EFFECT OF AMPICILLIN ADMINISTRATION
ON THE EXCRETION OF
TWELVE OESTROGENS IN PREGNANCY URINE

By

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ABSTRACT

The excretion of twelve oestrogens in urine, pooled daily from a group of pregnant women, was determined before, during and after ampicillin administration (2 g/day, for 3 days). On the second day of ampicillin administration total oestrogen excretion fell to 67% of the mean control value, oestriol excretion to 69% and that of the other eleven individual oestrogens to an average of 62% of the mean control values. In general, on the third day of treatment and on the two post-treatment days this decrease tended to be corrected. The patterns of change in the urinary levels of the individual metabolites provided no clear lead to the basic mechanism of ampicillin impairment of oestrogen excretion. However, as the drug affected all their excretion in more or less the same way as it did that of oestradiol, it is possible that ampicillin interferes primarily with their enterohepatic circulation in the mother as has been established with reasonable certainty in the case of oestradiol.

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Ampicillin administration has been shown to decrease the urinary excretion of oestriol in normal human pregnancy (Willman & Pulkkinen 1971). The administration of this antibiotic has also been reported to decrease the excretion of a number of C\textsubscript{19}- and C\textsubscript{21}-steroid metabolites under similar conditions (Trybuchowski 1973). However, the nature of the ampicillin-induced changes responsible for the impairment of urinary steroid excretion remains to be established with certainty.

A number of observations: (a) that neomycin, which undergoes little intestinal absorption, causes a similar decrease in urinary oestriol excretion (Pulkkinen & Willman 1973a), (b) that almost the entire ampicillin-induced decrease in urinary oestriol excretion could be accounted for by the decrease in oestriol-3-glucuronide (Tikkanen et al. 1973), which is known to be formed exclusively in the intestinal mucosal cells (Dahm & Breuer 1966; Stoa & Levitz 1968) and (c) that the ampicillin-induced decrease in urinary progesterone metabolites in pregnancy was transitory and confined to glucuronide conjugated metabolites (Martin et al. 1974) suggest that ampicillin interferes primarily with the enterohepatic circulation of steroids at the level of the gastro-intestinal tract. In an effort to corroborate this hypothesis the effect of ampicillin administration on the urinary excretion of 12 oestrogens in a group of normal pregnant women has been determined. Recently, characteristic changes in groups of oestrogen metabolites have been found in other clinical situations involving interrupted enterohepatic circulation of oestrogen (Adlercreutz et al. 1974).

**MATERIAL AND METHODS**

*Patients.* – Six fully informed, non-infected, hospitalized mothers, 33–37 weeks pregnant, participated in the study. Twenty-four hour urine samples were collected from each patient on seven consecutive days (days 1–7). On days 3, 4 and 5 each patient received 500 mg ampicillin (Doktacillin®, Astra, Sweden) 4 times daily. The urine samples were stored at -20°C until analyzed. The analyses were carried out on pooled samples formed by mixing 1 per cent of the total volume of each 24 h sample with the other 5 one per cent samples for the corresponding day.

*Oestrogen determinations.* – The determination of 12 urinary oestrogens was carried out by gas-liquid chromatography according to the method of Adlercreutz & Luukkainen (1967, 1968) using the modifications described recently (Adlercreutz et al. 1974). A critical assessment of the accuracy and precision of this method has recently been published (Adlercreutz 1975). Urinary oestriol, in the individual samples, was determined by a short procedure (Adlercreutz & Luukkainen 1965). All determinations were carried out in duplicate.

*Steroid nomenclature.* – The following trivial names have been used: oestrone, 3-hydroxy-1,3,5(10)-oestratrien-17-one; oestradiol-17β,1,3,5(10)-oestratriene-3,17β-diol; oestriol, 1,3,5(10)-oestratriene-3,16α,17β-triol; 16α-hydroxyoestrone, 3,16α-dihydroxy-.
RESULTS

The levels of the 12 urinary oestrogens in the 7 daily pools are given in Table 1. The values obtained on control days 1 and 2 are for the most part similar to the mean values obtained for the urinary excretion of the same oestrogens in 19 normal subjects, 30-40 weeks pregnant (Adlercreutz et al. 1974). How-

Table 1.
The excretion of twelve oestrogens in pregnancy urine during ampicillin administration.

<table>
<thead>
<tr>
<th></th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Oestriol</td>
<td>15.83</td>
</tr>
<tr>
<td>16α-Hydroxyoestrone</td>
<td>1.93</td>
</tr>
<tr>
<td>16-Oxo-oestradiol</td>
<td>1.59</td>
</tr>
<tr>
<td>Oestrone</td>
<td>0.95</td>
</tr>
<tr>
<td>16β-Hydroxyoestrone</td>
<td>0.44</td>
</tr>
<tr>
<td>15α-Hydroxyoestrone</td>
<td>0.14</td>
</tr>
<tr>
<td>2-Methoxyoestrone</td>
<td>0.10</td>
</tr>
<tr>
<td>Oestradiol-17β</td>
<td>0.22</td>
</tr>
<tr>
<td>11-Dehydro-oestradiol-17α</td>
<td>0.10</td>
</tr>
<tr>
<td>Oestradiol-17α + unknown oestradiol</td>
<td>0.03</td>
</tr>
<tr>
<td>17-Epooestriol</td>
<td>0.14</td>
</tr>
<tr>
<td>16-Epooestriol</td>
<td>0.61</td>
</tr>
<tr>
<td>Total oestrogens measured</td>
<td>22.08</td>
</tr>
</tbody>
</table>

Values are expressed as mg/24 h and each value represents the mean of two determinations. On each day 1% of the total 24-h urine from each of the 6 patients was pooled for oestrogen determination.

* On days 3, 4 and 5 each patient received ampicillin (2 g/day) orally.
Table 2.
Individual urinary oestriol excretion (mg/24 h) by the six subjects on the two control
days and on the second and third days of ampicillin administration¹).

<table>
<thead>
<tr>
<th>Subject</th>
<th>Day 1²)</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 5</th>
<th>% Ampicillin induced decrease³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. R.-L. P.</td>
<td>13.78</td>
<td>n. d.⁴)</td>
<td>7.12</td>
<td>10.0</td>
<td>48.3</td>
</tr>
<tr>
<td>3. S. V.</td>
<td>24.75</td>
<td>23.49</td>
<td>10.72</td>
<td>n. d.</td>
<td>55.6</td>
</tr>
<tr>
<td>5. M. L.</td>
<td>22.8</td>
<td>16.6</td>
<td>14.8</td>
<td>n. d.</td>
<td>24.8</td>
</tr>
<tr>
<td>6. A. R.</td>
<td>n. d.</td>
<td>12.72</td>
<td>9.02</td>
<td>8.77</td>
<td>29.0</td>
</tr>
</tbody>
</table>

¹) Ampicillin was administered orally at a dose of 2 g/day.
²) Days 1 and 2 are the control days and days 4 and 5 the second and third days of
ampicillin administration.
³) These values are calculated from the differences between the excretion on day 4
and the mean excretion on days 1 and 2.
⁴) n. d., not determined; lack of sample precluded determination in these cases.

ever, the levels of the most abundant metabolites, oestriol, 16α-hydroxyoestrone
and 16-oxo-oestradiol-17β were in the lower part of the normal range.

On the second day of ampicillin administration the total excretion of the
oestrogens measured was clearly decreased below control values. On the final
day of ampicillin administration and on the two post-treatment days total
oestrogen excretion tended to increase towards control levels (Table 1). A de-
crease similar to that seen in total oestrogen excretion on the second day of
ampicillin administration was also a feature of six of the individual oestrogen
excretion patterns (oestriol, 16α-hydroxyoestrone, 16-oxo-oestradiol, oestrone,
16β-hydroxyoestrone and 15α-hydroxyoestrone) (Table 1).

The excretory pattern of the individual oestrogens on the third day of drug
administration and on the two post-treatment days varied somewhat.

In order to test the validity of the pooling procedure oestriol was also
determined in the individual urine samples from the 6 patients on control
days 1 and 2, on day 4 and in 3 of the 6 subjects on day 5. A lack of sample
precluded determination in a number of cases. The results are presented in
Table 2. The mean oestriol excretion for control days 1 and 2 calculated from
these values, 16.05 mg/24 h (where day 1 and 2 values were not available,
subjects 1 and 6 (Table 2), the single control value was used), is very similar
to the value obtained from the pooled samples, 15.25 mg/24 h (Table 1). De-
creased oestriol excretion was seen in all 5 subjects studied on day 4 and in
the 3 subjects studied on day 5. The mean per cent decrease in oestriol excretion on the second day of ampicillin administration (day 4) in the 5 subjects studied, 33.3 ± 18.9 (mean ± sd), was very significant (P < 0.005). The per cent decrease on day 4 obtained with the pooled samples was 30.9%. It would thus seem that pooling the samples, which was necessary on account of the highly complex and time consuming nature of the procedure for the determination of the 12 oestrogens did not usually affect the overall pattern of the results.

DIscussion

The suppressive effect of ampicillin administration on the urinary excretion of the principal oestrogen metabolite, oestriol (Tables 1 and 2), was consistent with the findings of Willman & Pulkkinen (1971) and Tikkanen et al. (1973). The former investigators (Willman & Pulkkinen 1971) also showed that ampicillin reduces plasma oestriol levels in parallel with the urinary levels, thus eliminating the possibility that ampicillin influences the rate of oestriol clearance by the maternal kidneys. On the basis of the available evidence it also seems unlikely that ampicillin affects placental oestriol biosynthesis adversely. In this regard, Pinkus et al. (1973) have shown that ampicillin had a stimulatory rather than an inhibitory effect on placental dehydroepiandrosterone sulphate hydrolysis and had no effect on placental aromatization of unconjugated dehydroepiandrosterone. In addition, unchanged umbilical cord plasma oestriol levels were observed during ampicillin administration (Pulkkinen & Willman 1973b). As stated in the introduction a number of findings eventually led to the proposal that ampicillin depresses urinary oestriol excretion in pregnancy by interfering with its enterohepatic circulation. In particular, it was shown that the bulk of the decrease in urinary oestriol excretion could be accounted for by the decrease in oestriol-3-glucuronide excretion (Tikkanen et al. 1973). The latter metabolite is exclusively of intestinal origin (Dahm & Breuer 1966; Stoa & Levitz 1968). It was also shown that neomycin, which undergoes little intestinal absorption has an effect similar to ampicillin on urinary oestriol excretion (Pulkkinen & Willman 1973a). On the basis of this evidence it seems that ampicillin may act by inhibiting the hydrolysis of the biliary oestriol conjugates in the gut, thus impairing their reabsorption and reconjugation and resulting in faecal loss.

The individual excretory patterns of the other 11 oestrogens studied, however, are very difficult to interpret on the basis of the hypothesis presented above or in any other unified manner. Ampicillin administration resulted in a suppression in urinary excretion of five of these metabolites (16α-hydroxyoestrone, 16-oxo-oestradiol, oestrone, 16β-hydroxyoestrone and 15α-hydroxy-
oestrone) on day 4. On the 3 subsequent days the excretory pattern of the individual metabolites differed but in general the excretion tended to normalize but at differing rates. The fact that the depression on day 4 is of the same order as that fraction of circulating oestrogens which are secreted in bile may be of some significance. It is interesting to note that when the effect of ampicillin on urinary progesterone metabolite excretion was studied in the same subjects changes were seen only on day 4 and were confined to the glucuronide conjugated metabolites (Martin et al. 1974).

If ampicillin results in the faecal loss of steroids due to an inhibition of their enterohepatic circulation one might expect to see a preferential loss of those metabolites most prone to biliary secretion (see Table 3 in Adlercreutz et al. 1973), but, this was not the case. However, intestinal interconversion of the various metabolites, about which little is known, could mask such preferential changes. It has been proposed that the conversion of 16α-hydroxyoestrone to oestriol and of 16β-hydroxyoestrone to 16-epioestriol in the maternal organism partly depends on an intact enterohepatic circulation of oestrogens (Adlercreutz et al. 1974); but the changes observed showed no significant trend with regard to these particular metabolic relationships. Similarly, no correlation seems to exist between the effect of ampicillin and the predominant mode of conjugation of particular metabolites in bile.

Further studies, now in progress, on the effects of ampicillin on faecal oestrogen excretion may help to clarify the situation.

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