Contribution of Estriol to Total Urinary Estrogens during Pregnancy

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Estriol accounts for an average of 74% of the principal Kober-positive steroids in the urine of 13 women during uncomplicated pregnancies. This proportion is independent of the stage of pregnancy, from 20 weeks until term. The average ratio of estriol to ring-D α-ketols (mainly 16α-hydroxyestra-1,3,5(10)-trien-17-one), estradiol-17β, and estriol. It is now known that several other phenolic steroid metabolites contribute significantly to total urinary estrogens (13).

Our paper establishes the contribution of estriol to total estrogen excretion and supplements data already obtained in this laboratory (14, 15). In view of the interest in the urinary excretion of estriol and total estrogens during pregnancy complicated by diabetes (16–18), some comparison is given for normal and diabetic women during their pregnancies.

Materials and Methods

Total 24-h urines were collected without preservative in polyethylene bottles and, unless analyzed immediately, were stored at -15°C until processed.

Reagents

All reagents and organic solvents were purified where necessary by published procedures (19, 20). “Glusulase,” a molluscan enzyme preparation containing β-glucuronidase (β-d-glucuronide glucuronohydrolase, EC 3.2.1.31), and sulfatase was purchased from Endo Labs., Inc., Garden City, N. Y. A bacterial β-glucuronidase preparation was purchased from Sigma Chemical Co., St. Louis, Mo.

Crystalline steroids (Mann Research Labs., Inc., N. Y.) were checked for purity by thin-layer chromatography and melting point determination.

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Analytical Methods

Suitable volumes of urine (usually 50 ml) were analyzed by a modification (21) of a procedure published elsewhere (14). In some instances, “total conjugated” estrogens were hydrolyzed by Glusulase (22) while in others steroid glucosiduronates were hydrolyzed by bacterial β-glucuronidase (21) and the residual conjugates (mainly sulfates) were cleaved by solvolysis in ethyl acetate and H2SO4 (23). The various urinary estrogen fractions were purified and separated by solvent partition, Girard separation, and partition chromatography on a Celite column.

The ring-D α-ketolic fraction, consisting mainly of 16α-hydroxyestrone [3,16α-dihydroxyestra-1,3,5(10)-triene-17-one] and 16-ketostriadiol-17β[3,17β-dihydroxyestra-1,3,5(10)-triene-17-one], together with some 16β-hydroxyestrone [3,16β-dihydroxyestra-1,3,5(10)-triene-17-one], was measured by two methods. First, the portion of the column eluate that contained the latter fraction was measured spectrophotometrically in terms of 16α-hydroxyestrone (14). Secondly, the fraction was chemically reduced with NaBH4, 16α-hydroxyestrone being converted mainly to estriol (24) and 16-ketooestradiol-17β (+16β-hydroxyestrone) to 16-epiestriol [estra-1,3,5(10)-triene-3,16β,17β-triol] (24). The two steroid triols were then separated on Celite and measured individually (24). For our purposes these were added to yield “total ring-D α-ketols.” The spectrophotometric procedure of Ittrich (10) was used throughout, except that tetrachloroethylene was used as the solvent for extraction of the chromat (20). Absorbance was measured at 506, 538, and 570 nm in each case and corrected by the method of Allen (25). The corrected absorbance was related to the value obtained from known amounts of the appropriate standards. Supporting evidence for the identity of various fractions from urine was obtained by applying aliquots to thin (0.25 mm) layers of Silica Gel H on glass plates, which were then developed in suitable solvent systems (26). Spots on the plates were made visible either by spraying with diazotized sulfanilic acid or by spraying with a mixture of 2 ml of H2SO4 per 100 ml of ethanol and then heating the plates at 110°C. Mobilities of the various fractions were compared with those of the appropriate pure steroids. Values given in the tables below refer to fractions with Rf values corresponding to the standard steroids in question.

To measure recovery, known amounts of pure steroids, in the approximate proportions expected in pregnancy (14), were added to nonpregnancy urine and taken through the experimental procedure. The results were similar to those already published (14) but, when the ring-D α-ketolic fraction was reduced, with subsequent extraction and chromatography, about 10% less of it was recovered than by direct spectrophotometry. The average recoveries were estrone, 81%; estriol-17β, 83%; estradiol-17β, 81%; 16-epiestriol, 84%; ring-D α-ketols (direct method), 65%; and ring-D α-ketols (reduction method), 55%. All of the values reported below have been corrected for these average recoveries.

Results

Note that the excretion given for any one steroid represents “total conjugated forms” of that compound.

Table 1 contains data on five fractions from urine obtained during 13 uncomplicated pregnancies (51 analyses). The results are subdivided into four time intervals, 20–24, 25–29, 30–34, and 35 or more weeks of pregnancy. Estriol averaged 74% of the total estrogens measured during each interval. Ring-D α-ketols amounted to about 20% of the estriol concentrations.

Table 2 shows similar data for 17 diabetic

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**Table 1. Amount of Five Estrogen Fractions in the Urine of 13 Women during Uncomplicated Pregnancies**

<table>
<thead>
<tr>
<th>Weeks pregnant</th>
<th>No. analyses</th>
<th>E1 (mg/24 h)</th>
<th>E2 (mg/24 h)</th>
<th>Ring-D α-ketols</th>
<th>E3 (mg/24 h)</th>
<th>16epiE3 (mg/24 h)</th>
<th>Ratio of E1 to ring-D α-ketols</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–24</td>
<td>9</td>
<td>0.90 a</td>
<td>(0.39–1.5)</td>
<td>(0.16–0.34)</td>
<td>0.26</td>
<td>1.7</td>
<td>8.8</td>
</tr>
<tr>
<td>25–29</td>
<td>11</td>
<td>0.98</td>
<td>(0.32–1.5)</td>
<td>(0.21–0.44)</td>
<td>0.34</td>
<td>2.4</td>
<td>11.6</td>
</tr>
<tr>
<td>30–34</td>
<td>12</td>
<td>1.4</td>
<td>(0.65–2.4)</td>
<td>(0.34–0.76)</td>
<td>0.48</td>
<td>3.2</td>
<td>16.3</td>
</tr>
<tr>
<td>35+</td>
<td>19</td>
<td>1.3</td>
<td>(0.21–3.0)</td>
<td>(0.11–0.74)</td>
<td>0.46</td>
<td>4.6</td>
<td>24.0</td>
</tr>
</tbody>
</table>

* E1 is estrone; E2; estradiol-17β; E3; estriol; 16epiE3, 16epiestriol.
* a Average, range in parentheses.

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women during their pregnancies (54 analyses). Estradiol averaged 63% of the total and, once more, did not change markedly between 20 weeks and term, although individual values varied considerably. The ratio of estradiol to ring-D α-ketols averaged about 3:1. Once again, however, there was a scatter of individual values, reaching as low as 1:3:1.

Ring-D α-ketols averaged higher in the diabetic state than in normal pregnancy, a finding reflected in the ratio of the estradiol fraction to ring-D α-ketols. Estriol levels also appear higher in the diabetics. However, none of these differences was statistically significant (Table 3).

No particularly low estriol levels were encountered in the diabetic group, a finding perhaps attributable to the fact that no patient showed signs of intra-uterine death during the period of investigation.

Discussion

The steroids we measured are by no means the only estrogen metabolites present in human pregnancy urines, but they represent the principal steroids showing a strong extinction at 538 nm in the Ittrich modification of the Kober color reaction. It has already been shown that the fractions measured by our analytical procedure compare well as a whole with values obtained by the direct Ittrich method for total estrogens (14).

Our data show that between 20 weeks and term, estriol is a fairly constant fraction of the total estrogens. This might suggest that it is immaterial clinically whether one measures estriol or total estrogens. However, we show here that as little as 61% and 46% of the total estrogens measured may be estriol in uncomplicated pregnancies and those with concurrent diabetes, respectively. The greater part of the remainder is ring-D α-ketols. Cohen recently claimed that even smaller proportions of estriol may be found in some toxemic pregnancies (87).

The rationale for measuring urinary estriol is that during the second half of pregnancy, about 90% of it originates in the fetus (67) and thus it may be useful as an index to fetal viability. Recent work, however, suggests that the ring-D α-ketols, particularly 16α-hydroxyestrone, present in pregnancy urine, originate from the mother rather than the fetus (81, 88). For this reason it may be argued that the determination of the amount of estriol in urine would be more valuable than determination of total estrogens. Cohen suggests the opposite, because estriol may sometimes be a very low fraction of the total (87); therefore it might not accurately reflect the total output of steroid by the fetus. The answer to this question may depend on the origin of the urinary ring-D α-ketols, particularly in a condition such as pregnancy complicated by diabetes where these...
steroids appear to be of considerable quantitative significance. Cohen also presented indirect evidence for the presence of high levels of these latter steroids in the total estrogen of diabetic pregnancy urines (27).

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References


