THE METABOLIC FATE OF ESTRONE IN BILE FISTULA DOGS*

By W. H. PEARLMAN,† A. E. RAKOFF, K. E. PASCHKIS, A. CANTAROW, AND A. A. WALKLING

(From the Departments of Biochemistry, Obstetrics and Gynecology, Physiology, Medicine, and Surgery, Jefferson Medical College, Philadelphia)

(Received for publication, October 14, 1947)

It has been repeatedly demonstrated that, following the administration of estrogen to human or animal subjects, the hormone disappears rapidly from the organism and only a small quantity of the original compound or its metabolites can be recovered in crystalline form from the urine (2). On the other hand, Cantarow et al. (3) detected in the bile of dogs with external bile fistula as much as 90 to 95 per cent of the biological activity of the estrone or α-estradiol which had been injected intravenously. Since bioassay had been performed directly on the bile without resort to fractionation procedure, it was thought desirable to pursue the problem of biliary excretion of estrogens along chemical lines. Accordingly, a total quantity of 1.476 gm. of estrone acetate (containing 1.278 gm. as estrone equivalent to 18,000,000 mouse units of estrogenic activity) was dissolved in oil and injected intramuscularly into three external bile fistula dogs. The bile, urine, and feces were collected and extracted; 79 mg. of estrone and 18 mg. of α-estradiol were isolated in crystalline form from the bile; considerably less estrogenic material as determined by bioassay was contained in the urine or feces. A detailed report is given below.

Table I lists the estrogenic activity of the phenolic material derived from the bile, urine, and feces respectively; bioassay of unfractionated bile was also performed. Inspection shows that (a) considerably more estrogen was excreted in the bile than in the urine; a small but significant amount of estrogen appeared in the feces; (b) most of the estrogenic material of the bile and of the feces was present in a free or uncombined form; much of the estrogenic material in the urine was present in conjugated form, but the ratio of free to conjugated estrogen varied considerably.

Table II indicates the distribution of the estrogenic activity of the various extracts between the ketonic and non-ketonic weakly acidic phenols and the strongly acidic phenols. Hydrolysates of the conjugated phenolic material of the bile and feces were not partitioned because of the*

* This work was supported by grants-in-aid from the United States Public Health Service, under the National Cancer Institute Act, and from the Ciba Pharmaceutical Products, Inc., Summit, New Jersey. A preliminary report (1) was presented before the meeting of the American Society of Biological Chemists at Chicago, May, 1947.

† In the Department of Biochemistry.
low content of estrogen. It may be seen from Table II that (a) almost all of the estrogenic activity of the extracts (bile, urine, and feces) resided in the weakly acidic phenols, and (b) a variable proportion (22 to 88 per cent) of the total biological activity was due to the non-ketonic fraction of the weakly acidic phenols.

Table III gives the percentage recovery in the bile of the biological activity (mouse units) of the hormone injected, as well as that of estrogenic material (mg. of estrone plus α-estradiol).
TABLE II

Distribution of Estrogenic Activity*

The results are expressed in per cent for each collection period.

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Source extracted</th>
<th>Post injection collection period</th>
<th>Weakly acidic phenols</th>
<th>Strongly acidic phenols†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ketonic</td>
<td>Non-ketonic</td>
</tr>
<tr>
<td>69</td>
<td>Bile (free phenols)</td>
<td>1 + 2</td>
<td>78</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 + 4</td>
<td>42</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Urine &quot; &quot;</td>
<td>1 + 2</td>
<td>34</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 + 4</td>
<td>33</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>&quot; (conjugated phenols after hydrolysis)&quot;</td>
<td>1 + 2</td>
<td>21</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 + 4</td>
<td>16</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Feces (free phenols)</td>
<td>1 + 2 + 3 + 4</td>
<td>10</td>
<td>88</td>
</tr>
<tr>
<td>B72</td>
<td>Bile &quot; &quot;</td>
<td>1 + 2</td>
<td>71</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 + 4</td>
<td>37</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Urine &quot; &quot;</td>
<td>5</td>
<td>11</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 + 2</td>
<td>73</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Feces &quot; &quot;</td>
<td>3 + 4</td>
<td>25</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 + 3 + 4</td>
<td>7</td>
<td>88</td>
</tr>
<tr>
<td>B75</td>
<td>Bile &quot; &quot;</td>
<td>1</td>
<td>26</td>
<td>71</td>
</tr>
</tbody>
</table>

* Determined by bioassay (see foot-note, Table I).
† The actual values may be even lower, since after further partitioning of pooled strongly acidic phenols (8200 mouse units) of the bile, the 0.3 M Na₂CO₃-soluble phenols assayed only 1200 mouse units. Similarly, the corresponding fractions of the urine after pooling assayed 7500 mouse units, but after further partitioning assayed only 3200 mouse units.

TABLE III

Percentage Recovery in Bile of Estrogenic Activity and Estrogenic Substance (for Entire Collection Period)

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Based on bioassay of</th>
<th>Based on calculated* content of estrone and α-estradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bile directly (aqueous injections)</td>
<td>Total phenolic material of bile (oil injections)</td>
</tr>
<tr>
<td>69</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>B72</td>
<td>35</td>
<td>29</td>
</tr>
<tr>
<td>B75</td>
<td>15</td>
<td>15 16</td>
</tr>
</tbody>
</table>

* It is assumed that the biological activity of the bile is due entirely to the above substances. These figures may be calculated from those given in Tables I and II and from the bioassay values for 17 each of estrone and α-estradiol in oil which are 14 and 33 mouse units respectively in this laboratory.

It is evident from Tables I and II that most of the biological activity of the bile was due to the weakly acidic phenols (unhydrolyzed). This ma-
terial appeared to be suitable for isolation work. Accordingly, the ketonic fractions which were derived from the bile were pooled; 123 mg. of crystalline material (1,500,000 mouse units equivalent to 107 mg. of estrone) were obtained. The material was recrystallized from alcohol, yielding 79 mg. of impure estrone, m.p. 252–256°, which, on subsequent purification, proved to be identical with an authentic specimen. From the non-ketonic weakly acidic phenols (unhydrolyzed bile) there were obtained 118 mg. of a semi-crystalline product (1,600,000 mouse units equivalent to 48 mg. of α-estradiol). It yielded 29 mg. of digitonin-precipitable material which, on crystallization from alcohol, gave 18 mg. of α-estradiol. The identity of this product was established by ascertaining its specific optical rotation and by a determination of the melting point on admixture with an authentic specimen of α-estradiol; a dibenzoyl derivative was also prepared which gave the expected carbon and hydrogen values on analysis. The non-digitonin-precipitable material was submitted to chromatographic analysis, but β-estradiol could not be isolated.

EXPERIMENTAL

Collection and Extraction of Bile, Urine, and Feces Bile was collected in 24 hour periods, and after adjusting the pH to 6.5 to 7.5, it was immediately extracted by gentle shaking with n-butanol; for each 100 ml. of bile, 50 ml. of solvent were used in the initial extraction, followed by four extractions each with 10 ml. portions. The butanol extracts were combined, washed twice with 10 ml. portions of water, and evaporated in vacuo. The butanol residue was dissolved in 25 ml. of water, made distinctly acid to litmus with concentrated HCl, and extracted once with 100 ml. and then twice with 50 ml. of ether. The ether extracts were combined, back-washed with 10 ml. of water, washed successively with dilute sodium bicarbonate and water, and evaporated. The ether residue was dissolved in benzene and extracted four times with equal volumes of NaOH; the alkaline extracts were back-washed with 0.2 volume of benzene. The benzene solution was washed with water and evaporated to give the neutral fraction (unhydrolyzed bile). The alkaline extracts were made acid to Congo red with concentrated HCl and thoroughly extracted with ether in order to obtain the phenolic fraction (unhydrolyzed bile). The acid fraction (unhydrolyzed bile) was obtained from the residual bile (after butanol extraction) by acidification to Congo red with concentrated HCl followed by extraction with ether. Some acidic material was also obtained from the sodium bicarbonate washes of the ether-soluble, water-insoluble fraction of the butanol residue described above. The water-soluble, ether-insoluble fraction of the butanol residue was heated on the water bath to remove dissolved ether; after the addition of 10 per cent by volume of concentrated
HCI, the solution was refluxed for 10 minutes, rapidly cooled, and extracted with ether. The phenolic fraction (hydrolyzed bile) was obtained from the ether extract in the usual way.

Urine was collected in 24 hour periods and immediately extracted with butanol; the phenolic fractions were obtained as above. Feces were ground with sand and extracted with liberal amounts of alcohol at room temperature. The residue obtained on evaporation of the alcohol extracts was partitioned into "free" phenols and conjugated phenols; the latter were subsequently hydrolyzed.

Fractionation of Phenolic Material—The strongly acidic and weakly acidic phenolic fractions were obtained by the method of Mather (4) by distributing the phenols between benzene and 0.3 M Na₂CO₃. Repeated partitioning was necessary in order to achieve effective separations, especially with bile extracts. Troublesome emulsions were often encountered which were broken by long centrifugation. The weakly acidic phenols were separated into ketonic and non-ketonic moieties with the aid of Girard’s Reagent T (5).

Isolation and Identification of Estrone (Unhydrolyzed Bile)—The ketonic, weakly acidic phenols (unhydrolyzed bile) excreted by Dog 69 (1 + 2 + 3 + 4 days), Dog B72 (1 + 2 + 3 + 4 + 5 days), and by Dog B75 (1 day) were pooled. Since the estrogenic content of bile collected subsequently was comparatively small, this material was not included. The pooled material was crystalline, weighed 123 mg., and assayed 1,500,000 mouse units (equivalent by our method of bioassay to 107 mg. of estrone). It was recrystallized from alcohol to give 79 mg. of impure estrone, m.p. 252–256°; an additional crop of crystals, m.p. 225–232°, was obtained from the mother liquors. Repeated crystallization of the former product yielded 45 mg., m.p. 258–260°; [α]₂⁰ = +170° (0.752 per cent in dioxane); admixture with authentic estrone, m.p. 259–260°, [α]₂⁰ = +163° (in dioxane), gave no depression in melting point. A benzoyl derivative was prepared which melted at 219.5–221° and gave no depression in melting point on admixture with authentic estrone benzoate, m.p. 222–223°.

Isolation and Identification of α-Estradiol (Unhydrolyzed Bile)—The non-ketonic, weakly acidic phenols corresponding to the ketonic phenols above were pooled. There were obtained 118 mg. of a semicrystalline product which assayed 1,600,000 mouse units (equivalent by our method of bioassay to 48 mg. of α-estradiol). After treatment with 400 mg. of digitonin by a method described by Huffman et al. (6), there were obtained 29 mg. of digitonin-precipitable material. It crystallized readily from aqueous alcohol to give 18 mg., m.p. 173–175°, [α]₃¹ = +77° (0.710 per cent

1 All melting points reported here are corrected.
in absolute ethanol). Further recrystallization from the same solvent raised the melting point to 175°; no melting point depression was observed on admixture of this product with authentic α-estradiol, m.p. 176°, [α]_D = +78° (in absolute ethanol).

A dibenzoyl derivative was prepared from 14 mg. of the above product, m.p. 173°-175°. It was repeatedly crystallized from chloroform-ethanol to give 9 mg., m.p. 169.5°-170°; admixture with authentic α-estradiol dibenzoate, m.p. 170°-170.5°, gave no depression in melting point.

C_{12}H_{10}O_4. Calculated, C 79.96, H 6.72; found, C 79.80, H 6.74

Chromatographic analysis of the non-digitonin-precipitable fraction (58 mg.) failed to yield any crystalline products.

**DISCUSSION**

Whereas previous evidence (2) for the in vivo conversion of estrone into α-estradiol has been entirely circumstantial, the evidence presented here is based on the isolation of the metabolite in crystalline form. It cannot be assumed, however, that the reduction of estrone proceeds in this direction in all mammalian species, especially since β-estradiol but no α-estradiol was isolated from the urine of rabbits injected with estrone (7); the β isomer was likewise isolated in similar experiments (8, 9) in which α-estradiol was injected. In the present study, β-estradiol could not be isolated from the bile.

The reverse process, i.e. the biological conversion of α-estradiol into estrone, has been clearly demonstrated (2); however, in the case of the dog, the evidence (10, 11) is of an indirect nature. It might be inferred that these processes take place in the liver, since estrogens of endogenous and exogenous origin have been isolated from bile; some support for this hypothesis has been obtained from in vitro experiments (13, 14).

It appears questionable from our data that the dog is capable of transforming estrone into estriol to any significant extent. On the other hand, Longwell and McKee (15) detected a significant degree of estrogenic activity in the 0.3 M Na_2CO_3-soluble, benzene-insoluble phenolic fraction of bile obtained from dogs injected with small quantities of estrone. (Of the native estrogens, estriol alone is partitioned in this manner (4, 16).) Pearlman et al. (11) likewise detected some biological activity in similar material obtained from dogs injected with α-estradiol.

* Estrone has recently (12) been isolated from the bile of pregnant cows; it is the major estrogen of the bile.
* See foot-note, Table II. It is quite likely that most of the biological activity exhibited by the strongly acid phenols was, in our experiments, due to slight contamination with the highly active, weakly acidic phenols.
Our experience, in common with that of most investigators working in the field of estrogen metabolism, indicates that a major portion of the estrogenic substance administered cannot be accounted for in the excreta as biologically active material. Chromatographic analysis of the neutral material and of the free acids of the pooled specimens of unhydrolyzed bile was undertaken, but none of the hypothetical products of estrogen inactivation was isolated.

Our data (see Table III) on the percentage recovery in the bile of the biological activity of the injected hormone are in closer agreement with the data of Longwell and McKee (15) than with those of Cantarow et al. (3). The former authors observed a biliary excretion ranging from 1.3 to 8.0 per cent, whereas the latter authors reported an almost quantitative recovery of hormonal activity. Pearlman et al. (11) reported that 10 per cent or possibly 26 per cent (bioassays performed independently in the two laboratories) of the biological activity of the injected α-estradiol was detected in the bile. In a study which apparently was the first of its kind on this subject, Stamler (18) reported a biliary excretion of "not less than 13 per cent" of the estrone injected. It is difficult to explain these discrepancies. Cantarow et al. (3) have emphasized the necessity of insuring a satisfactory state of nutrition and liver function in bile fistula dogs, since otherwise relatively insignificant hepatic functional defects may have a profound influence upon the metabolism of the steroid hormones.

Considerably more estrogen is excreted in the bile than in the urine following the administration of estrone acetate (see Table I). On the other hand, Longwell and McKee (15) reported a biliary excretion ranging between 1.3 to 8.0 per cent of the injected hormone and a urinary excretion between 6.4 and 13.5 per cent. Perhaps no great significance can be attached to this point of difference if one considers the unphysiological dose of estrogen employed in our experiments which, in this instance, were designed primarily to ascertain the nature of the metabolites of estrone rather than to obtain information concerning the physiological transport of estrogen. Yet, in a study by Cantarow et al. (21) in which a comparatively small amount of estrone (250,000 i.u.) was injected intravenously, it

4 The neutral material of unhydrolyzed bile contained less than 20,000 mouse units. This precludes the possibility that estrone acetate was present and attests to the quantitative removal of unesterified estrogen from this fraction.

5 The free acids of unhydrolyzed bile contained only 3300 mouse units. This makes it appear unlikely that any but trace amounts of the highly active compounds of the doisyionic acid series were present. It had been suggested that such compounds might occur naturally (17).

6 The subject of nutrition and endocrinology has recently been reviewed by Hertz (19) and also by Biskind (20).
was reported that the amount of estrogen in the urine was much less than that in the bile.

The fact that a small but none the less significant degree of estrogenic activity is exhibited by the phenolic material of the feces of bile fistula dogs (all the bile draining externally) is an indication that estrogen entered the bowel from the bloodstream by passage through the intestinal wall. Perhaps this mechanism operates under normal physiological conditions; it would account, in part at least, for the estrogen content of the feces of pregnant women (22) or of pregnant cows (23).

Our findings with regard to the chemical nature of the estrogens of dog feces parallel (perhaps fortuitously) those obtained by Levin (23) in his investigation of the fecal estrogens of pregnant cows in that (a) the estrogen is present chiefly in a free or unconjugated form, and (b) the biological activity resides chiefly in the non-ketonic, weakly acidic phenols.

**SUMMARY**

Massive doses of estrone, as the acetate, were injected intramuscularly into three external bile fistula dogs. A small quantity of estrone and of \( \alpha \)-estradiol was isolated from the pooled bile specimens. By comparison, the urine and feces contained much less estrogen as determined by bioassay. Most of the estrogenic substance administered cannot be accounted for in the excreta.

The implications of these findings are discussed.

The technical assistance of Miss Emily Cerceo, Miss Edith Goldberg, and Miss Dorothy Ozer is gratefully acknowledged. We are indebted to Mr. James Rigas for the microanalysis.

Part of the estrone used was kindly furnished by the Schering Corporation, Bloomfield, New Jersey.

**BIBLIOGRAPHY**

7. Stroud, S. W., *J. Endocrinol.*, 1, 201 (1939).
THE METABOLIC FATE OF ESTRONE IN BILE FISTULA DOGS
W. H. Pearlman, A. E. Rakoff, K. E. Paschkis, A. Cantarow and A. A. Walkling


Access the most updated version of this article at http://www.jbc.org/content/173/1/175.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/173/1/175.citation.full.html#ref-list-1