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THE METABOLISM OF ESTRADIOL; ORAL COMPARED TO INTRAVENOUS ADMINISTRATION

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Summary—We administered [6,7-^{3}H]estradiol p.o. and [4-^{14}C]estradiol i.v. simultaneously to 5 women 22–35 years of age. Fourteen blood samples were collected over 480 min, and all urine was collected for 96 h. The blood samples were analyzed for radioactivity as estradiol, estrone, estrone-sulfate and estradiol glucuronide. The urine samples were analyzed for radioactivity as the glucuronide and pH 1 hydrolyzable conjugates of estradiol, estrone, estriol, 16α-hydroxy-estrone, 2-hydroxy-estrone, 2-hydroxy-estradiol, 2-methoxy-estradiol and 2-methoxy-estrone. The major circulating estrogen, after either estradiol, p.o. or i.v. administration, was estrone sulfate; ~ 50% of estradiol administered by either route being converted to and measured as estrone sulfate in the blood. Following oral administration about twice as much estradiol was converted to and measured as estradiol glucuronide in the blood as after i.v. administration. Of the estradiol administered p.o., only 10% was absorbed into the blood as estradiol the rest being metabolized prior to absorption. After estradiol, p.o., the major radioactive compounds in the urine were the glucuronides of estrone and estradiol, but after estradiol, i.v., the conjugates of estrone, estradiol and estriol were present to about the same extent as the conjugates of the 2-oxygenated compounds. Following p.o., considerable metabolism of estradiol administration occurs in the splanchnic tissue, much of it in the intestinal wall.

INTRODUCTION

The daily blood production rate of estradiol ranges from 70–500 μg/day in young women depending on the time of their menstrual cycle [1]. However, to achieve effects on the uterus or bones similar to this production rate, estradiol must be administered orally at levels of 2–4 mg/day [2, 3]. This discordance between the normal production rate and the oral dose has been thought to be due to the extensive initial metabolism by splanchnic tissue of the orally administered estradiol.

Beer and Gallagher [4] noted that the fraction of the radioactive dose measured in the urine was similar after oral as compared to the intramuscular administration of estradiol indicating that the oral dose entered the circulation. However, they did not identify the circulating estrogens and compare them after the different doses.

Fishman *et al.*[5] administered [^{3}H]estradiol i.v. and [^{14}C]estradiol p.o. simultaneously to 2 elderly women. They noted rapid and efficient absorption of the orally administered estradiol. They noted that the urinary glucosiduronates of estrone and estradiol were relatively enriched with ^{14}C from the oral estradiol. The ^{3}H/^{14}C ratios in the other urinary metabolites were not so enriched and the ratios were similar among them. They concluded that a portion of the orally administered estradiol was conjugated with glucuronic acid in the splanchnic bed, but that most of the estradiol mixed with the intravenously administered estradiol.

There have been a number of recent studies [1, 6, 7] measuring the circulating levels of estradiol and estrone after oral administration of estradiol. These studies have shown that following the oral administration of estradiol, the major unconjugated estrogen in the blood is estrone.

Despite these studies, the fractional absorption of orally administered estradiol and the degree of splanchnic metabolism in young women remain uncertain. The present studies were done to characterize the absorption of orally administered estradiol, to compare the metabolism of estradiol administered p.o. to that of estradiol administered i.v. and to provide baseline data for subsequent studies on how these parameters might be affected by alterations in diet.

EXPERIMENTAL

The subjects were 5 women whose mean ± SD age was 27 ± 5 years, mean weight was 58.2 ± 5.4 kg and mean height was 100.1 ± 4.6 cm. All were having regular menstrual cycles as characterized by duration, rise in body temperature and/or rise in progesterone in the

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luteal phase. They were taking no medicines, and were eating a regular diet containing 25–40% fat. The studies were started on days 5–7 of the menstrual cycle. All subjects signed informed consent which had been approved by the Institutional Review Boards.

Organic solvents, except those used in HPLC, were used without further purification. Solvents for HPLC were obtained as HPLC Grade from J. T. Baker Co. [6, 7^{3}H]Estrone (47 Ci/mmol) and [^{14}C]estradiol (51 mCi/mmol) were obtained from New England Nuclear Corporation, Boston, MA, and were more than 98% pure by thin layer chromatography [8]. Estrone and estradiol were obtained from Steraloids, Wilton, NH, and crystallized from methanol prior to use. 16α-Hydroxyestrone was obtained from Steraloids and used without further purification. Estrone sulfate was obtained from Sigma Co., St Louis, MO, and used without further purification. 2-Hydroxyestrone, 2-hydroxyestradiol, 2-methoxyestrone and 2-methoxyestradiol were made as previously described [9, 10].

All studies were started between 7:30–8:00 a.m. with the subjects fasting and supine. A small Teflon catheter was placed in a vein in one arm. The subject then received 1.5 μCi [4-^{14}C]estradiol in 10 ml of 8% ethanolic saline in a vein of the arm opposite to the catheter. At the same time, she ingested a small sugar cube onto which had been placed 10 μCi [6, 7-^{3}H]-estradiol and 8 μg estradiol in 0.1 ml ethanol. The mass of estradiol administered p.o. and i.v. was similar since we wished to compare metabolism by these routes and did not wish to risk variations due to differences in mass. Blood samples, 4–20 ml, were obtained via the catheter at 3, 6, 10, 15, 30, 45, 60, 90, 120, 180, 240, 360 and 480 min after receiving the radiolabeled steroids. All samples were centrifuged and the plasma frozen until analyzed. All urine was collected for 96 h after receiving the radiolabeled estradiol.

Plasma samples were thawed and to them were added 200 μg estrone, 200 μg estradiol and 300 μg estrone sulfate. Samples were extracted twice with 80 ml cyclohexane–ethylacetate (2:1, v/v). The solvent phase was washed with 5 ml vol of H_2O and dilute $NaHCO_3$ and the solvent removed under vacuum. Estrone and estradiol were then purified by multiple chromatographic derivatization steps as described [10].

The spent plasma was adjusted to pH 5.0 with hydrochloric acid and 300 U/ml of β-glucurase (Sigma Co., St Louis, MO). The plasma was incubated at 37°C for 48 h and 200 μg estrone and 200 μg estradiol were added. The plasma was then extracted with cyclohexane–ethylacetate as described above. The solvent was pooled, washed and removed under vacuum. The estrone and estradiol, hydrolyzed from their respective glucuronides, were then purified by multiple chromatographic and derivatization steps as described [11]. The estrone and estradiol so purified represented the glucuronide conjugates.

The remaining plasma was adjusted to pH 1 with H_2SO_4 and made 20% by vol with NaCl solvolyzed for 24 h using ethylacetate at room temperature. The ethylacetate fraction was washed and the solvent removed under vacuum. The estrone hydrolyzed from estrone sulfate was then purified by multiple chromatographic and derivitization steps as described [10, 11]. The estrone so purified represented the pH 1 conjugate (estrone sulfate).

Following the final HPLC chromatography, the mass of steroid was measured using u.v. spectrophotometry and the losses through the procedures were calculated for each of the unconjugated-glucuronide and sulfate fractions. Then the radioactivity in each fraction was measured using a liquid scintillation spectrometer and corrected for losses through the procedures.

The extraction, and purification of the estrogens from the urine was done essentially as described [9, 10]. Briefly, a 25% aliquot of the pooled 96 h urine was extracted once with an equal volume and twice with $\frac{1}{2}$ vol of ethylacetate. The organic phase was backwashed twice with a 10% volume of water. The washes were added to the residual urine. The ethylacetate extract was evaporated but not worked up further, and represented the unconjugated estrogens which amounted to less than 1% of the urinary radioactivity.

The residual urine was made 0.005 M in sodium acetate and brought to pH 5 with 50% H_2SO_4. The carriers, 2-hydroxyestrone and 2-hydroxyestradiol (700 μg of each in ethanol, less than 1% vol), and β-glucuronidase (500 Fishman U/ml of urine) were added, and the urine was incubated at 37°C for 48 h. Then the remaining carriers estrone, estradiol, estriol, 2-methoxyestrone, 2-methoxyestradiol and 16α-hydroxyestrone (400 μg of each in ethanol) were added and the urine extracted once with an equal volume and twice with $\frac{1}{2}$ vol of ethylacetate. The organic phase was backwashed twice with a 10% vol of water. The washes were added to the residual urine, and the organic phase further washed with 5% $NaHCO_3$ and water until neutral.

The solvent was removed under vacuum and the residue chromatographed on Silica Gel HF_{254} (Brinkman Instruments, Westbury, NY) in the system benzene–ethylacetate–acetic acid (80:20:2, by vol). Following development 4 zones were separately eluted; those containing (1) estrone and 2-methoxyestrone, (2) 2-hydroxyestrone, 2-methoxyestradiol, 16α-hydroxyestrone and estradiol, (3) 2-hydroxyestradiol and (4) estriol. The individual steroids were then purified by multiple chromatographic and derivatization steps [9, 10]. The estrogens recovered from this analysis represented the glucuronide conjugates.

Then the residual urine following enzyme hydrolysis and extraction was made 20% w/v with NaCl, brought to pH 1 with 5 N HCl, shaken with an equal volume of ethylacetate containing all the previously

noted carriers (400–700 μg of each) and allowed to stand overnight at 25°C. After a second shaking, the organic phase was removed and, using successive 10% vol, washed once with water, repeatedly with 5% $NaHCO_3$ until the aqueous phase remained basic and then with water until the washes were neutral.

The solvent was removed under vacuum and the residue treated as above with multiple chromatographic and derivatization steps to separate and purify the individual estrogens which represented the pH 1 (sulfate) conjugates.

For each estrogen in each fraction, following the final HPLC, the mass was determined by u.v. spectrometry and the losses through the procedure calculated for each estrogen. The radioactivity in each estrogen for each fraction was measured in a liquid scintillation spectrometer and corrected for losses through the procedures.

For the plasma, the concentration of both ^3H and ^{14}C radioactivity corrected for losses through the procedure were plotted against time of sampling and the areas under the curves (AUC) determined using a mathematical approach [11, 12]. The metabolic clearance rates (MCR) were calculated as:

$$MCR = \frac{R}{AUC}$$

where R = administered dose of radioactivity. The fraction of the orally administered [^3H]estradiol which was measured in the blood as [^3H]estradiol was calculated as:

$$\text{fraction absorbed} = \frac{S_o^2}{S_s^2}$$

where S_o^2 = area under the curve for the disappearance of radioactive estradiol from the blood after oral administration. S_s^2 = area under the curve for the disappearance of radioactive estradiol from the blood after i.v. administration.

The conversion ratios ($CR^{PRE-PRO}$) were calculated as: $CR^{PRE-PRO} = area^{PRO}/area^{PRE}$ where the superscript stands for the steroids measured and the subscript stands for the method of administration and the pool sampled (S = systemic and O = oral), e.g. $CR_{OS}^{2,2-gluc}$ = ratio of radioactivity as estradiol glucuronide to estradiol measured in blood following oral administration of estradiol. The error in calculating a conversion ratio will not exceed 15–20% by this method [11].

For the radioactivity in the urine the data are presented as % of the administered dose in each fraction and as the $^3H/^{14}C$ ratio in each fraction compared to the $^3H/^{14}C$ ratio of the administered dose. In order to compare the ratios among the subjects, the $^3H/^{14}C$ ratios of each fraction have been normalized to a $^3H/^{14}C$ ratio of 1.0 for the administered dose.

RESULTS

The MCR's and conversion ratios following estradiol i.v. are shown in Table 1. Most of the radioactivity in the plasma appears as E_1SO_4 with E_2-glucuronide a relatively minor component. In one individual (subject 5) E_1-glucuronide was also measured and the $CR_{SS}^{E_2,E_1gluc}$ was 0.25 which was greater than the $CR_{SS}^{E_2,E_2gluc}$ of 0.09 in that subject. In subjects 1 and 2 radioactivity as estriol and 2-$MeOE_1$ were determined but only background activity was found.

The data for radioactivity in the plasma estrogens following estradiol p.o. are shown in Table 2. The MCR's are considerably higher than the MCR for estradiol i.v. The conversion ratios indicate that most of the radioactivity appears as E_1SO_4. With one exception, CR^{E_2,E_1} in subject no. 1, each CR_{OS} is greater than the respective CR_{SS}. The mean (±SE) fractional absorption is 0.11 ± 0.02. The CR^{E_2,E_1gluc} in the one subject measured was 4.2.

The pattern of urinary metabolites appearing as glucuronides is shown in Table 3. Following oral administration, estrone is the major glucuronide in the urine, while after estradiol i.v. the glucuronide of 2-hydroxyestrone is usually the major conjugate although the percent of administered dose measured as catechol estrogens is similar to the percent measured as estrone, estradiol, estriol and 16α-hydroxyestrone.

As shown in Table 4, excretion as a pH 1 hydrolyzable conjugate is generally a minor pathway for metabolites of estradiol administered either p.o. or i.v. However in 2 subjects (2 and 4) the excretion of E_1SO_4 was much greater than the others, but was still less than excretion as the glucuronides.

The $^3H/^{14}C$ ratios are shown in Table 5. Since they are normalized to an administered $^3H/^{14}C$ ratio of 1.00, any ratio >1.0 indicates that the orally administered estradiol is metabolized via that pathway to a greater degree than the i.v. administered estradiol. Ratios <1.0 indicate that the pathway favors the i.v. estradiol. For only 2 metabolites E_1 glucuronide and E_2 glycuronide are the ratios >1.0. For all other metabolites the ratios are <1.0, and are essentially identical.

Table 1. Metabolic Clearance Rates (MCR_S) and Conversion Ratios ($CR_{SS}^{PRE-PRO}$) measured in plasma after administration of [4-^{14}C]-estradiol i.v.

Subject	MCR_S l/day	$CR_{SS}^{E_2,E_1}$	$CR_{SS}^{E_2,E_1SO_4}$	$CR_{SS}^{E_2,E_2gluc}$
1	1,040	0.44	7.42	—
2	1,310	0.21	1.66	0.16
3	1,100	0.27	5.14	0.09
4	810	0.28	10.9	0.07
5	1,920	0.35	3.22	0.09
$\bar{X}$	1,240	0.31	5.67	0.10
SE ±	190	0.04	1.62	0.02

Table 2. Metabolic Clearance Rates (MCR_O) and Conversion Ratios ($CR_{OS}^{PRE-PRO}$) and absorption measured in plasma after administration of [6,7-^{3}H]estradiol p.o.

Subject	MCR_O l/day	$CR_{OS}^{E_2 \cdot E_1}$	$CR_{OS}^{E_2 \cdot E_1SO_4}$	$CR_{OS}^{E_2 \cdot E_2\,gluc}$	Fract. Absorp.
1	5,780	0.35	14.2	—	0.18
2	20,635	1.67	17.6	5.76	0.06
3	6,630	0.90	32.2	2.00	0.16
4	12,810	2.11	109.5	3.90	0.06
5	18,310	1.33	19.4	1.30	0.10
$\bar{X}$	12,800	1.27	38.6	3.24	0.11
SE ±	3,000	0.30	18.0	1.00	0.02

Table 3. Urinary metabolites as glucuronides after administration of [^{3}H]estradiol by mouth and [^{14}C]estradiol IV expressed as percent of administered dose

	Subject 1		2		3		4		5	
	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
E1	19.5	6.36	29.7	10.3	15.9	6.44	17.5	10.3	13.5	6.37
E2	7.52	3.50	10.3	4.43	5.29	3.03	5.28	3.32	5.26	2.90
E3	5.88	9.66	3.83	5.60	3.25	4.21	2.04	3.09	3.71	5.54
16α-OH-E1	1.39	2.17	2.55	3.79	2.92	3.76	1.71	2.57	0.99	2.58
2-OHE1	2.56	5.26	9.13	16.2	10.1	13.9	7.05	11.2	5.04	8.50
2-OHE2	0.58	0.97	1.15	1.99	1.15	1.53	1.02	1.57	1.44	2.11
2-MeOE1	0.36	0.59	2.18	3.80	1.37	1.81	2.42	3.60	1.18	1.68
2-MeOE2	0.05	0.06	0.10	0.18	0.10	0.14	0.13	0.18	0.11	0.16

Table 4. Urinary metabolites as pH 1 conjugates after administration of [^{3}H]estradiol by mouth and [^{14}C]estradiol IV expressed as percent of administered dose

	Subjects 1		2		3		4		5	
	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
E1	0.46	0.74	2.90	5.01	0.51	0.61	3.26	4.84	0.70	1.02
E2	0.05	0.11	0.20	0.36	0.04	0.08	0.29	0.51	0.07	0.12
E3	0.21	0.35	0.32	0.45	0.16	0.20	0.42	0.65	0.21	0.31
16α-OH-E1	0.42	0.73	0.76	1.19	0.51	0.71	1.00	1.51	0.43	0.66
2-OHE1	0.28	0.44	1.28	2.10	0.44	0.60	1.76	2.75	0.49	0.77
2-OHE2	0.005	0.008	0.024	0.036	0.012	0.020	0.022	0.040	0.013	0.023
2-MeOE1	0.29	0.51	0.99	1.57	0.41	0.55	1.54	2.34	0.38	0.54
2-MeOE2	0.01	0.02	0.05	0.08	0.02	0.03	0.10	0.15	0.03	0.04

Table 5. ^{3}H/^{14}C ratios of the urinary estrogen metabolites: estrone (E_1), estradiol (E_2), estriol (E_3), 16α-hydroxyestrone (16α-OHE_1), 2-hydroxyestrone (2-OHE_1), 2-hydroxyestradiol (2-OHE_2), 2-methoxyestrone (2-$MeOE_1$), 2-methoxyestradiol (2-$MeOE_2$), excreted as glucuronides (G), and pH 1-hydrolyzable conjugates (pH 1)

	E_1		E_2		E_3		16α-OH-E_1		2-OH-E_1		2-OH-E_2		2-MeO-E_1		2-MeO-E_2	
Subject	G	pH 1	G	pH 1	G	pH 1	G	pH 1	G	pH 1	G	pH 1	G	pH 1	G	pH 1
1	2.72*	0.62	2.15	0.50	0.61	0.60	0.64	0.57	0.49	0.64	0.60	0.62	0.61	0.57	0.73	0.52
2	2.82	0.58	2.32	0.55	0.68	0.71	0.67	0.64	0.56	0.61	0.58	0.67	0.57	0.63	0.55	0.61
3	2.34	0.84	1.74	0.53	0.77	0.80	0.78	0.72	0.73	0.73	0.75	0.60	0.76	0.74	0.71	0.53
4	1.70	0.67	1.59	0.57	0.66	0.65	0.66	0.66	0.63	0.64	0.64	0.55	0.67	0.66	0.72	0.66
5	2.12	0.68	1.81	0.56	0.67	0.68	0.38	0.65	0.59	0.64	0.68	0.56	0.70	0.70	0.69	0.62

*In all subjects, the ^{3}H/^{14}C ratios have been normalized to an administered ^{3}H/^{14}C ratio of 1.00.

DISCUSSION

The values for MCR_S^2, and $CR_{SS}^{E_2}$, E_1SO_4 for estradiol following i.v. administration are similar to those values that we reported previously after pulse injections [12]. The mean value for $CR_{SS}^{E_2 E_1}$, is somewhat higher than we noted before and the reasons for this difference are unclear. The techniques were similar in the two studies which were both done in the early follicular phase of the cycle. The $CR_{SS}^{2,2gluc}$ represents conversion of estradiol to estradiol-3-glucuronide, estradiol-17-glucuronide and the diglucuronides since we did not separate them prior to hydrolysis. There are no data for the MCR of estradiol glucuronide in the literature but in 1 young woman in whom we infused [^{3}H]estradiol glucuronide the MCR_S was calculated as 810 l/day (Longcope C., unpublished data). Therefore, we can estimate that the conversion of estradiol to estradiol glucuronide (i.e. $[\rho]_{SS}^{E_2 \cdot E_2 gluc}$) will be in the range of 10%, far less

than value of 42% for the conversion of estradiol to estrone sulfate [13].

Following the administration of estradiol p.o. the mean MCR_0^2 is about 10 times that of the MCR_S^2 indicating extensive metabolism has occurred in the splanchnic tissue confirming the conclusions of Fishman *et al.*[5] based on urinary data. Although the CR's for the estradiol p.o. are considerably greater than the corresponding CR's for estradiol i.v. the actual percent of estradiol given p.o. which enters the blood as each product ($[\rho]_{OS}$ v. $[\rho]_{SS}$) is increased only for the conversion of estradiol to estradiol glucuronide and perhaps estrone glucuronide.

While there is considerable individual variation, the mean absorption of orally administered estradiol into the estradiol pool is only 10%. Using the MCR's for estrone and estrone sulfate determined by pulse injection which we have reported previously [12, 13], we calculate that an additional 15% of the estradiol administered p.o. will be adsorbed as estrone, 25% will be absorbed as estrone sulfate and 25% as estradiol glucuronide. In the one individual in whom estrone glucuronide was measured the percent of estradiol absorbed and appearing as estrone glucuronide was ~25%. Thus while only a small amount of orally administered estradiol enters the blood pool of estradiol, there is entry into the blood pool of estrone and estrone sulfate which can contribute to estrogenic activity in target tissues, either directly or after conversion to estradiol [14, 15].

The urinary data reflect that found in plasma and are similar to those reported by Fishman *et al.*[5]. We also note a marked increase in the fraction of the oral dose excreted as estrone and estradiol glucuronide. That these are the favored pathways for the metabolism of estradiol p.o. is borne out by the normalized $^3H/^{14}C$ ratios which are >1.0 only for those conjugates. The 2- and 16-oxygenated estrogen and all the sulfate conjugates that we measured have normalized ratios <1.0. The fact that the ratios are <1.0 for these other compounds probably reflects the extensive metabolism to estrone and estradiol glucuronidation that occurs before these other pathways of metabolism can be entered.

The site of this extensive metabolism of estradiol p.o. must be the intestinal wall, the liver or a combination of these tissues. It has been shown, *in vitro*, that intestinal wall can convert estradiol to estrone and estradiol glucuronides [16] but there was no evidence for conversion to 2- or 16-hydroxylated estrogens, or sulfates. Human liver, however, has been shown to convert estradiol to estrone, and the 2- and 16-oxygenated estrogens [17, 18]. In the dog, Longcope *et al.*[11] concluded that much of the first-pass metabolism of estradiol p.o. was due to metabolism and conjugation in the intestinal wall.

It is therefore likely that 20–25% of the estradiol p.o. is converted to estrone and estradiol glucuronide in the intestinal wall and that these metabolites do not undergo further changes. Thus, only 70–75% of the estradiol is available for hepatic metabolism along pathways available to the estradiol i.v.

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