Studies of the Metabolism of 17α-Estradiol[1] in Man

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ABSTRACT. When 50 mg of 17α-estradiol was given orally to each of 2 male subjects, estrone was isolated as metabolite from the urine; only trace amounts, at most, of 17β-estradiol could be detected. Of the total dose of 17α-estradiol administered, only 2.2 and 3.4%, respectively, were recovered in the estrone, estradiol and estriol fractions, as determined by the method of Brown, Bulbrook and Greenwood (4). In comparative experiments with 17β-estradiol, 20% of the administered dose was recovered. Similarly, 17α-estradiol was metabolized to a greater extent than 17β-estradiol during incubation with human liver slices. The results obtained show that a 17α-hydroxysteroid oxidoreductase is present in man and that the metabolism of 17α-estradiol *in vivo* as well as *in vitro* differs from that of 17β-estradiol. (*J Clin Endocr* **26:** 533, 1966)

RECENTLY, Schott and Katzman (1) described a method for the separation and quantitative estimation of 17α-estradiol. During the course of these studies, the possible presence of 17α-estradiol in human pregnancy urine was also investigated. In confirmation of a previous statement by Marrian (2), no 17α-estradiol could be isolated from pooled urines of pregnant women; strongly suggestive evidence, however, for the presence of this estrogen was obtained in two different specimens of urine collected from an individual during the last week of pregnancy. The substance behaving like 17α-estradiol was liberated by β-glucuronidase in amounts estimated to be from 40 to 80 μg/liter. It was suggested that the failure to detect regularly 17α-estradiol in human urine may partly be due to its rapid metabolic alteration. It seemed therefore of interest to study the fate of 17α-estradiol in the human.

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[1] 17α-Estradiol [estra-1,3,5(10)-trien-3,17α-diol]. The systematic names of the other steroids mentioned in this manuscript are stated in brackets after the trivial names as follows: 17β-estradiol [estra-1,3,5,(10)-trien-3,17β-diol]; estrone [estra-1,3,5(10)-trien-3-ol,17-one]; estriol [estra-1,3,5(10)-trien-3,16α,17β-triol]; 16-epi-estriol [estra-1,3,5(10)-trien-3,16β,17β-triol]; 17-epi-estriol [estra-1,3,5(10)-trien-3,16α,17α-triol]; 16,17-epi-estriol [estra-1,3,5(10)-trien-3,16β,17α-triol]; 16α-hydroxyestrone [estra-1,3,5(10)-trien-3,16α-diol, 17-one]; 16β-hydroxyestrone [estra-1,3,5(10)-trien-3,16β-diol-17-one]; 11-dehydro-17α-estradiol [estra-1,3,5(10), 11(12)-tetraen-3,17α-diol]; cis-testosterone [androst-4-en-17α-ol-3-one].

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Materials and Methods

The experiments were carried out on 4 healthy male subjects (age: 35–40 years). Urines were collected at 24-hr intervals, diluted to 1200 ml and stored at −10 C until used. Aliquots of 200 ml were filtered through a Sephadex column (G-25) according to Beling (3). The filtrates were acidified to pH 5.2 with acetic acid, buffered to this pH by the addition of 0.1 vol of m-acetate buffer and incubated at 37 C with an extract from *Helix pomatia* (1 ml, containing 50,000 Fishman units of β-glucuronidase and 25,000 Whitehead units sulfatase) for 48 hr. The hydrolyzed mixture was extracted twice with 0.67 vol of ether; the extracts were combined and processed following the method of Brown, Bulbrook and Greenwood (4). The methylated estrone, estradiol and estriol fractions thus obtained were measured by the Kober reaction under the optimal conditions as described by Nocke (5); in some experiments, the Kober color was extracted according to Ittrich (6). The methylated estradiol fractions obtained after oral administration of 17α-estradiol were also subjected to the 2 Kober reagents as described by Haenni (7). One of these (reagent B) is specific for 17α-estradiol, whereas reagent A yields the Kober color with both 17α- and 17β-estradiol; for further details, see (1). In control experiments, it was found that after addition of 20 μg of estrone, 17β-estradiol, 17α-estradiol and estriol to male urine and subsequent enzymatic hydrolysis, 85, 78, 82 and 83%, respectively, could be recovered. All

TABLE 1. Urinary excretion of estrogens by two male subjects following oral administration of 50 mg of 17α-estradiol. 17α-Estradiol was administered on day 2. The estrogen fractions were determined by the method of Brown, Bulbrook and Greenwood (4).

Subject	Day	Amounts of estrogens in urine (μg/24 hr)		
		Estrone	Estradiol*	Estriol
A	1	4	2	5
	2	38	657	15
	3	30	532	17
	4	16	279	11
	5	6	92	5
	6	5	59	0
B	1	2	2	7
	2	15	565	25
	3	11	460	23
	4	7	355	14
	5	8	190	12
	6	5	73	18

* In this fraction, only 17α-estradiol could be identified with certainty.

values given in the paper are corrected for methodological losses.

For subsequent qualitative experiments, separation of the nonpolar and the polar fractions was carried out by distribution between water and benzene-petroleum ether (1:1), according to Brown (8). The nonpolar fractions obtained from 200 ml of urine were treated at room temperature for 24 hr with 100 mg of trimethylammonium hydrazide chloride in 10 ml of ethanol and 1.5 ml of acetic acid. The ketonic and nonketonic fractions were obtained in the usual way.

Free estrone was chromatographed on an alumina column, using benzene with 0.5% ethanol as eluent (9). Paper chromatography of the nonmethylated estrogen fractions was carried out at room temperature on formamide-impregnated paper, using, as mobile phases, monochlorobenzene and chloroform, respectively [for details, see (10)]. Microsublimation under atmospheric pressure and melting point determination were performed as described previously (11).

Samples of liver tissue were obtained from 2 patients at laparotomy. The liver tissue was taken immediately to the laboratory and sliced according to Deutsch (12). In each experiment, 100 μg of steroid, dissolved in 0.025 ml of ethanol, was incubated with 200 mg of liver slices in 3 ml of Krebs-Ringer-phosphate saline, pH 7.4, containing 20 mM glucose, at 37 C for 60 min. At the end of incubation, the mixtures were extracted 3 times with equal volumes of ether, and the ether extracts were combined and evaporated *in vacuo*. The residues were then chromatographed on formamide-impregnated paper with monochlorobenzene. The estrogens [estrone (R_F value 0.76), 17α-estradiol (R_F value 0.46) and 17β-estradiol (R_F value 0.39)] were eluted, the eluates were methylated and the 3-methyl ethers were chromatographed on alumina columns (4, 8). Quantitative determinations were carried out by the Kober reaction, as described by Breuer and Nocke (13).

Results

After oral administration of 50 mg of 17α-estradiol to each of two male subjects, an increase in the excretion of the estrone, estradiol and estriol fractions in urine was observed (Table 1). The increase was most pronounced in the estradiol fraction, with small but significant increases in the estrone and estriol fractions. Highest levels of excretion occurred on the day of administration. The total recovery in the three fractions was 2.2% for subject A and 3.4% for subject B. These values are low by comparison with those found for 17β-estradiol (Fig. 1). When the latter was given orally under similar experimental conditions, recovery as estrone, 17β-estradiol and estriol amounted to 20%.

Qualitative experiments were carried out to obtain further information concerning the nature of the estrogens present in the estrone, estradiol and estriol fractions after oral administration of 17α-estradiol. For this purpose, aliquots of the urines from the first and second days following ingestion of 17α-estradiol were separated into the polar ("estriol") fraction, and into the nonpolar ketonic ("estrone") and nonpolar nonketonic ("estradiol") fractions.

The nonpolar ketonic fraction contained a Kober chromogen which behaved like estrone when chromatographed on an alumina column. After sublimation, the melting point of the estrone-like material (254–256 C) was identical with that of authentic estrone. When the Kober chromogen was treated with dimethyl sulfate, the methylated compound showed, after chromatography on alumina, the same

elution pattern as authentic estrone 3-methyl ether.

The nonpolar nonketonic fraction contained two Kober chromogens which, after paper chromatography on formamide-impregnated paper with monochlorobenzene, had the same R_F value as 17α-estradiol (0.46) and 17β-estradiol (0.39); however, the compound with the mobility of 17β-estradiol was present only in minute quantities. Furthermore, the material was subjected to the two Kober reactions as described by Haenni (7). By the use of these two reactions, 17α-estradiol and 17β-estradiol may be detected in the presence of one another (1, 7). In the nonpolar nonketonic fractions thus investigated, evidence was obtained for the presence of 17α-estradiol, whereas 17β-estradiol could not be detected with certainty.

Owing to the large amounts of impurities present, it was necessary to purify the polar fraction before further investigation. After saponification and chromatography on formamide-impregnated paper with chloroform, various phenolic substances were detected. The one present in highest amounts had the same R_F value (0.04) as estriol; when this substance was treated with dimethylsulfate, the methylated compound showed, after chromatography on alumina, the same elution pattern as authentic estriol 3-methyl ether. Two additional polar substances were found which, in their chromatographic behavior, resembled 16-epi-estriol and 17-epi-estriol. Their identity, however, could not be established with certainty.

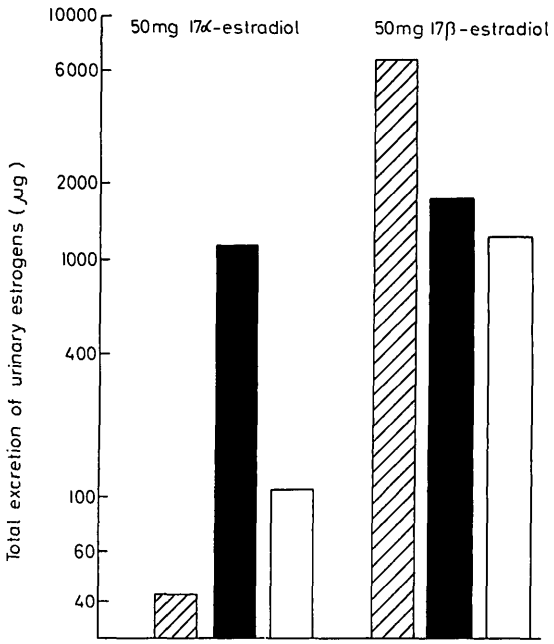

FIG. 1. Total excretion of urinary estrogens after oral administration of 50 mg of 17α-estradiol (2 male subjects) and of 50 mg of 17β-estradiol (2 male subjects). The columns represent mean values of estrogens excreted by 2 subjects over a 5-day period. Striped bar, estrone fraction; solid bar, estradiol fraction; open bar, estriol fraction.

To find whether differences also exist *in vitro* between the metabolism of 17α-estradiol and 17β-estradiol, both steroids were incubated with human liver slices. The results of these experiments are summarized in Table 2. It can be seen that during 60 minutes of incubation 17α-estradiol is metabolized to a greater extent than 17β-estradiol; no estrone could be detected in the experiments with 17α-estradiol.

TABLE 2. Metabolism of 17α-estradiol and 17β-estradiol by human liver slices obtained at laparotomy. In each experiment, 100 μg of steroid was incubated with 200 mg of liver slices at 37 C for 60 minutes.

Estrogen incubated	Estrogen found	Amount of estrogen (μg)	
		Patient I	Patient II
17α-estradiol	17α-estradiol	7.4	13.5
	estrone	0	0
17β-estradiol	17β-estradiol	22.5	28.1
	estrone	3.5	1.2

Discussion

The results reported here show that 17α-estradiol is oxidized to estrone in the human organism. This oxidation is catalyzed by a 17α-hydroxysteroid oxidoreductase whose presence in man has previously been demonstrated both *in vitro* and *in vivo*. Thus, on incubation with human liver tissue, 17-epi-estriol was formed from 16α-hydroxyestrone (14) and 16,17-epi-estriol from 16β-hydroxyestrone (15). Further, 17-epi-estriol and 16,17-epi-estriol were isolated from the urine of men injected with 16α-hydroxyestrone and 16β-hydroxyestrone, respectively (16).

Two marked differences in the metabolism of 17α-estradiol and 17β-estradiol in man have been found in the comparative studies described in the present communication. Under the experimental conditions employed, 17α-estradiol is metabolized both *in vitro* and *in vivo* to a greater extent than 17β-estradiol. Moreover, only small amounts of estrone are formed from 17α-estradiol *in vivo*, whereas 17β-estradiol yields large amounts of estrone; similar observations were made for the estriol fractions. Consequently, the total recovery of 17α-estradiol as estrone, estradiol and estriol is much lower (approximately 3%) than that of 17β-estradiol (20%) in similar experiments. The rapid disappearance of estrone and estriol-like compounds shows that 17α-estradiol, in contrast to 17β-estradiol, is metabolized to a large extent to as yet unidentified compounds. Accordingly, a search for these degradation products might well be rewarding.

Thus far, the following 17α-hydroxylated C_{18}- and C_{19}-steroids have been isolated from human urine: 17-epi-estriol (17), 16, 17-epi-estriol (18) and *cis*-testosterone (19–21). More recently, evidence for the presence of 11-dehydro-17α-estradiol has been reported by Luukaainen and Adlercreutz (22). The physiological significance of these 17α-hydroxysteroids is difficult to envisage since their biological properties have not yet been studied in detail. On the basis of the present findings, two reasons may be given for the failure to isolate 17α-estradiol from human urine. First, 17α-estradiol may be formed, owing to the relatively low activity of the 17α-hydroxysteroid oxidoreductase, only in very small amounts. Second, 17α-estradiol is subjected to rapid metabolism to as yet unidentified products. It may well be, then, that, by processing huge amounts of urine and employing special techniques, 17α-estradiol can be isolated from human urine.

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