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METABOLISM OF [4-^{14}C]ESTRONE IN HAMSTER AND RAT HEPATIC AND RENAL MICROSOMES: SPECIES-, SEX- AND AGE-SPECIFIC DIFFERENCES

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Summary—The metabolism of [4-^{14}C]estrone (E_1) was examined in liver and kidney microsomes of adult castrated male and ovariectomized female hamsters and rats and in neonatal and immature hamster renal microsomes. In castrated male hamster liver microsomes, E_1 was metabolized extensively to six major metabolites; 15β-hydroxyestrone, 7α-hydroxyestrone, 6α-hydroxyestrone, 6β-hydroxyestrone, 2-hydroxyestrone, and Δ(9,11)-dehydroestrone, and a nonpolar fraction. Six minor metabolites of E_1 were also detected. In contrast, kidney microsomes derived from castrated male hamsters metabolized E_1 to mainly 17β-estradiol, 2- and 4-hydroxyestrone, 6α-hydroxyestrone, 6β-hydroxyestrone and one monohydroxyestradiol metabolite. However, 16α-hydroxyestrone was not detected. A variable, but low amount of estriol was also found. Interestingly, the quantity of 2-hydroxyestrone found in kidney microsomes of the hamster represented 26% of the total amount of metabolites formed, whereas in liver microsomes, only 9% of the overall metabolism resulted in the formation of 2-hydroxyestrone. The ability of kidney microsomes of female ovariectomized hamsters and two different rat strains to metabolize E_1 was 5.9- and 9.4-fold lower, respectively, compared to renal microsomes of male castrated hamsters. The onset of oxidative metabolism in newborn hamster kidneys during development was also assessed. The results indicate that the oxidative metabolism of [^{14}C]E_1 in renal microsomes of newborn hamsters was 20-fold less than in kidney microsomes of adult hamsters. While catechol E_1 metabolites were essentially negligible in hamster kidneys of these ages, it was evident that the conversion of E_1 to estradiol via 17β-hydroxysteroid dehydrogenase resembles levels seen in the adult animals. Between the age of one and two months, the male hamster kidney exhibited the capacity to metabolize E_1 at levels seen in fully mature adult hamsters.

INTRODUCTION

It has been established that the oxidative metabolism of estrogens involves specific *P*-450 monooxygenases and proceeds via the formation of catechol estrogens [1–3]. In recent years, evidence has accumulated indicating that catechol estrogens are not merely inactive products of estrogen metabolism, but may regulate hormonal action in the hypothalamus, pituitary, brain and uterus [4–6]. While, the presence and some characteristics of an estrogen 2-/4-hydroxylase (ESH), has been reported for the hamster liver and kidney [7–8], no detailed data are available concerning the identification and quantity of normal estrogen metabolites found in this species at these organ sites. Moreover, the generation of oxidative estrogen metabolites during the development of these tissues has also not been investigated.

We have previously shown that the hamster kidney is a unique estrogen-dependent and responsive tissue, which exhibits many pertinent characteristics of a *bona-fide* estrogen target tissue [9–13]. Additionally, the kidney is of particular interest because of the 100% tumor incidence which can be produced at this organ following chronic estrogen treatment of castrated as well as intact hamsters [12, 14]. A number of studies have suggested that estrogen metabolism may play a role in the tumorigenic process in which reactive intermediates are generated [12, 15, 16]. Evidence that estrogen metabolism can generate electrophiles capable of binding to cellular macromolecules in the hamster liver has been demonstrated [17] and this observation is consistent with this hypothesis.

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Therefore, we have examined in detail the metabolism of radiolabeled estrone (E_1), a major substrate for ESH, in liver and kidney microsomes of normal untreated male hamsters at different ages and compared these findings to similar studies carried out in the kidney of two strains of untreated adult male rats. In addition, the ability of hamster kidneys at birth to metabolize E_1 has also been examined.

MATERIALS AND METHODS

Chemicals and reagents

[4-^{14}C]E_1 (56 mCi/mmol, 99% radiochemical purity) was obtained from New England Nuclear (Boston, MA). E_1, 17β-estradiol (E_2), 11β-hydroxyestrone (11β-OH-E_1), 16α-hydroxyestrone (16α-OH-E_1), 2-hydroxyestriol (2-OH-E_3), NADPH (Type III), and L-ascorbic acid were purchased from Sigma Chemical Co. (St Louis, MO). Methanol, acetone, ammonium sulfate and magnesium chloride were purchased from Fisher Scientific (Stassen, IL). The estrogen metabolites 2-hydroxyestrone (2-OH-E_1), 4-hydroxyestrone (4-OH-E_1), 6-dehydroestrone (6-d-E_1) and 6-ketoestrone (6-keto-E_1) were obtained from Steraloids (Wilton, NH). The 6α-hydroxyestrone (6α-OH-E_1), 6βhydroxyestrone (6β-OH-E_1) and 7α-hydroxyestrone (7α-OH-E_1) were a gift from Dr P. N. Rao, Southwest Biomedical Foundation, San Antonio, TX. Water used for HPLC analysis was deionized and double-distilled. All other chemicals used were reagent grade.

Animals and treatment

Adult castrated male and female Syrian golden hamsters, Wistar–Furth, and Sprague–Dawley rats weighing 90–100 g were purchased from Harlan Sprague–Dawley, Indianapolis, IN. Animals were acclimatized for at least 1 week prior to use and were maintained on a 12-h light–12-h dark cycle and fed certified rodent chow (Ralston-Purina 5002) and tap water *ad libitum*. Pregnant female hamsters were housed individually. The animal studies were performed in adherence to the guidelines established in the "Guide for the Care and Use of Laboratory Animals," U.S. Department of Health and Human Resources (NIH 1985). Animals were housed in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care.

Microsomal preparation

Microsomes were prepared as described previously [16]. Briefly, immediately after decapitation, liver and kidneys were perfused with 150 mM Tris–HCl, pH 7.4, removed, blotted on filter paper, weighed, minced, and homogenized in glass–Teflon homogenizers with 3 vol/g (liver), 2 vol/g (kidney) in 250 mM sucrose. For the identification of E_1 metabolites, microsomes prepared from individual livers were used but it was necessary to pool kidneys from 4 to 6 animals in order to generate sufficient metabolites from microsomes for isolation and GC/MS analyses. Tissue homogenates were centrifuged at 12,000 *g* for 20 min, the pellets discarded, and the resultant supernatant fluids were then centrifuged at 165,000 *g* for 60 min. Microsomal pellets freed of glycogen, were washed in the same volume of 1.15% KCl, and 5 ml aliquots centrifuged again at 165,000 *g* for 60 min. The final microsomal pellets, containing about 20 mg protein each, were stored at −80°C until further use. Microsomal protein was determined according to the method of Lowry *et al.* [18] using bovine serum albumin as the standard.

Metabolism of [^{14}C]E_1 in liver and kidney microsomes

The metabolism studies were carried out according to a modified method described previously [16]. Briefly, microsomal pellets were diluted with 150 mM Tris–HCl, pH 7.4, to a final concentration of either 0.6 mg/ml (liver) or 2 mg/ml (kidney). A 5.0-ml aliquot was incubated with 50 μM [^{14}C]E_1, 1 mM ascorbic acid, and 5 mM $MgCl_2$ for 5 min at 37°C under gentle agitation. The reaction was initiated by the addition of 2 mM NADPH. The samples were incubated for 30 min (liver) or 1 h (kidney) at 37°C. Initial studies indicated that maximal estrogen metabolite formation, particularly 2-hydroxylation, was observed at these time periods. For control incubations, NADPH was omitted. Radioactive E_1 was used as a substrate since it was shown to generate substantially more catechol metabolites in microsomal preparations derived from kidney and similar catechol levels compared to E_2 in livers of hamsters using the radio-enzymatic estrogen hydroxylase assay [7, 8]. The reaction was terminated by adding 35 ml cold methanol–acetone (1:1, v/v), containing 0.5% acetic acid. The precipitated protein was sedimented at 3000 *g* for 30 min and

then extracted successively with 25 ml methanol, methanol–ether (1:1, v/v), and ether.

The combined supernatants were transferred into round bottomed flasks and evaporated under nitrogen. The residues were redissolved in 10 ml distilled water containing 5 g ammonium sulfate and extracted twice with 60 ml diethyl ether. After evaporation of the ether, the metabolites were dissolved in 200 μl methanol containing 1 mg ascorbic acid and stored under argon at −20°C until HPLC analysis.

HPLC separation of oxidative estrogen metabolites

Separation of the [^{14}C]-labeled E_1 metabolites was accomplished using a Waters model 840 liquid chromatograph equipped with a radioactive flow detector, model IC (Radiomatic Instruments, Tampa, FL). Samples were eluted on two tandem octadecyl (C_{18}) columns, 0.45 × 25 cm (Jones Chromatography, Littleton, CO) using a linear gradient from 52 to 72% methanol in water for 42 min at a flow rate of 1 ml/min and at a column temperature of 35°C set under 100% methanol for 10 additional min. Scintillation fluid used for detecting the radioactivity was Biofluor (New England Nuclear) at a flow rate of 3 ml/min. When available, retention times of the radioactive metabolites were compared with those of unlabeled reference compounds. Results are expressed as percentage metabolite of the total recovered radioactivity.

Identification of metabolites by GC/MS

HPLC fractions used for GC/MS analysis were evaporated to dryness and derivatized with *O,N*-bis(trimethylsilyl)acetamide (10–50 μl). GC/MS was performed on a Finnigan 4510 GC/MS/Data System using a fused silica capillary column (Durabond DB-1, ICT) (15 m long, 0.32 mm i.d.) 1.0 μm thick. The column was set at a temperature range of 150–310°C at 10°C/min and maintained at 310°C for 15 min. Electron impact mass spectra were taken at an electron energy of 70 eV scan time per mass spectra was 1 s, and scanning intervals were 50–650 *m/e*. E_1 metabolites were identified by both their mass spectra and their retention times on the GC column and were compared with available reference standards. If no standard was available, a tentative structure was proposed from the mass spectrum only. Mass spectral data were retained in the data system and compared, when appropriate, to mass spectra file of identified compounds. Mass spectra were obtained after background subtraction and corrected for the most intensive ion peak (= 100).

RESULTS

Metabolism of [^{14}C]E_1 in hamster liver and kidney microsomes

Initial studies determined the optimal incubation conditions for the oxidative metabolism of radiolabeled E_1 in hamster liver and kidney microsomes. To determine the optimal conditions for oxidative metabolism of [4-^{14}C]E_1 in these tissues, either the protein concentration (2–10 mg), substrate concentration (5–100 μM), or time (15–60 min) was varied. Since there are differences in the amount of *P*-450 activity in hamster liver (1.2 ± 0.01 nmol/mg protein) and kidney (0.13 ± 0.01 nmol/mg protein) microsomes and since these tissues possess different activities of estrogen hydroxylating enzyme, the optimal incubation conditions for liver and kidney microsomes were expectedly dissimilar. The optimal metabolism of [^{14}C]E_1 in hamster liver microsomes occurred at a protein concentration of 3–4 mg, using 50 μM substrate, 1 mM ascorbate, 2 mM NADPH, and an incubation time of 30 min, whereas in kidney microsomes a protein concentration of 7–8 mg, 5 μM substrate and 60 min incubation time resulted in optimal oxidative metabolite formation. In preliminary studies, the tissue distribution of the E_1 metabolism in the hamster kidney was investigated in microsomal fractions of cortex and medulla. At least 95% of E_1 metabolism was located in the renal cortex (data not shown).

Figure 1 depicts the typical HPLC profiles for [^{14}C]E_1 metabolites in liver and kidney microsomes from castrated male hamsters, as well as kidney microsomes of castrated male rats. In the hamster liver, at least eleven metabolites and a large nonpolar fraction were detected. These E_1 products included five major and six minor metabolites. The conversion of E_1 to E_2 was low. These metabolites were fractionated from pooled HPLC separations and applied to GC/MS. Because of the limited availability of reference compounds, the structures of some E_1 metabolites were proposed on the basis of their mass spectra alone. In contrast, five major and two minor E_1 metabolites were separated by HPLC employing hamster kidney microsomes. In contrast to hamster liver microsomes, there

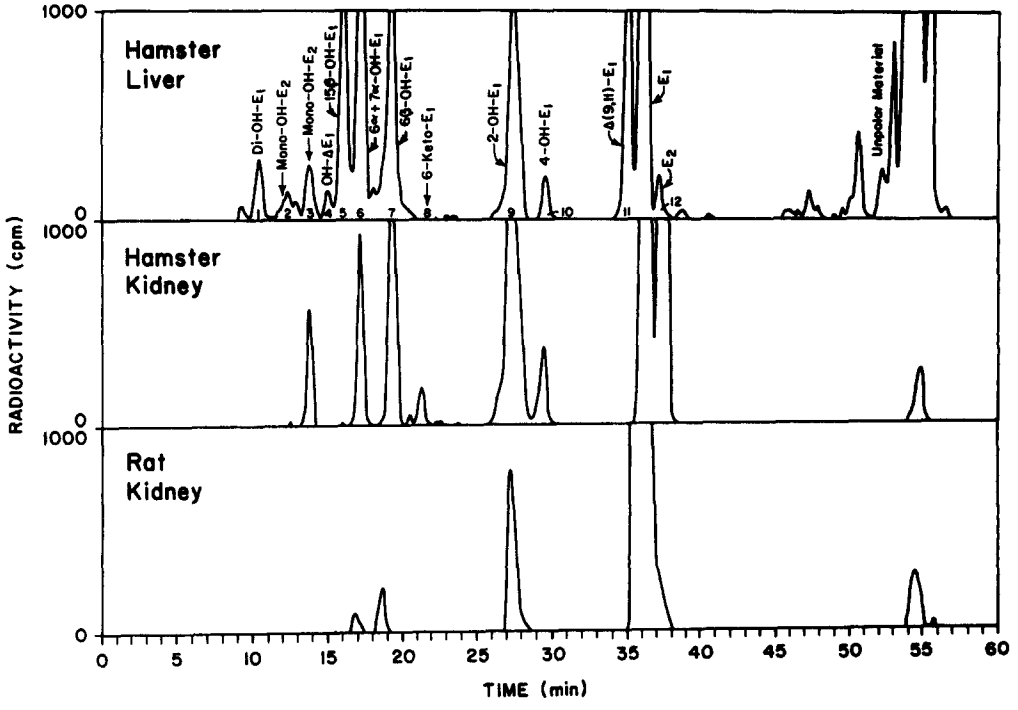

Fig. 1. HPLC profiles of [4-^{14}C]E_1 metabolites in castrated hamster liver and kidney, and castrated rat kidney microsomes. Incubations consisted of 3–4 mg (liver) or 7–8 mg (kidney) microsomal protein, 50 μM (liver) or 5 μM (kidney) [4-^{14}C]E_1, 1 mM ascrobate and 2 mM NADPH. HPLC separation was carried out using a linear gradient of 52–72% methanol in water in 42 min, followed by 10 min elution with 100% methanol.

was a substantial conversion of E_1 to E_2 and the nonpolar material was considerably smaller in the hamster kidney. Figure 2 depicts the mass spectra of the major metabolites of estrone in castrated hamster liver microsomes. Interestingly, metabolism of radiolabeled E_1 in renal microsomes derived from castrated rats revealed only 2-OH-E_1 and a very small quantity of Δ(9,11)-dehydroestrone metabolite formation. Figure 2 also depicts the mass spectra of the major metabolites of E_1 in castrated hamster liver microsomes. The metabolites 7α-OH-E_1, 6α-OH-E_1, 6β-OH-E_1, 2-OH-E_1, (Fig. 2, mass spectra 2–5), 6-keto-E_1, 4-OH-E_1 and E_2 (data not shown) were conclusively identified on the basis of their mass spectra and retention time from known standards. For metabolites 5 and 11 (Fig. 1) no standards were available. On the basis of their mass spectra (Fig. 2, spectra 1 and 6) and a computer based search in a mass spectra library, we have proposed 15β-hydroxyestrone for peak 5 and Δ(9,11)-dehydroestrone for peak 11. In addition to these nine metabolites, we have also identified two monohydroxyestradiol metabolites (Fig. 1, peaks 2 and 3), a dihydroxyestrone and a dehydrohydroxyestrone (Fig. 1, peaks 1 and 4, mass spectra not shown). A variable, but low amount of E_3 was also detected.

Table 1 summarizes the metabolic pattern of [^{14}C]E_1 in hamster liver and kidney microsomes. The overall metabolism of [^{14}C]E_1 in hepatic microsomes was 16-times greater compared to kidney microsomes and correlated well with their capability to bind [^{14}C]E_1 metabolites in a covalent manner. The amount of covalently bound [^{14}C]E_1 metabolites to hamster liver microsomes after 30 min at 37°C was 7.94 ± 0.14 nmol/mg protein, whereas kidney microsomes only exhibited 0.47 ± 0.01 nmol bound per mg protein. Although 7α-OH-E_1 and 6α-OH-E_1 were not separable on the reverse-phase column, they could be separated by GC/MS and the ratio 7α-OH-E_1:6α-OH-E_1 is approx. 2:1. Moreover, if the total protein concentration exceeded 4 mg, no 2-OH-E_1 or 4-OH-E_1 could be detected and was reflected as an increased amount of unpolar material, and the very polar material also increased. In contrast, kidney microsomes converted E_1 mainly to 2-OH-E_1 and E_2 and smaller amounts of 6α-OH-E_1 and 6β-OH-E_1. Major E_1 metabolites found in the liver such as 15β-OH-E_1, 7α-OH-E_1, Δ(9,11)-dehydroestrone were detected.

Table 1. Comparison of the metabolism of [4-^{14}C]E_1 in hepatic and renal microsomes of castrated male Syrian hamsters[a]

[4-^{14}C]E_1 metabolites	Liver		Kidney	
	% of total recovered radioactivity	ESH activity (pmol/mg/min)	% of total recovered radioactivity	ESH activity (pmol/mg/min)
Dihydroxyestrone	1.0 ± 0.10		ND	
Mono-OH-E_2-1	0.6 ± 0.06		ND	
Mono-OH-E_2-2	0.6 ± 0.03		1.6 ± 0.60	
Mono-OH-d-E_1	0.4 ± 0.10		ND	
15β-OH-E_1	5.1 ± 0.40		ND	
7α + 6α-OH-E_1[b]	8.8 ± 0.50[c]		3.4 ± 0.10	
6β-OH-E_1	5.4 ± 0.80		3.2 ± 0.90	
6-Keto-E_1	ND[d]		1.0 ± 0.50	
2-OH-E_1	6.7 ± 0.80	186.1 ± 22.0	12.9 ± 0.90	6.7 ± 0.5
4-OH-E_1	0.2 ± 0.06	5.5 ± 1.7	1.3 ± 0.30	0.7 ± 0.2
Δ(9,11)-dehydro-E_1	6.9 ± 0.70		ND	
E_1	20.5 ± 3.10		50.0 ± 0.20	
E_2	0.5 ± 0.10		25.3 ± 0.30	
Unpolar material	40.3 ± 6.90		1.1 ± 0.10	

[a]Incubations consisted of 3–4 mg (liver) or 7–8 mg (kidney) microsomal protein, 50 μM (liver) or 5 μM (kidney) [4-^{14}C]E_1, 1 mM ascorbate and 2 mM NADPH. HPLC separation was carried out using a linear gradient of 52–72% methanol in water, in 42 min.
[b]The hydroxy group is located at the 6α- or 7α-position.
[c]Ratio 7α-OH-E_1:6α-OH-E_1 in liver microsomes = 2:1. Kidney microsomes contained 6β-OH-E_1 only.
[d]ND = not detected.

Metabolism of [4-^{14}C]E_1 in hamster kidney microsomes at different ages

Table 2 shows the age-dependent development of the metabolism of [^{14}C]E_1 in Syrian hamster kidney microsomes. Interestingly, the oxidative metabolism of [^{14}C]E_1 in kidney microsomes of newborn hamsters was 20-fold less than in kidney microsomes of adult hamsters.

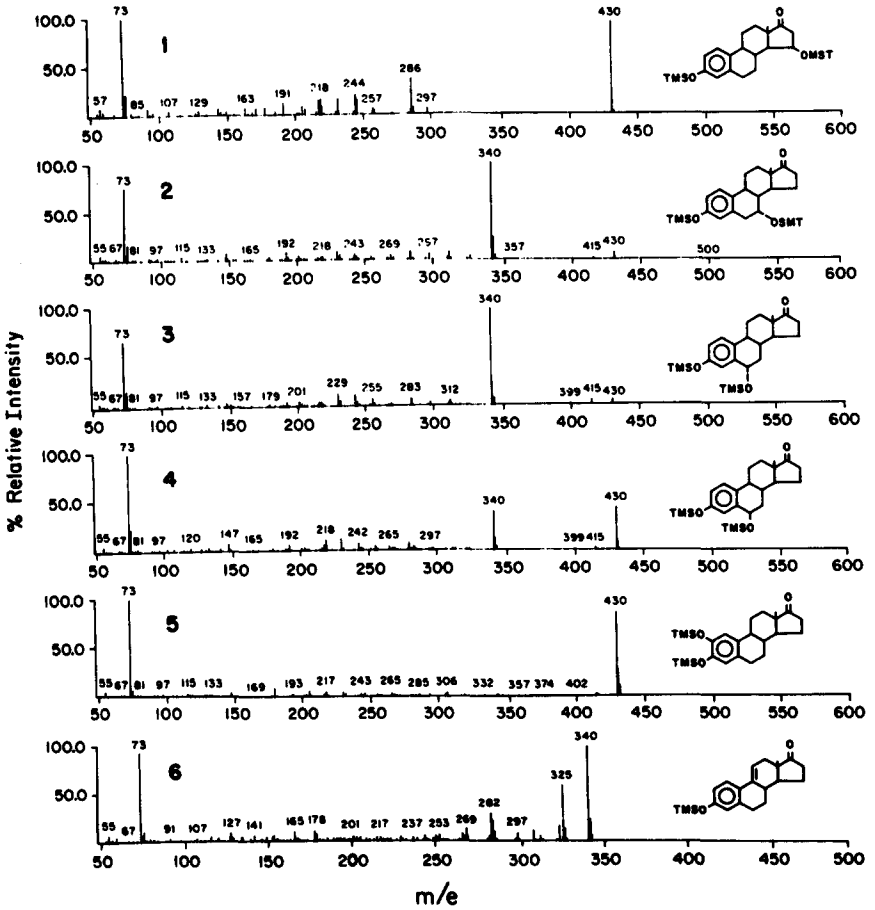

Fig. 2. Mass spectra of the major *in vitro* metabolites of E_1 in hamster liver microsomes. Names of the metabolites are (from top to bottom): 15β-OH-E_1, 7α-OH-E_1, 6α-OH-E_1, 6β-OH-E_1, 4-OH-E_1, and Δ(9,11)-dehydro-E_1.

Table 2. Age dependent development of the metabolism of [4-^{14}C]E_1 in kidney microsomes of Syrian hamsters[a]

[4-^{14}C]E_1 metabolites	Newborn	22-Days-old	30-Days-old	2 and 3 Months old
Mono-OH-E_2-2	ND[c]	ND	0.30 ± 0.07	1.1 ± 0.1
Metabolite 1[b]	0.3 ± 0.1	ND	ND	ND
7α + 6α-OH-E_1	0.4 ± 0.2	0.40 ± 0.04	0.70 ± 0.08	3.1 ± 0.2
6β-OH-E_1	0.2 ± 0.1	0.20 ± 0.01	0.40 ± 0.08	4.6 ± 1.3
6-Keto-E_1	0.2	ND	0.10 ± 0.03	0.9 ± 0.2
2-OH-E_1	0.2 ± 0.1	0.30 ± 0.04	1.00 ± 0.20	13.2 ± 2.5
4-OH-E_1	ND	ND	ND	1.2 ± 0.1
E_1	78.6 ± 5.5	70.00 ± 0.20	68.60 ± 2.70	55.8 ± 0.4
E_2	16.2 ± 4.1	24.90 ± 0.80	24.20 ± 2.30	25.0 ± 0.8
Unpolar material	0.5 ± 0.2	0.70 ± 0.10	1.00 ± 0.10	1.3 ± 0.2

[a]Incubations consisted of 10 mg microsomal protein, 5 μM [4-^{14}C]E_1, 1 mM ascrobate, and 2 mM NADPH. HPLC separation was carried out using a linear gradient of 52–72% methanol in water for 45 min. $n = 3$, except for newborn ($n = 2$), 60 fetuses each. Values are expressed as percentage of total recovered radioactivity ± SE.
[b]Metabolite 1 has the same retention time as 15β-OH-E_1.
[c]ND = not detected.

Moreover, the formation of total catechol metabolites was also essentially negligible (72-fold lower) and the dehydration of E_1 to E_2, however, was only 1.5-fold less than adult levels. After the hamsters were weaned at an age of 22 days the catechol metabolites in the kidney were still 48-fold lower compared to adults but the conversion of E_1 into E_2 was already fully developed. The metabolism of E_1 in hamster kidney microsomes of 30-day-old hamsters resembled the pattern of E_1 metabolism found in the adult hamster but catechol formation was still 14-fold less compared to the adult hamster kidney. At an age of 60 days the metabolism of [^{14}C]E_1 was fully developed as also shown in Tables 1 and 3 and was not altered through 6 months of age (data not shown).

Metabolism of [4-^{14}C]E_1 in kidney microsomes of Syrian golden hamsters of both sexes and different rat strains

Table 3 shows the differences in the metabolism of [^{14}C]E_1 between sexes and species. Kidney microsomes from male castrated Syrian golden hamsters were able to metabolize [^{14}C]E_1 to 2-OH-E_1, 6α-OH-E_1, 6β-OH-E_1, and E_2 as the main metabolites as previously shown in Table 1. In contrast, the ability of female ovarectomized hamster kidney microsomes to convert [^{14}C]E_1 to oxidative metabolites was 5.9 times lower compared to the castrated male hamster kidney. Strikingly, the formation of E_2 however was increased 2-fold. The oxidative metabolism of [^{14}C]E_1 in Wistar–Furth or Sprague–Dawley castrated rat kidney microsomes was even lower than in ovariectomized female hamster kidney microsomes and 9.4 times lower compared to the castrated male hamster kidney. No E_2 metabolite could be detected. The covalent binding of [^{14}C]E_1 metabolites to rat kidney microsomes was also 2.5-fold less compared to hamster kidney microsomes of castrated animals (data not shown).

DISCUSSION

It has been postulated that reactive estrogen semi-quinone or quinone intermediates generated via catechol formation, may be involved in either the cytotoxic effects of estrogens resulting

Table 3. Metabolism of [4-^{14}C]E_1 in Syrian hamster and Wistar–Furth or Sprague–Dawley rat kidney microsomes[a]

[4-^{14}C]E_1 metabolites	Hamster, female ovariectomized	Hamster, male castrated	Wistar–Furth rat, male castrated[b]
Mono-OH-E_2	0.5 ± 0.10	1.8 ± 0.5	ND[c]
7α + 6α-OH-E_1	0.8 ± 0.10	3.5 ± 0.5	0.1 ± 0.01
6β-OH-E_1	2.5 ± 0.50	4.2 ± 1.3	0.4 ± 0.04
6-Keto-E_1	0.2 ± 0.07	0.8 ± 0.1	ND
2-OH-E_1	2.6 ± 0.20	13.9 ± 1.9	2.2 ± 0.20
4-OH-E_1	0.3 ± 0.04	1.3 ± 0.3	ND
E_1	45.1 ± 0.20	44.7 ± 3.0	94.3 ± 0.20
E_2	44.8 ± 0.80	24.8 ± 0.4	ND
Unpolar material	1.1 ± 0.10	1.2 ± 0.1	0.8 ± 0.10

[a]Incubations consisted of 10 mg microsomal protein, 5 μM [4-^{14}C]E_1, 1 mM ascorbate, and 2 mM NADPH. Incubations were carried out for 1 h at 37°C. HPLC separation was achieved using a linear gradient of 52–72% methanol in water for 45 min. Values are expressed as percentage of total recovered radioactivity ± SE.
[b]Studies with Sprague–Dawley rats gave the same results.
[c]ND = not detected.

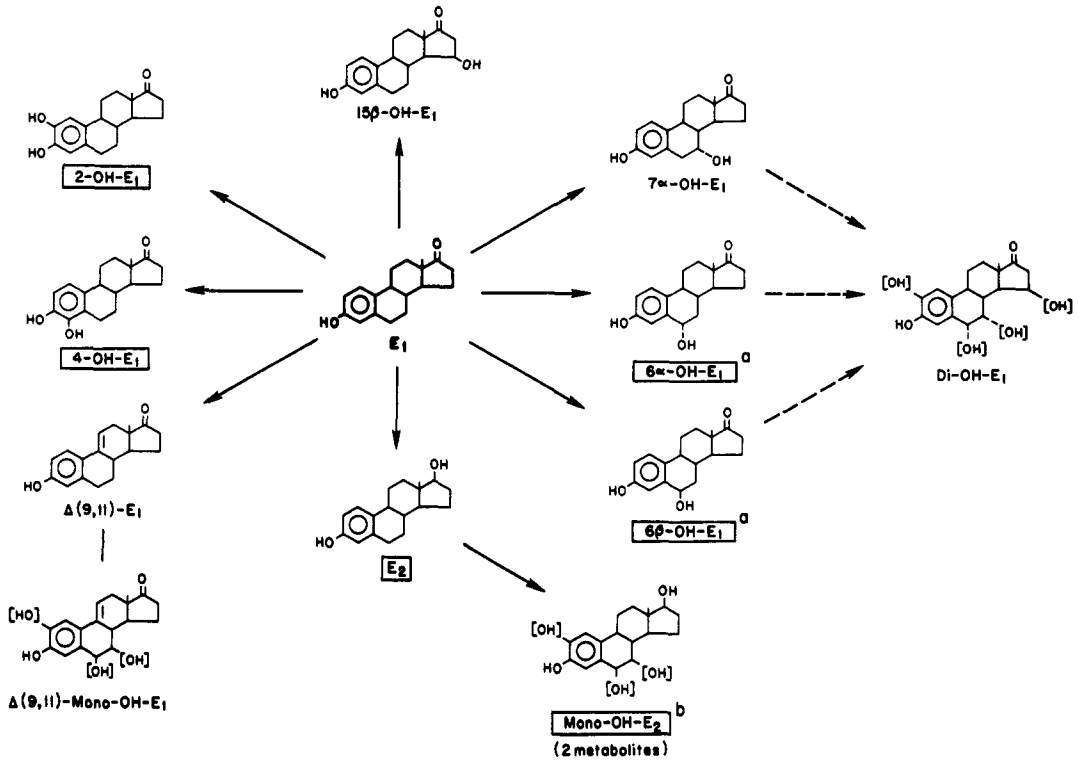

Fig. 3. Metabolic pathway of E_1 in hamster liver and kidney microsomes. Hydroxygroups in brackets indicate that the hydroxygroups may be located at one of the positions indicated. Metabolites contained in boxes are common to both kidney and liver, whereas metabolites not contained in boxes are formed by liver microsomes only. [a]In addition to these metabolites, kidney microsomes formed 6-keto-E_1 most probably by dehydrogenation of 6α-OH-E_1 or 6β-OH-E_1. [b]Kidney microsomes formed only one mono-OH-E_2 metabolite.

in tubular cell damage and/or estrogen carcinogenicity in the hamster kidney [12, 16, 19]. Since renal tumorigenesis in response to estrogenic hormones is unique to this species, it seemed obvious that detailed information was needed, not heretofore shown, regarding the ability of the hamster kidney and liver to metabolize estrogens. E_1 was used as a substrate in these studies since it was shown previously to be the most actively metabolized estrogen in the hamster kidney relative to other natural or synthetic estrogens examined [7, 12]. Early studies have shown that hamster urinary metabolites of radiolabeled E_1 or E_2 yielded mainly 2-OH-E_1 and small quantities of 2-methoxyestrone [20]. This finding is not surprising since Fishman *et al.* [22] suggested that if E_2 is administered, at least to humans, the real substrate of 2-hydroxylation is E_1 rather than E_2. The data presented herein clearly demonstrates that [^{14}C]E_1 is metabolized extensively in hamster liver and kidney microsomes to at least twelve and seven oxidative metabolites in addition to nonpolar fractions, respectively. Moreover, 29% of the metabolized radioactive E_1 yielded 2- and 4-hydroxycatechol products in hamster kidney microsomal incubations. In contrast, Brueggemeier *et al.* [21] reported that radiolabeled E_2 incubations of primary kidney cell cultures derived from untreated or DES-treated hamsters yielded only small amounts of E_1 and appreciable quantities of unidentified polar metabolites, but no catechol intermediates. With the exception of 15β-hydroxylation in the hamster liver, little E_1 metabolism occurred in the D ring of either hamster liver or kidney microsomal incubations. It is notable that both 16α-E_1 and E_3, present in the human [22], is absent in the hamster, indicating that oxidative metabolism of natural estrogens occurs almost entirely in rings A and B. Since recent studies strongly implicate a primitive blastema-like interstitial cell as the origin of the estrogen-induced hamster kidney tumor [23, 24], we have studied the metabolism of E_1 in the early stages of kidney development (i.e. newborn, 22- and 30-day-old). Interestingly, only traces (1.4–7.0%)

of oxidative E_1 catechol metabolites were found in these early stages of developing kidney compared to kidneys obtained from mature castrated adult hamsters.

It is well established that catecholestrogen products are capable of binding irreversibly to cellular macromolecules, most notably with microsomal proteins [17, 25–27]. Whether these findings play any role in estrogen carcinogenicity in the hamster kidney remains unknown. However, the extremely poor metabolism of 11β-methoxyethinylestradiol (Moxestrol) recently shown in hamster kidney microsomes, mitochondria, and in freshly prepared intact proximal tubules [28, 29], particularly in estrogenized animals, seriously weakens the belief that estrogen metabolite activation and irreversible binding to cellular constituents has a significant role in estrogen-induced carcinogenicity in this system. Since a progressive and hence appreciable hormonally-induced renal tubular damage has been observed [24] following chronic exposure to estrogens at high doses and for prolonged periods, it is possible that this in turn may result in a secondary release of lipid hydroperoxides, reactive oxygen species, etc. from damaged cells [30]. These reactive species then could contribute to events leading to transformed renal phenotypes in the hamster.

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