan, Gynaecologist, Air Force Hospital, Trimalgherry, in this study. Mr. Syed Mohammed rendered technical assistance.

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