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OXIDATIVE METABOLISM OF ESTROGENS IN RAT INTESTINAL MITOCHONDRIA

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Abstract—Intestinal epithelial cells are capable of metabolizing a wide variety of exogenous substrates. To determine how this metabolic capacity may affect endogenous substances such as steroid hormones, we examined the ability of rat gut epithelial preparations to hydroxylate estradiol at the C-2 position. Utilizing a site-specific tritium exchange assay, an active estrogen 2-hydroxylase system was shown to be localized to gut mitochondria throughout the intestine, with enzymatic activities comparable to the activity in crude hepatic homogenates of non-induced animals (0.2 nmol/min/mg protein). Gas chromatography–mass spectrometry confirmed the formation of C-2 hydroxylated estrogens by these mitochondrial preparations. The enzyme system was shown to involve a saturable monooxygenase, utilizing NADH (preferably) or NADPH in a protein- and time-dependent fashion. The Michaelis–Menten constant for this pathway was approximately 150 μM. Enzyme activity decreased by 20% in the presence of carbon monoxide, and was largely unaffected by organic P450 inhibitors such as α-naphthoflavone, metyrapone, and SKF-525A. These studies suggest that intestinal mitochondria are able to contribute to the oxidative metabolism of endogenous estrogens circulating within the enterohepatic pool.

The endogenous metabolism of estrogens in humans is largely oxidative in nature, involving cytochrome P450-dependent hydroxylations primarily at the C-2 or C-16α positions [1, 2]. Catechol estrogens (CEs§), such as 2-hydroxyestrone, are formed to a greater extent in men and women than 16α-hydroxyestrone and estriol [2]; furthermore, CEs are essentially inactive in peripheral tissues in contrast to the latter two metabolites [3]. The formation of CEs takes place in numerous tissues, such as liver, intestines, lung, brain and kidney [4, 5], as well as in reproductive tissues of the breast and uterus [6, 7]. Modulation of these oxidative pathways by diet, genetics, or environmental factors has been directly linked to altered risk for developing hormone-dependent cancers of the breast and endometrium [8].

The extent of estradiol 2-hydroxylation is highly variable in humans, and has been shown to be catalyzed by at least three major cytochrome P450 gene subfamilies, including the aryl hydrocarbon-inducible P450IA, phenobarbital-inducible P450IIB, and glucocorticoid-inducible P450IIIA isozymes [9–11]. These isozymes are abundant in the liver, and are present to a lesser extent in extrahepatic tissues, including the epithelial cells of the small intestine [12–16]. The highest concentrations of dietary agents capable of modulating these P450 enzymes are likely to be found within the lumen of the small intestine following ingestion. Estrogen substrates are also delivered directly into the intestinal lumen via the bile, and thereafter circulate largely as glucuronide and sulfate conjugates throughout the gut. In many species, bacterial deconjugation of steroids within the intestinal lumen allows reentry into the circulation, constituting the enterohepatic circulation.

In these studies, we examined the capacity of the small intestinal epithelial cells to form catechol estrogens. We report here the finding that the small intestine exhibits a modest capacity for the formation of these steroid metabolites, and that this metabolic activity is associated primarily with the mitochondria.

MATERIALS AND METHODS

Materials. [2-^{3}H]Estradiol was purchased from the New England Nuclear Corp. (Boston, MA; specific activity 25 Ci/mol). Unlabeled estrogens were purchased from Steraloids (Wilton, NH), while bis(trimethylsilyl)trifluoroacetamide (BSTFA) used in gas chromatography–mass spectrometry (GC-MS) analysis was obtained from Supelco (Bellefonte, PA). All other buffers and chemicals were obtained from the Sigma Chemical Co. (St. Louis, MO). Male Sprague–Dawley rats (200–250 g) used in these studies were purchased from Taconic Farms (Germantown, NY). Animals were housed in the Rockefeller University Laboratory Animal Research Center in temperature-controlled (23 ± 1°), light-cycled (12 hr light/dark cycle) quarters for at least 1 week prior to study. Animals were maintained on standard Purina Rodent Laboratory Chow 5001 (St Louis, MO) and were allowed free access to food and water up to the time of being killed.

Preparation of subcellular fractions. Following sacrifice, the liver was removed and the small

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§ Abbreviations: CEs, catechol estrogens; BSTFA, bis(trimethylsilyl)trifluoroacetamide; TCA, trichloroacetic acid; and CO, carbon monoxide.

intestine cut at the pyloric junction. The entire length of the intestine was irrigated *in situ* with ice-cold 0.9% NaCl to remove intestinal contents. Except when evaluating distal colon, the first 30 cm of small intestine was removed and irrigated once more with 30 mL of cold saline. The initial 5-cm segment was discarded, and the remaining tissue placed onto an ice-cold watch glass and cut longitudinally to expose the mucosal surface. The epithelial cells were scraped off gently with a stainless steel scalpel, weighed, and placed into an ice-cold isolation medium (10% tissue w/v), containing 0.25 M sucrose, 10 mM Tris base, 10 mM KCl, and 1 mM EDTA, pH 7.2 [17]. Trypsin inhibitor (5 mg/mL) and heparin (3 U/mL) were included in the intestinal isolation medium to aid in subcellular fractionation [18]. The homogenate was centrifuged at 600 *g* for 10 min. The resultant postnuclear supernatant was then centrifuged at 8800 *g* for 10 min. The mitochondrial pellet obtained was resuspended gently to the original volume in 0.25 M sucrose and recentrifuged at 600 *g* for 10 min and then at 7500 *g* for another 10 min. The final mitochondrial pellet was washed once in 0.25 M sucrose and resuspended in 0.5 mL of isolation medium to a protein concentration of approximately 2 mg/mL and used for subsequent assays. To document the purity of this preparation, the final mitochondrial pellet was examined under electron microscopy. Figure 1 shows that mitochondria were, in fact, the predominant organelle in this subcellular fraction; very little of the endoplasmic reticulum not associated with ribosomes was present.

The postmitochondrial supernatant was centrifuged at 105,000 *g* for 60 min in a Beckman L5-50 ultracentrifuge to obtain the microsomal fraction. The microsomal pellet was carefully rinsed and resuspended in an appropriate volume of potassium phosphate buffer (0.1 M, pH 7.4) to a protein concentration of approximately 5–10 mg/mL.

Estradiol radiometric assays. Unless otherwise noted, the radiometric assay was performed using 0.05 to 0.2 mL of tissue in buffer, 0.7 to 0.85 mL potassium phosphate buffer (0.05 M, pH 7.5), 0.1 mL buffer containing 2.5 mg/mL of NADPH, and 20 μL of ethanol containing 8 nmol unlabeled estradiol with 10^5 dpm [2-^{3}H]estradiol in a final incubation volume of 1 mL. The reaction was initiated by adding NADPH, followed by shaking for 20 min in a water bath at 37°. After incubation, the reaction vial was placed on ice and mixed with 1.0 mL of ice-cold water containing 2% charcoal for 10 min, followed by centrifugation at 3000 *g* for 15 min. C-2 hydroxylation released ^3H_2O, which was collected for scintillation counting by lyophilization *in vacuo*.

Gas chromatography–mass spectrometry. Estrogen metabolite analysis following incubation with unlabeled estradiol was carried out using GC–MS according to a variation of a published method [19]. Briefly, following incubations as described above, estrogens were collected in the presence of ascorbic acid on a C18 Sep-pak column and eluted with methanol (5 mL). Following evaporation under nitrogen, estrogens were derivatized using 50 μL of BSTFA and 10 μL pyridine at room temperature overnight. The injected samples (1 μL) were scanned primarily in the selected-ion mode, using deuterated estradiol (d_3-E_2) and deuterated 2-hydroxyestradiol (d_5-2OHE_2) as internal standards, Analyses were conducted using a Hewlett–Packard model 5980 gas chromatograph and model 5971A mass selective detector. The following mass ions and GC elution times were routinely monitored: 8.1 to 8.5 min, *m/z* 342 and 257 (E_1); 8.7 to 9.1 min, *m/z* 416 and 285 (E_2); 9.4 to 9.8 min, *m/z* 430 and 431 (2OHE_1). The ions with *m/z* 419 and 509 correspond to the mass ions of the deuterated internal standards, (d_3-E_2) and (d_5-2OHE_2). These are the mass ions previously reported for trimethylsilylated estrogens [20].

Statistical analysis. Where appropriate, the data were analyzed by Student's *t*-test, and a P value of < 0.05 was regarded as statistically significant.

RESULTS

Differential centrifugation was used to obtain various subcellular fractions of rat intestine and liver, as indicated in Table 1. Assay conditions for estradiol 2-hydroxylase were identical for all fractions in both tissues (8 μM unlabeled estradiol with 10^5 dpm of [2-^{3}H]estradiol). As expected, 2-hydroxylase activity in the liver homogenate exceeded activity present in the intestine. Upon fractionation of the intestinal homogenate, however, the highest activity in this tissue was found in the mitochondria, whereas intestinal microsomes had little activity. In contrast to the small intestine, liver 2-hydroxylase activity was clearly localized to the microsomes (Table 1).

Estradiol 2-hydroxylase activity in the intestinal mitochondria was dependent upon mitochondrial protein added (Fig. 2a), and was linear with respect to time for 20 min at 37° (Fig. 2b). A Lineweaver–Burk plot of 2-hydroxylase for intestinal mitochondria and liver microsomes revealed that the apparent K_m for the intestinal enzyme was approximately 150 μM, larger than that of liver microsomes (35 μM, data not shown).

We compared the estradiol-metabolizing effects of incubating gut mitochondria in the presence of either NADH or NADPH alone. In gut mitochondria, the ratio of activity with NADH alone compared to that with NADPH alone was 2.2 ± 0.4; in the gut microsomes, the ratio of estradiol 2-hydroxylation with NADH to that with NADPH was 0.16 ± 0.05 (mean ± SD of three experiments).

The effects of addition or deletion of various components of the reaction mixture, as well as the addition of other known monooxygenase inhibitors, were examined in intestinal mitochondria (Table 2). Compared with the complete system (which included both NADH and NADPH), activity was reduced (57% of control) when incubating with NADPH only. Preincubation with trichloroacetic acid (TCA) greatly reduced enzyme activity. Carbon monoxide (CO) was only minimally effective in reducing enzyme activity, while activity increased slightly in the presence of metyrapone and SKF-525. The inclusion of α-naphthoflavone was without effect.

Mitochondria obtained from colonic epithelial cells were also capable of the 2-hydroxylation of

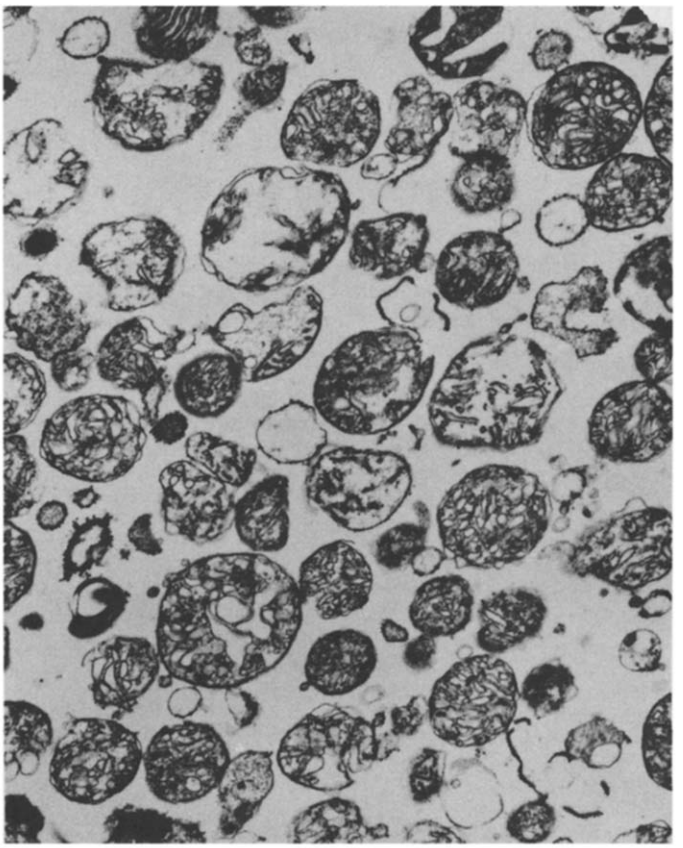

Fig. 1. Electron micrograph of a subcellular mitochondrial preparation obtained from intestine. Mitochondria were prepared as described under Materials and Methods, and electron micrographs were obtained at 15,200× magnification.

Table 1. Subcellular localization of intestinal estradiol 2-hydroxylase

Tissue fraction	Activity of 2-hydroxylase (pmol formed/min/mg protein)	
	Gut	Liver
Homogenate	34.5 ± 4.1	201.3 ± 17.2
9,000 *g* Pellet	90.3 ± 10.7	29.6 ± 4.2
Mitochondria	224.8 ± 41.6	13.0 ± 2.0
9,000 *g* Supernatant	1.9 ± 0.5	288.1 ± 22.5
Microsomes	3.7 ± 0.3	394.7 ± 32.7
105,000 *g* Supernatant	2.2 ± 0.2	5.4 ± 2.2

The ^{3}H-labeled steroids were incubated for 20 min at 37° with NADPH and tissue protein from either gut or liver samples, as described in Materials and Methods. Results are the means ± SEM of three experiments.

estradiol (Fig. 3), although activity was lower when compared with mitochondria obtained from the small intestine (average 97.4 vs 209.5 pmol/mg/min, respectively, N = 3). In addition, estradiol 2-hydroxylase activity was considerably lower in colonic microsomes compared with the mitochondria.

While the radiometric assay has been shown to be largely stoichiometric when compared with product isolation assays [21, 22], GC–MS has been used to confirm the production of catechol estrogens in radiometric assays [23]. To demonstrate the formation of catechol estrogens in these experiments, we thus performed GC–MS analysis of extracts of the mitochondrial reaction mixture. Using incubation conditions identical to those described above, the entire reaction mixture was applied to a Sep-pak cartridge, eluted with methanol, and derivatized as described in Materials and Methods. Figure 4 shows the results of selected-ion monitoring of this extract, in which a peak appeared at 9.5 min. Both principal ion and qualifier ion (m/z 430 and 431, respectively) as well as retention time corresponded to that of authentic 2-hydroxyestrone, thus providing unequivocal evidence for the formation of catechol estrogens during these mitochondrial incubations.

DISCUSSION

These studies present evidence that intestinal epithelial cells are capable of catalyzing the 2-

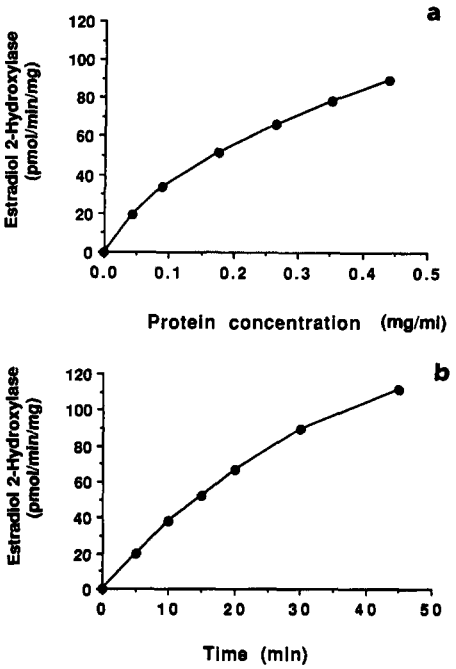

Fig. 2. Protein- and time-dependence of the gut mitochondrial estradiol 2-hydroxylase assay. (a) Incubations were conducted with estradiol (8 μmol/L) containing 10^5 dpm [2-^{3}H]estradiol, NADH (0.3 mmol/L), and rat intestinal mitochondrial protein (between 0.05 and 0.5 mg/mL). Results are the averages of two experiments (range <10% of averages). (b) Incubations were performed as above, using 0.2 mg/mL intestinal mitochondrial protein, incubated for varying lengths of time.

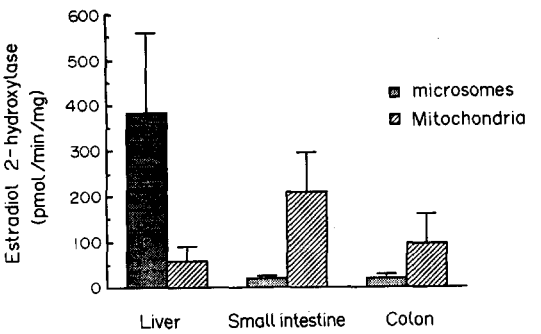

Fig. 3. Estradiol 2-hydroxylase activity in different rat tissues. Enzyme activity is shown for both microsomes and mitochondria from liver, and from small and large intestine. Assays were performed as described in the text; results are the means ± SD of four experiments.

hydroxylation of estrogens, and that this enzymatic activity is found primarily within the mitochondria of these cells. The diverse functions of mitochondrial cytochrome P450 systems have been well established in various tissues. For example, $P450_{scc}$ and $P450_{11\beta}$ are located within the mitochondria of steroidogenic tissues of the adrenal gland [24]. Further, in the liver and kidney, the endogenous activity of mitochondrial cytochrome P450 is found in the oxidation of physiologically important compounds, including cholesterol, vitamin D_3 and the bile acids [25–27]. In the liver, as much as 25% of the total cytochrome P450 is present within mitochondria [28]. Although the small intestine is not considered to be a steroidogenic tissue, the subcellular distribution of cytochrome P450 in intestinal cells is unusual in that nearly a 3-fold excess of spectrally measurable hemoprotein is reportedly present in mitochondria compared with microsomes [29].

The intestinal mitochondrial system described in these studies appears to involve a saturable monooxygenase enzyme, utilizing NADH (preferably) or NADPH in a protein- and time-dependent manner. Niranjan *et al.* [28] have shown that the hepatic mitochondrial cytochrome P450 system is supported by an electron transport system different from that of microsomes, and has specific requirements for a mitochondrial type ferrodoxin/reductase. When normalized for protein, the mitochondria reported

Table 2. Effects of components in the incubation medium on intestinal mitochondrial estradiol 2-hydroxylase activity

Incubation mixture	Estradiol 2-hydroxylase (% of complete system)
Complete	100
NADH only	105
NADPH only	57
TCA (10 mmol/L)	6
CO-bubbled	79
Metyrapone (300 μmol/L)	141
SKF-525 (200 μmol/L)	138
α-Naphthoflavone (300 μmol/L)	95
No tissue	3
No cofactors	24

Estradiol 2-hydroxylase activity was determined in gut mitochondria, as described in Materials and Methods. Activity of the complete system was 178.5 pmol/mg/min. Results are the averages of two experiments (range < 10% of averages). The individual components were added to the test tubes in 20–50 μL of ethanol, which was evaporated under nitrogen to yield the final concentration reported above.

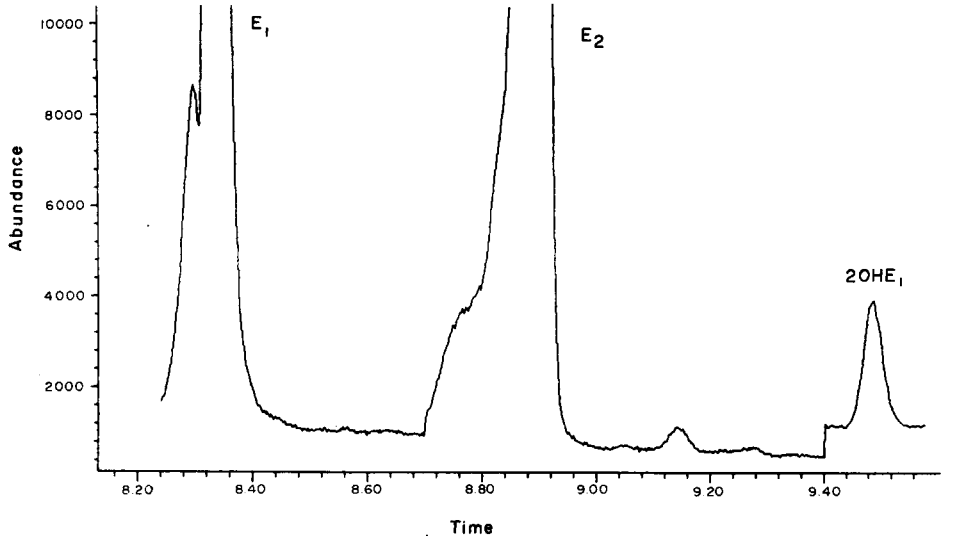

Fig. 4. Analysis by gas chromatography–mass spectrometry of rat gut mitochondria following incubation with estradiol. Selected-ion monitoring was used to evaluate production of various metabolites by mitochondria, using incubation conditions as described in the text. Estradiol (E_2) was the most abundant peak, followed by estrone (E_1) and 2-hydroxyestrone ($2OHE_1$).

here exhibit levels of activity that are comparable to liver microsomes from untreated female rats.

These studies also confirm and extend earlier observations describing estradiol 2-hydroxylation in murine extrahepatic tissues, including the small intestine [14]. This enzymatic activity was attributed to cytochrome P_3450 (IA2) on the basis of a similar pattern of induction of acetanilide 4-hydroxylase. Furthermore, activity was found to be inducible (2.5-fold) in the small intestine by either 3-methylcholanthrene or isosafrole [14]. In these earlier studies, however, intestinal microsomes were used as the source of enzymatic activity. Therefore, it is possible that the capacity of the small intestine to engage in the oxidative 2-hydroxylation of estradiol was underestimated.

The biochemical nature of this mitochondrial estrogen pathway in the small intestine is presently unknown. Upon coincubation with the isozyme-selective organic inhibitors of phenobarbital-inducible cytochrome P450, metyrapone and SKF-525A, a slight activation of estradiol 2-hydroxylase activity was observed. Metyrapone has been shown previously [25] to produce a 2-fold stimulation in the 24-hydroxylation of 25-hydroxyvitamin D_3. The presence of α-naphthoflavone, an inhibitor of 3-methylcholanthrene-inducible cytochrome P450, was without effect.

We also observed a relative insensitivity of this system to CO inhibition. The effects of CO on cytochrome P450-catalyzed reactions are complex. A similar lack of inhibition of testosterone 7α-hydroxylation by CO in testicular microsomes was observed in a previous study [30]. On the basis of this refractoriness to CO inhibition, the authors suggested the involvement of cytochrome P450a (IIA1). In fact, the lack of requirement for NADPH and insensitivity to CO inhibition prompted Warner [25] to suggest that the mitochondrial 25-hydroxylation of vitamin D_3 in kidney occurs via a P450-catalyzed peroxygenase reaction. On the other hand, CO has been shown to inhibit by 80% the activation of aflatoxin B_1 in rat liver mitochondria [28]. Thus, the observations in the present study on the effects of cytochrome P450 inhibition do not conclusively establish or exclude the possibility that intestinal mitochondrial estradiol 2-hydroxylase functions via a cytochrome P450-dependent mechanism. For other steroid-metabolizing systems (notably the aromatase complex), lack of CO inhibition was not by itself definitive in excluding the participation of cytochrome P450 [31].

In conclusion, these studies describe a pathway for the formation of catechol estrogens in the rat intestinal epithelium, and represent the first report of an estrogen catabolic pathway occurring within mitochondria. The potential physiological significance of this enzymatic activity is linked to the fact that in many mammalian species large concentrations of estrogens are excreted via the bile directly into the intestinal lumen, followed in many cases by bacterial deconjugation [32]. These estrogens contact epithelial cells in the course of their intestinal transit, and consequently may be further transformed by mitochondrial and/or microsomal enzymes. Our results suggest that the gut epithelium may serve as a metabolic gate, through which estrogens and other steroids must pass in order to return to the circulation following bacterial deconjugation.

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REFERENCES

1. Fishman J, Aromatic hydroxylation of estrogens. *Annu Rev Physiol* **45**: 61–72, 1983.
2. Fishman J, Bradlow HL, Schneider J and Kappas A, Radiometric analysis of biological oxidations in man: Sex differences in estradiol metabolism. *Proc Natl Acad Sci USA* **77**: 4957–4960, 1980.
3. Martucci CP and Fishman J, Impact of continuously administered catechol estrogens in uterine growth and luteinizing hormone secretion. *Endocrinology* **105**: 1288–1292, 1979.
4. Barbieri RL, Canick JA and Ryan KJ, Estrogen 2-hydroxylase: Activity in rat tissues. *Steroids* **32**: 529–538, 1978.
5. Tuteja N, Gonzalez FJ and Nebert DW, Developmental and tissue-specific differential regulation of the mouse dioxin-inducible P_1-450 and P_3-450 genes. *Dev Biol* **112**: 177–184, 1985.
6. Hoffman AR, Paul SM and Axelrod J, Catecholestrogen synthesis and metabolism by human breast tumors *in vitro*. *Cancer Res* **39**: 4584–4587, 1979.
7. Bunyagidj C and McLachlan JA, Catechol estrogen formation in mouse uterus. *J Steroid Biochem* **31**: 795–801, 1988.
8. Bradlow HL and Michnovicz JJ, A new approach to the prevention of breast cancer. *Proc R Soc Edinb* **95B**: 77–86, 1989.
9. Graham MJ, Lucier GW, Linko P, Maronpot RR and Goldstein JA, Increases in cytochrome P-450 mediated 17β-estradiol 2-hydroxylase activity in rat liver microsomes after both acute administration and subchronic administration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a two-stage hepatocarcinogenesis model. *Carcinogenesis* **9**: 1935–1941, 1988.
10. Waxman DJ, Interactions of hepatic cytochromes P-450 with steroid hormones. *Biochem Pharmacol* **37**: 71–84, 1988.
11. Yang CS and Yoo J-SH, Dietary effects on drug metabolism by the mixed-function oxidase system. *Pharmacol Ther* **38**: 53–72, 1988.
12. Traber PG, Chianale J, Florence R, Kim K, Wojcik E and Gumucio JJ, Expression of cytochrome P450b and P450e genes in small intestinal mucosa of rats following treatment with phenobarbital, polyhalogenated biphenyls, and organochlorine pesticides. *J Biol Chem* **263**: 9449–9455, 1988.
13. Watkins PB, Wrighton SA, Schuetz EG, Molowa DT and Guzelian PS, Identification of glucocorticoid-inducible cytochromes P-450 in the intestinal mucosa of rats and man. *J Clin Invest* **80**: 1029–1036, 1987.
14. Kimura S, Gonzalez FJ and Nebert DW, Tissue-specific expression of the mouse dioxin-inducible $P_1$450 and $P_3$450 genes: Differential transcriptional activation and mRNA stability in liver and extrahepatic tissues. *Mol Cell Biol* **6**: 1471–1477, 1986.
15. Bonkovsky HL, Hauri H-P, Marti U, Gasser R and Meyer UA, Cytochrome P_{450} of small intestinal epithelial cells. Immunochemical characterization of the increase in cytochrome P_{450} caused by phenobarbital. *Gastroenterology* **88**: 458–467, 1985.
16. Lindeskog P, Haaparanta T, Norgård M, Glaumann H, Hansson T and Gustafsson J-Å, Isolation of rat intestinal microsomes: Partial characterization of mucosal cytochrome P-450. *Arch Biochem Biophys* **244**: 492–501, 1986.
17. Rosenberg DW and Kappas A, Characterization of heme oxygenase in the small intestine epithelium. *Arch Biochem Biophys* **274**: 471–480, 1989.
18. Stohs SJ, Grafström RC, Burke MD, Moldéus PW and Orrenius SG, The isolation of rat intestinal microsomes with stable cytochrome *P*-450 and their metabolism of benzo(α)pyrene. *Arch Biochem Biophys* **177**: 105–116, 1976.
19. Spink DC, Lincoln DW II, Dickerman HW and Gierthy JF, 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin causes an extensive alteration of 17β-estradiol metabolism in MCF-7 breast tumor cells. *Proc Natl Acad Sci USA* **87**: 6917–6921, 1990.
20. Adlercreutz H, Martin F, Wahlroos Ö and Soini E, Mass spectrometric and mass fragmentographic determination of natural and synthetic steroids in biological fluids. *J Steroid Biochem* **6**: 247–259, 1975.
21. Jellinck PH, Quail JA and Crowley CA, Normal and recombinant human growth hormone administered by constant infusion feminize catechol estrogen formation by rat liver microsomes. *Endocrinology* **117**: 2274–2278, 1985.
22. Jellinck PH and Bradlow HL, Peroxidase-catalyzed displacement of tritium from regiospecifically labeled estradiol and 2-hydroxyestradiol. *J Steroid Biochem* **35**: 705–710, 1990.
23. Porubek DJ and Nelson SD, A gas chromatographic/mass spectrometric assay for catechol estrogens in microsomal incubations: Comparison with a radiometric assay. *Biomed Environ Mass Spectrom* **15**: 157–161, 1988.
24. Guengerich FP, Enzymatic oxidation of xenobiotic chemicals. *Crit Rev Biochem Mol Biol* **25**: 97–153, 1990.
25. Warner M, 25-Hydroxyvitamin D hydroxylation. Evidence for a dioxygenase activity of solubilized renal mitochondrial cytochrome P-450. *J Biol Chem* **258**: 11590–11593, 1983.
26. Hollis BW, 25-Hydroxyvitamin D_3-1α-hydroxylase in porcine hepatic tissue: Subcellular localization to both mitochondria and microsomes. *Proc Natl Acad Sci USA* **87**: 6009–6013, 1990.
27. Javitt NB, 26-Hydroxycholesterol: Synthesis, metabolism, and biologic activities. *J Lipid Res* **31**: 1527–1533, 1990.
28. Niranjan BG, Raza H, Shayiq RM, Jefcoate CR and Avadhani NG, Hepatic mitochondrial cytochrome P-450 system. Identification and characterization of a precursor form of mitochondrial cytochrome P450 induced by 3-methylcholanthrene. *J Biol Chem* **263**: 575–580, 1988.
29. Jones DP, Grafstrom R and Orrenius S, Quantitation of hemoproteins in rat small intestinal mucosa with identification of mitochondrial cytochrome P-450. *J Biol Chem* **255**: 2383–2390, 1980.
30. Sonderfan AJ, Arlotto MP and Parkinson A, Identification of the cytochrome P-450 isozymes responsible for testosterone oxidation in rat lung, kidney, and testis: Evidence that cytochrome P-450a (P450IIA1) is the physiologically important testosterone 7α-hydroxylase in rat testis. *Endocrinology* **125**: 857–866, 1989.
31. Thompson EA Jr and Siiteri PK, The involvement of human placental microsomal cytochrome P-450 in aromatization. *J Biol Chem* **249**: 5373–5378, 1974.
32. Adlercreutz H, Martin F, Järvenpää P and Fotsis T, Steroid absorption and enterohepatic recycling. *Contraception* **20**: 201–223, 1979.