URINARY OESTRIOL EXCRETION IN DIABETIC PREGNANCY

By

V. Aasted Frandsen, Jørgen Pedersen and Georg Stakemann

ABSTRACT

The urinary excretion of oestriol has been investigated in 30 cases of diabetic pregnancy. The mean excretion was low, although most of the values found were within the range of excretion in normal pregnancies. No correlation was found between the severity of the diabetes and the amounts of excretion oestriol.

For many years Priscilla White and collaborators (White 1952; Nelson et al. 1953) in Boston have claimed that a hormonal imbalance is present in many diabetic pregnancies, especially in complicated cases and those which end with foetal death. White claims that the imbalance can be corrected by stilboestrol and progesterone treatment resulting in a diminished incidence of toxaemia and a higher foetal survival. Although the results have been confirmed by others (Moreau et al. 1955; Buck & Day 1955), most workers to-day believe that the improved results obtained by White and collaborators must be due to other factors than the hormone treatment. In a carefully conducted study by the Medical Research Council in England (1955) no benefit from ethisterone or stilboestrol treatment was found, and others have reported similar results (Peel 1955; Clayton 1956).

In spite of the marked interest in determining the possible value of oestrogen treatment in diabetic pregnancies, amazingly few investigations have been carried out on the urinary oestrogen excretion in such pregnancies. Rubin et al. (1946) found in a small series a normal urinary excretion of oestrogen in diabetic pregnancies, but Jayle & Crépy (1954) reported a diminished excretion while Ten Berge (1959) found 80 per cent of the oestriol estimations to be less than the normal average. Recently Hobkirk et al. (1960) have reported on
the urinary excretion of oestrone, oestriol and oestriol in 14 diabetic pregnancies without toxaemia; on the whole the excretion was lower than in normal pregnancies, but even then half of the values were within the normal range. They did not correlate the oestrogen level with the severity of the diabetes.

We have investigated the urinary oestriol excretion in cases of diabetic pregnancies and compared the amounts of excreted oestriol with the severity of the diabetes.

**M A T E R I A L**

The treatment of pregnant, diabetic women in Copenhagen is concentrated in a single hospital department, and in the ward they are all supervised by the same internist and team of obstetricians. The general management of these patients has been described elsewhere (Pedersen & Brandstrup 1956); no hormone treatment except insulin is used, and the patients are generally admitted to hospital when 30–32 weeks pregnant. In the present investigation 24-hour specimens of urine were collected twice weekly during the stay in hospital. Altogether 36 consecutive diabetic patients were examined; six developed toxaemia and are omitted from this study, leaving 30 patients on whom a total of 226 analyses were performed, from 3 to 15 per patient.

**METHOD AND ANALYSIS**

The presence of glucose in the urine is known to interfere with the estimation of oestrogens (Hobkirk et al. 1959). Probably due to the aldehyde group of the glucose molecule, some destruction of the oestrogens takes place during acid hydrolysis. The destruction increases with increasing concentration of glucose in the urine (Fig. 1). Hobkirk et al. minimized the destruction by dilution of the urine prior to acid hydrolysis, in this way reducing the concentration of glucose. This procedure is efficient but is inconvenient from a practical point of view because of the large volumes that have to be handled. We therefore looked for other ways of eliminating the effect of glucose. Experiments in which we tried to destroy the glucose with the aid of yeast as employed in the method for estimating 17-ketogenic steroids (Jørgensen 1957) seemed promising. In some experiments we noticed, however, a destruction of the oestriol due to the addition of yeast, a destruction which could be just as marked as the one due to glucose; consequently we abandoned this method.

We next tried the principle recommended by Edwards et al. (1953) which has also been applied to methods for the estimation of 17-ketogenic steroids for eliminating glucose. The results were satisfactory and after preliminary experiments it was decided to use this technique:

1/200 of a 24-hour urine specimen is filled up to 10 ml with distilled water, acidified with 2 drops of 50 per cent sulphuric acid, and after the addition of 5 g ammonium sulphate extracted with 3 volumes of a mixture of ethyl acetate and ethanol (23 + 7).
The destruction of oestriol due to the presence of glucose in the urine. Glucose is added to the urine before hydrolysis.

Table 1.
The effect of extracting the conjugated oestriol prior to hydrolysis.
Method A: Hydrolysis performed on the untreated urine.
Method B: Extraction of the conjugated oestriol and hydrolysis.

<table>
<thead>
<tr>
<th>Urine No.</th>
<th>mg oestriol/24 h</th>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1447</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>B 16</td>
<td>2.2</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>B 17</td>
<td>2.4</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>B 18</td>
<td>2.7</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>1312</td>
<td>10.7</td>
<td>10.6</td>
<td></td>
</tr>
<tr>
<td>1196</td>
<td>11.3</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td>1320</td>
<td>15.1</td>
<td>16.4</td>
<td></td>
</tr>
<tr>
<td>1158</td>
<td>15.9</td>
<td>18.7</td>
<td></td>
</tr>
<tr>
<td>1164</td>
<td>20.1</td>
<td>21.8</td>
<td></td>
</tr>
<tr>
<td>1450</td>
<td>22.8</td>
<td>23.7</td>
<td></td>
</tr>
<tr>
<td>1190</td>
<td>35.2</td>
<td>31.4</td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>12.7</td>
<td>12.9</td>
<td></td>
</tr>
</tbody>
</table>

402
After removal of the aqueous phase the organic phase is washed with 2 ml of a 50 per cent solution of ammonium sulphate. The organic phase is evaporated and the residue dissolved in 10 ml of 3 per cent sulphuric acid. The estimation of the oestriol is performed according to the method of Frandsen (to be published).

In this way the conjugated oestriol was separated from glucose so that only traces of glucose could be demonstrated in the residue after the evaporation of the organic phase. In order to see whether the extraction of the conjugate was quantitative and whether any destruction occurred during the evaporation an analysis was made of different samples of urine from normal pregnancies with the above mentioned technique and without extraction. As will be seen (Table 1), no difference exceeding the experimental error was found between the results.

RESULTS

The 30 patients were grouped according to White's classification, 11 fell into group B, 6 into group C, 12 into group D and 1 into group F.

In Fig. 2 the amounts of oestriol excreted in groups B and C are shown, and in Fig. 3 those of groups D and F; 95 per cent of the estimations of oestriol-excretion found in normal pregnancies fall inside the indicated area (Frandsen & Stakemann 1960 a). It is clearly seen from the charts, therefore, that in diabetic pregnancies there is a tendency to a lowered output of oestriol, though 80 per cent of the values fall within the normal range. Furthermore, a comparison of Figs 2 and 3 shows there is no difference between the amounts of oestriol excreted in the severe cases of diabetes and those in the less severe cases.

The 30 patients were re-grouped according to the size of their daily dose of insulin, group 1 having a daily dose of more than 40 IU and group 2 a dose of 40 IU or less per day (Figs 4 and 5). It is readily observed that in this grouping too, no difference between the oestriol output of the less and the more severe cases of diabetes can be demonstrated.

OUTCOME OF THE PREGNANCIES

In one case the foetus died 2 days before delivery and of the 29 babies born alive, 2 died within the first 24 hours.

Case 13 (B 1883/60): 27-year-old diabetic woman, White group C. No previous pregnancies. Present pregnancy was uneventful except for a slight hydramnion and tendency to oedema; she was admitted for prophylactic reasons eight weeks before the calculated term. During the following five weeks her oestriol output rose from 7 to 13 mg/24 h. Two days after the last urine specimen was
collected, heart sounds and foetal movements stopped abruptly and two days later she delivered a dead baby of 4600 g.

Case 22 (B 203/61): 26-year-old diabetic woman, White group D. In 1956 she had a caesarean section. Present pregnancy was uneventful, she was admitted for prophylactic reasons eight weeks before term. During the next three weeks her oestriol excretion rose from 8 to 11 mg/24 h. The day after the last urine specimen was collected, there were contractions of the uterus and as the heart sounds became irregular, a caesarean section was therefore performed. A live, male baby of 3100 g was delivered. The baby died suddenly two hours later, and at autopsy showed no abnormalities.

Case 2 (B 843/61): 19-year-old diabetic woman, White group D. No previous pregnancies. Present pregnancy was uneventful until the 35th week, when
Fig. 3.
Oestriol excretion in 13 cases of diabetic pregnancy classified according to White in groups D and F.

On April 10, 1961, she was admitted in diabetic precoma (serum HCO₃⁻ 12.5 mmol/l). During the next 24 hours she excreted only 2 mg of oestriol. Adequate treatment was immediately started and she recovered rapidly and during the following week her oestriol output rose to 10 mg/24 h. Except for the first few days in hospital the clinical condition of the patient and of the foetus seemed to be excellent; however, after the temporary rise, the oestriol excretion diminished and amounted on April 24 and 27 only to 5 mg/24 h. Because of the uneventful clinical condition an attempt to induce artificial labour with injections of pitocin was carried out but was unsuccessful; consequently a caesarean section was performed, and a live, male baby of 3300 g was delivered on May 4. The condition of the baby was poor right from the time of delivery and he died six hours later.
In a previous study (Frandsen & Stakemann 1960 b) we found that a foetal stress-situation was sometimes accompanied by a diminished oestriol excretion of the mother. In the first two of the above mentioned cases, the oestriol excretion was low but yet within the normal range, and therefore no warning of a dangerous situation of the foetus could be predicted from the hormone analyses. However, in Case 26 we found a very low oestriol excretion (2 mg/24 h) when the mother – and most probably also her foetus – was stressed by the diabetic coma. Afterwards the clinical condition of the mother and the foetus gave rise to no anxiety, yet the oestriol excretion only rose temporarily and the baby was born in such a poor condition that it lived for only six hours. In retrospect we think we should have been warned by the diminished oestriol
Fig. 5.
Oestriol excretion in 15 cases of diabetic pregnancy with an individual treatment of 40 IU of insulin per day or less.

output and performed the caesarean section at an earlier stage so as to give the child a better chance of survival.

DISCUSSION

The theoretical background of White's hormone treatment is the findings of Smith & Smith (1947) that many pathological pregnancies are associated with a high urinary excretion of chorionic gonadotrophin and a low excretion of pregnanediol. These results have been partly confirmed in this country by Pedersen (1951) who found an elevated level of the average urinary excretion of chorionic gonadotrophin during the last three months of diabetic pregna-
cies. Loraine (1949) found an increased excretion of chorionic gonadotrophin in some diabetic pregnancies, but no correlation between this and the development of complications and only a temporary fall of the chorionic gonadotrophin excretion during treatment with stilboestrol even in large doses.

In the present investigation we found, in agreement with Hobkirk et al. (1960), that in diabetic pregnancies the mean urinary excretion of oestriol is somewhat diminished; yet 80 per cent of our values were within the normal range, whereas Hobkirk et al. found only 50 per cent to be within this range. This is partly due to the fact that their lower limit of the normal excretion is a little higher than ours.

From the results in the literature and from our own investigation it seems clear that diabetic pregnancies can be associated with a hormonal imbalance: increased urinary excretion of chorionic gonadotrophin and decreased excretion of pregnanediol and oestriol. This seems to make therapy with stilboestrol and progesterone logical as advocated by White. The results of Loraine, however, indicate that even continuous treatment with stilboestrol does not alter the hormonal imbalance for more than a short period, and in several studies no benefit was found clinically from the hormone treatment. The reason for this discrepancy could be that no relation exists between, on the one hand the diabetic condition of the mother and the complications and outcome of the pregnancy, and on the other hand the altered excretion of chorionic gonadotrophin, pregnanediol and oestriol; this seems to be confirmed both by our own results and by those of Loraine.

In our experience, however, oestriol analyses may sometimes be of value to the clinician in cases of diabetic pregnancies as a method of deciding if the foetus is in a stress-situation.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the technical assistance of Mrs. Bente Mønsted and Miss Hanne Laustsen, and to thank Mrs. Grethe Rasmussen for the careful control of the sampling of the urines.

REFERENCES

Pedersen J.: Ugeskr. Læg. 113 (1951) 1771.
  & Marble A., Philadelphia (1952) 676.

Received on November 15th, 1961.