Oxidative Metabolism of Estradiol*†

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The metabolism of estrone and estradiol was simultaneously examined in three different patients. The ratio of weight as studied. Urine and blood collections were obtained at successive intervals after injection. Portions of each injection solution were removed and the ratio of C14 to H3 determined.

All subjects were women who were catheterized, and urine collections were made at specific intervals starting with 4 hour after injection. Portions of each injection solution were removed and the ratio of C14 to H3 determined.

The urine collected at each interval was processed individually by the procedure used in these laboratories (6). Glycerin was incubated for 5 days at pH 5 was followed by continuous extraction with ether; the ether extract was washed with 9% NaHCO3 solution. The crude phenolic and neutral extract thus obtained was subjected to a 99-tube countercurrent distribution in System I composed of 70% methanol-carbon tetrachloride. The distribution was examined by measurement of radioactivity.

Tubes 70 to 99 were combined and redistributed through 99 tubes in System II composed of ethanol-water-ethyl acetate-cyclohexane, 1:1:1:1. This distribution in the two systems resolved the estrogen metabolites of interest into well defined areas with the following peak tubes: System I, 2-methoxyestrone in tube 22 and estrone in tube 56; System II, estriol in tube 28 and estradiol in tube 62. In addition, a region with a peak at tube 48 contained the epiestriol and the ring D ketol metabolites.

The appropriate regions in the countercurrent distribution were mixed with carrier steroid followed by crystallization to constant specific activity. Estradiol and epiestriol were converted to the triacetates before counting because of the insolubility of the free steroids in the scintillant mixture. Aliquots of the peak countercurrent tube of each metabolite and each of the adjacent tubes were counted before carrier addition.

With Subject R, Table III, the countercurrent region containing 16-epiestriol (tubes 37 to 56) was chromatographed on paper in the system toluene-isooctane-methanol-water, 15:5:16:4. Standards of epiestriol and 16-ketoestradiol were chromatographed simultaneously. The 16-epiestriol area, as determined by the radioactivity measured with an Aquebogue scanner, was eluted and inert 16-epiestriol added. After acetylation the material was recrystallized from methanol to constant specific activity and the ratio of C14 to H3 determined.

* Known as Ketodase and obtained from the Warner-Chilcott Laboratories, a division of Warner Lambert Pharmaceutical Company.
The collected blood (60 cc) was diluted with 10 volumes of 1:1 ethanol-acetone mixture, filtered, and the precipitate washed well with ethanol-acetone. The filtrate and washings were concentrated to dryness and the residue was made to 500 cc with water and extracted continuously with ether for 24 hours. The ether extract was washed with a 9% sodium bicarbonate solution saturated with sodium chloride. The dry residue (40,700 c.p.m.) was dissolved in 70% ethanol and washed with petroleum ether. The aqueous ethanol was removed and the "free" steroid (33,700 c.p.m.) was dissolved in 70% ethanol and washed with petroleum ether. The aqueous residue (33,700 c.p.m.) were subjected to countercurrent separation. The aqueous ethanol was removed and the "free" steroid (33,700 c.p.m.) was dissolved in 70% ethanol and washed with petroleum ether. The aqueous residue was treated with a 9% sodium bicarbonate solution and the ether was removed. The dry residue (66,000 c.p.m.) was submitted to countercurrent separation.

The isotope ratios of each metabolite were measured differentially with a Packard Tricarb liquid scintillation counter equipped with the Blau modification (8). It should be noted that the measured isotope ratios are not the true ratio of the two isotope but rather the ratio of that fraction of the total disintegrations from each of the two isotopes measured by the instrument under the experimental conditions employed.

**RESULTS**

**Estrone-Estradiol**—The first study was primarily designed to establish whether the approach was feasible. The mixture administered contained estrone-16-C\(^{14}\) and estradiol-6,7-H\(^3\) in a mass ratio of 330 and a measured isotope ratio of 0.54. Estrone obtained from the urine during the first half hour after administration had a measured isotope ratio of 0.59 and this value remained virtually constant for estrone throughout the study (Table I). During the first half hour the excreted estradiol contained so little C\(^{14}\) that this isotope could not be measured. Even by the 12th hour the measured isotope ratio for estradiol was different from the estrone isolated during this period; by the second day after the injection, however, the excreted estradiol had a ratio of C\(^{14}\) to H\(^3\) indistinguishable from the injected mixture.

In the second study a mixture containing equal weights of the two hormones and a measured isotope ratio of 1.16 was administered to a different patient (Table II). Again estrone during the first half hour after administration had nearly the isotope ratio of the injected mixture since the ratio of C\(^{14}\) to H\(^3\) for the metabolite was 1.32. By the end of the first hour this value was experimentally indistinguishable from that of the injected mixture. As in the initial study, the estradiol during this same interval had a measured ratio of 0.05 and 0.28 for the first two 30-minute periods. Even during the ninth hour after administration of the mixture of hormones, estradiol had an appreciably lower measured isotope ratio than either estrone or the injection mixture.

In the third experiment a mixture containing a 7 to 1 mass ratio of estrone to estradiol and a measured C\(^{14}\) to H\(^3\) ratio of 0.92 was administered. Again the estrone rapidly approached the isotope ratio of the injected mixture since during the first half hour after administration the ratio of C\(^{14}\) to H\(^3\) was 1.15 for the urinary metabolite. By the end of the second half hour the isotope ratio of estrone was indistinguishable from the injection mixture. In contrast, estradiol with C\(^{14}\) to H\(^3\) 0.08 and 0.39 for the first two half hours had a clearly different isotope composition. Not until the sixth hour after administration had the estradiol become isotope-indistinguishable from the estrone samples (Table III). During the time of greatest disparity in the measured isotope ratio of these metabolites, i.e. from zero through the fourth hour, the amount of either isotope lost from the body by excretion was too little (2% of the dose) to have had any measurable effect upon the isotope ratio of the compounds within the body.

**Estradiol**—The estradiol samples were almost indistinguishable isotopically from the corresponding estrone samples in the earliest collection containing sufficient estradiol radioactivity to permit differential isotope analysis. There was no significant change in the measured ratio at later times. The ability to detect estradiol in patients W and R, at the times recorded was possible only because of the large amount of radioactivity given in these studies.

**Other Metabolites**—The intentionally large amount of radioactivity in the third study permitted isolation of 16-spiestriol early in the study when differences in isotope content between estrone and estradiol were still substantial. The isotope ratio

**TABLE I**

<table>
<thead>
<tr>
<th>Subject M (age 43, multiple sclerosis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose: Estrone-16-C(^{14}), 1 mg, 2.7 (\mu)g; estradiol-6,7-H(^3), 3 (\mu)g, 20 (\mu)c in 1 ml of propylene glycol; measured C(^{14}) to H(^3) = 0.54.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mid-time</th>
<th>Estrone</th>
<th>Estradiol</th>
<th>Estriol</th>
<th>2-Methoxyestrone</th>
</tr>
</thead>
<tbody>
<tr>
<td>hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3/4</td>
<td>0.59*</td>
<td>0*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/4</td>
<td>0.56*</td>
<td>0.12*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/4</td>
<td>0.51*</td>
<td>0.15*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24/4</td>
<td>0.49*</td>
<td>0.18*</td>
<td>0.50*</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.50</td>
<td>0.26</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.55</td>
<td>0.43</td>
<td>0.44*</td>
<td>0.57*</td>
</tr>
<tr>
<td>36</td>
<td>0.56</td>
<td>0.57</td>
<td>0.53</td>
<td>0.55*</td>
</tr>
</tbody>
</table>

* Only peak tubes counted.

**TABLE II**

<table>
<thead>
<tr>
<th>Subject W (age 67, carcinoma of breast, oophorectomized and adrenalectomized)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose: Estrone-16-C(^{14}), 2 mg, 5.4 (\mu)c; estradiol-6,7-H(^3), 2 mg, 20 (\mu)c in 2 ml of propylene glycol; measured C(^{14}) to H(^3) = 1.16.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mid-time</th>
<th>Estrone</th>
<th>Estradiol</th>
<th>Estriol</th>
<th>2-Methoxyestrone</th>
</tr>
</thead>
<tbody>
<tr>
<td>hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>1.32</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3/2</td>
<td>1.10</td>
<td>0.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>1.15</td>
<td>0.34</td>
<td>0.98</td>
<td>1.10*</td>
</tr>
<tr>
<td>2/2</td>
<td>1.08</td>
<td>0.49</td>
<td>1.06</td>
<td></td>
</tr>
<tr>
<td>3/2</td>
<td>1.14</td>
<td>0.65</td>
<td>1.05</td>
<td>0.86*</td>
</tr>
<tr>
<td>4/2</td>
<td>1.09</td>
<td>0.80</td>
<td>1.03</td>
<td></td>
</tr>
<tr>
<td>7/2</td>
<td>1.15</td>
<td>0.93</td>
<td>1.11</td>
<td>0.97*</td>
</tr>
</tbody>
</table>

* Only peak tubes counted.
The “free” blood steroids at 30 minutes in Subject R (Table III) did not have the same isotopic composition as the conjugated steroids. This is presumed to result principally from the persistence of small amounts of the injected estrogens not withdrawn into tissues. The important fact is that the conjugated, i.e. these were metabolites released from the tissues. The only possible means by which tritium could appear in estrone was the oxidation in vivo of the administered estradiol. The blood and urine measurements at the earliest sampling showed that at least 90% of the tritium injected had been incorporated into estrone.

The early achievement of a C\(^{14}\) to H\(^3\) ratio in urinary estrone, virtually but not completely identical with that of the injected mixture of steroids, indicates that estradiol is initially and almost completely converted to estrone. Thus, Reaction I is rapid

\[
\text{(I) } \text{Estradiol} \rightarrow \text{Estrone}
\]

even when the mass ratio of estrone was 333 to 1 of estradiol. Conversely, the later appearance of a C\(^{14}\) to H\(^3\) ratio approaching that of the mixture injected indicates that this estradiol was formed secondarily from estrone. Therefore, Reaction II

\[
\text{(II) } \text{Estradiol} \leftarrow \text{Estrone}
\]
is slow. These results are the same for the three widely varying mass ratios studied. A necessary consequence of these rate differences is that estrone occupies a central position in estrogen metabolism.

The purpose of this study was to obtain information about the immediate precursor of estradiol (16α-hydroxylation), epiestriol (16β-hydroxylation), and 2-methoxyestrone (hydroxylation to form a catechol and subsequent methylation). It seems clear, from comparison of the isotopic ratios of these metabolites at the earliest times that they appeared in measurable quantities, that their isotopic composition closely approximated that of estrone and deviated considerably from estradiol. The simplest explanation of these facts is that estrone was the immediate precursor that was oxidized in the A and D rings. The interesting conclusion, then, is that estrone is the only direct metabolite of estradiol and all the other estrogens isolated represent further alterations of estrone.

The conclusions drawn from this study are summarized in Scheme 1. It is evident that 16-ketoestradiol (13, 14) and 16-ketoestrone (15) present problems with respect to their route of biogenesis. It is possible that 16-ketoestrone may be derived from oxidation of either 16α- or 16β-hydroxyestrone and selective reduction at C-17 of the diketone might explain the presence of the third known ring D ketol metabolite. Experiments to study the pathway involved in the production of these compounds are now in progress.

The rapidity of two reactions observed in this study deserves comment. The fact that in less than 30 minutes more than 98% of a large unphysiological amount of hormone had been removed from the circulation implies a highly effective mechanism for the capture of both estrone and estradiol by tissues. The speed of this process together with the very rapid oxidation of estradiol may be interpreted as an indication that:

1. A single organ with a characteristic concentrating mechanism and oxidative enzyme system dominates, in a quantitative sense, the metabolic fate of the estrogenic hormone. This does not imply that this organ (probably liver) is the sole site for the immediate localization of estrogen since it is clear that many tissues must be able to obtain a supply of the hormone. However, there must either be a great difference in the capacity of one organ and its oxidative enzyme system or a similar enzyme must be widely distributed in organs and tissues.

2. The systems responsible for the further metabolic alteration of the estrogen molecule, e.g., transformation to estradiol, must have a similar tissue distribution since so little estradiol or estrone circulates in the blood.

3. The biological activity of estrone may be due to the relatively slow reaction by which it is transformed to estradiol. On the other hand, considering the speed with which estrone is produced and the fact that all other metabolites are formed from estrone, it may be that this substance is in fact the circulating hormone as distinct from the product secreted by the glands. In this view, the reaction that regenerates estradiol

Estradiol → Estrone

\[ \text{Scheme 1. Routes of estrogen metabolism in man} \]

may be a means for the conservation and prolongation of estrogenic action.

4. Since in some species, particularly the rat, estradiol has a greater biological activity than estrone, it may be suggested that the rapid oxidation observed in the human may not occur to the same extent in these species. It is perhaps significant to note in this connection that reduction of estrone to estradiol-17α is characteristic of many animals (16) but is an insignificant reaction in the human, if indeed it occurs at all.

These considerations which arise from this study of the transformation of two related steroids, simultaneously administered, provide a basis for many other investigations of the dynamics of estrogen metabolism. It is believed that further study of these basic biochemical facts may well provide the means for understanding how these hormones achieve their profound effects upon tissues and the metabolic systems under hormonal control.

**SUMMARY**

A mixture of estrone-16-C¹⁴ and estradiol-6,7-H² in varying mass and isotope ratio was administered to three patients. Comparison of the measured isotopic ratio in estrone and estradiol in successive urine collections demonstrated that oxidation of estradiol to estrone must be more rapid than reduction of estrone to estradiol. From the measured isotopic ratio of other metabolites isolated it was concluded that estradiol, epiestriol, and 2-methoxyestrone are derived from estrone and not from estradiol.

**Acknowledgments**—The authors wish to express their gratitude to Drs. Leon Hellman and Barnett Zumoff for their assistance with the patients. They also wish to thank Dr. Marcel Gut for his gift of estradiol-6,7-H². The technical assistance of Rosemarie Lehman and Ruth Jandorek is also gratefully acknowledged.

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