J. steroid Biochem. Vol. 36, No. 4, pp. 325–331, 1990
Printed in Great Britain.

0022-4731/90 $3.00 + 0.00

ESTROGEN METABOLISM IN PRIMARY KIDNEY CELL CULTURES FROM SYRIAN HAMSTERS

ROBERT W. BRUEGGEMEIER,* KO TSENG, NANCY E. KATLIC, MUSTAPHA A. BELEH and YOUNG C. LIN[1]

Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy and [1]Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210, U.S.A.

(*Received* 19 *September* 1989; *received for publication* 9 *March* 1990)

Summary—Estrogen metabolism was evaluated in freshly isolated kidney and liver microsomes and in primary kidney cell cultures from Syrian hamsters, a potential experimental model for examining the possible role(s) of estrogens in tumor initiation and development. Initial velocity studies of the conversion of estradiol to 2-hydroxyestradiol, as determined by the ^3H_2O release assay with the substrate [2-^{3}H]estradiol, resulted in similar apparent K_ms of estrogen 2-hydroxylase of 2.85 and 6.25 μM for liver and renal microsomes, respectively. The apparent V_{max} for freshly prepared liver microsomes was 0.13 nmol·mg^{-1}·min^{-1}, while that for renal microsomes was 0.040 nmol·mg^{-1}·min^{-1}. Evaluation of estrogen metabolism was also performed in primary cell cultures of hamster kidney cells, consisting of 75% epithelial cells. [6,7-^{3}H]Estradiol (10 μM) was incubated for 0, 24 and 48 h in primary kidney cell cultures, and the organic soluble metabolites analyzed by reverse-phase HPLC. The cultures from untreated, castrated hamsters metabolize [^{3}H]estradiol to yield small quantities of estrone and significant amounts of polar metabolites, while no catechol estrogens were isolated. Estrogen metabolism by diethylstilbestrol-treated (DES-treated) hamster kidney cell cultures also provided small quantities of estrone and no evidence of catechol estrogens. Additionally, larger amounts of additional polar metabolites were isolated in the cultures from DES-treated hamsters. Finally, levels of estrogen 2-hydroxylase were detected in these cultures using the ^3H_2O release assay. Thus, the short-term primary kidney cell cultures from the Syrian hamster are capable of metabolizing estrogens. Furthermore, the enzymatic processes appear to be available for the conversion of any catechol estrogens formed into more polar metabolites. These investigations in intact cells, capable of performing all biochemical processes, complement both *in vivo* and subcellular biochemical studies and may aid in elucidating the roles of estrogens and estrogen metabolism in the initiation and development of estrogen-induced, estrogen-dependent kidney tumors in the Syrian hamster.

INTRODUCTION

Cancer is the leading cause of death among women between the ages of 30 and 54, with breast and uterine cancers being common diseases. Numerous epidemiological studies suggest that steroid hormones may be involved in the formation and development of both mammary carcinoma and uterine cancer. The possible biochemical role(s) of estrogens and/or their metabolites in the development of estrogen-dependent cancers remains to be elucidated. Changes in the amount of estrogen or the metabolism of estrogen appear to be associated with the induction and growth of a variety of neoplasms [1–4], but the molecular mechanisms by which hormones such as estrogens may be involved in tumorigenesis are still unknown. Diethylstilbestrol (DES) and other synthetic estrogens appear to play a role in human vaginal cancer [4, 5] and in dysplasia of the cervix [6].

One potential experimental model for examining the possible role(s) of estrogens in tumor development is the Syrian hamster. The finding that prolonged estrogen treatment of the hamster results in renal adenocarcinoma was made by Matthews *et al.*[7]. The kidney tumors are malignant and estrogen-dependent, and appear in essentially all male hamsters that have been treated continuously with DES for more than 250 days [8]. The tumors can be induced in females by estrogen treatment only if the concentration of progesterone circulating in the blood is reduced by ovariectomy. Males can be protected against induction of the tumor by estrogens if they are treated simultaneously with progesterone. The tumors are not only estrogen-induced, but are estrogen-dependent and can be transplanted into

*To whom correspondence should be addressed: Dr Robert W. Brueggemeier, College of Pharmacy, 500 West 12th Avenue, Columbus, OH 43210, U.S.A.

other hamsters only if the host has been estrogen-treated. The kidney tumors are metastatic and new foci of tumor growth appear as metastases transported into the abdominal cavity or through the lymphatic and circulatory systems.

Evidence that estrogen plays a direct carcinogenic role in the initiation of the renal tumor has been accumulating. Recent reports demonstrate the importance of the oxidative metabolism of estrogens to the catechol estrogens to the development of cancer [9–11]. Cytochrome *P*-450 levels and estrogen 2-/4-hydroxylase activities are found primarily in hamster kidney cortex, the location of tumor formation [12, 13]. The metabolism of estradiol and other estrogens by estrogen 2-hydroxylase appears to be a critical step in the initiation of the hamster renal carcinoma. The question of whether it is the formation of the catechol estrogen themselves, their own action, or further metabolism of the 2-/4-hydroxy-estrogens via oxidative or peroxidative pathways [14, 15] also remains unanswered.

A short-term kidney primary tumor cell culture system has been developed and used to characterize the regulation of progesterone receptors in cultured tumor cells from a kidney tumor induced by long-term DES implanted in hamsters [16]. This manuscript describes the evaluation of estrogen metabolism in primary kidney cell cultures from untreated hamsters and from hamsters treated for 12 months with estrogen.

EXPERIMENTAL

Materials

Commercial steroids were obtained from Searle Laboratories, Skokie, Ill. and Steraloids, Wilton, N.H. and purified by recrystallizations. Chemical reagents were purchased from Aldrich Chemical Co., Milwaukee, Wis. and the purity determined by melting point and/or thin-layer chromatography. Biochemicals were obtained from Sigma Chemical Co., St Louis, Mo. [6,7-^{3}H]Estradiol (sp. act. 56 Ci/mmol) and [2-^{3}H]estradiol (sp. act. 22.7 Ci/mmol) were obtained from New England Nuclear, Boston, Mass. Radioactive samples were dissolved in NEN Formula 963 and counted by liquid scintillation in a Beckman LS 6800 scintillation counter. Reverse-phase high-pressure liquid chromatography was performed using an Altex Ultrasphere ODS column (4.5 × 250 mm) on a Beckman 421 gradient HPLC system.

Culture media RPMI 1640 was obtained in powdered form from GIBCO (Long Island, N.Y.). The sterilized liquid media was prepared by the Ohio State University Comprehensive Cancer Center by dissolving the powder in water containing sodium chloride (0.487 g/l), pyruvic acid (0.11 g/l), sodium bicarbonate (1.5 g/l) and Phenol Red (0.01%) and the pH adjusted to 6.8. Sterilized Hank's balanced salt solution and phosphate buffered saline were also prepared the Ohio State University Comprehensive Cancer Center. Fetal calf serum was obtained from KC Biological (Lenexa, Kans). Steroids were removed from the fetal calf serum by two treatments with dextran-coated charcoal [17]. Tissue culture flasks and supplies were obtained from Corning Glass Works (Corning, N.Y.).

Preparation of microsomal fractions

Young mature male Syrian hamsters (90–120 g.) were purchased from Harlan Industries Inc., Cumberland, Ind. Hamster liver and kidney microsomes were prepared by differential centrifugation procedures on homogenized tissue in the same manner as described for rat liver microsomes [18]. The microsomal pellet was stored at −70°C and remained stable for over 6 months.

Enzyme kinetic studies for estrogen 2-hydroxylase

The enzyme kinetics of estrogen 2-hydroxylase were examined under the initial velocity conditions, as previously described for rat liver microsomes [18, 19]. Briefly, varying concentrations of radiolabeled estradiol (2.0–10.0 μM/flask), 160,000 dpm ([2-^{3}H]estradiol/flask) were incubated with NADP (0.8 mM/flask), glucose-6-phosphate (2.0 mM/flask), glucose-6-phosphate dehydrogenase (2.5 units/flask), and the microsomal fraction (0.1–0.2 mg protein/flask) in 0.05 M Tris–HCl, pH 7.4 (2.6 ml total volume) containing 0.1 mM ascorbic acid at 37°C for 15 min. The assays were performed in triplicate, and blank samples were incubated with boiled microsomes. The incubations were stopped by the addition of 2 N HCl (5.0 ml) and the released ^3H_2O was separated from the steroids by elution through XAD-2 columns and counted by liquid scintillation.

Establishment of primary kidney cell cultures

The procedures of Lin *et al.*[16] were utilized to establish the primary cultures of kidney cells. Briefly, kidney tissue was removed from hamsters aseptically under ether anesthesia, placed on ice and dissected free of non-kidney tissues. Kidney tissue was then minced finely, washed in Hanks balanced salt solution and trypsinized for 30–45 min under a gentle stirring movement by a magnetic stirring bar at 37°C in RPMI media (0.5–1 g kidney tumor tissue/50 ml) containing 0.25% trypsin. After the trypsinization, the cell suspension in Hanks solution was filtered through a 100 μ pore size nylon mesh. Cells were collected by centrifugation at 200 *g* for 10 min, at room temperature, washed, resuspended in RPMI 1640 medium containing 10% fetal calf serum and antibiotics (Streptomycin, 100 μg/ml of medium), and inoculated at 10^6 cells/ml in T-75 (75 cm^2) flasks. The viability of the kidney cells prepared by this method was about 80–90% using trypan blue exclusion stain method [20].

Characterization of primary kidney cell cultures

The primary kidney cell cultures were characterized by standard histochemical methods for γ-glutamyl transpeptidase (GGT) and alkaline phosphatase (AP) [21–24]. Cells were isolated as described above and plated on glass cover slips in 9.2 cm^2 wells; after 6 days, cells reached approximately 80% confluency.

For GGT activity [21, 22, 24], cover slips were washed in PBS, dried, and incubated for 45 min with a solution of L-γ-glutamyl *p*-nitroanilide (0.08 g/l), glycylglycine (0.26 g/l), and Fast Garnet GBC (0.4 g/l) in 25 mM Tris–HCl buffer, pH 7.3. Cover slips were then washed twice with distilled water, incubated in 0.1 M $CuSO_4$ for 2 min, washed twice with distilled water, and viewed under phase contrast and bright field light microscopy. Cells containing GGT activity show bright red precipitates in the cytoplasm, while cells negative for GGT activity appear light brown.

For AP activity [23, 24], cover slips were washed in PBS, dried, and incubated for 3 h in 25 mM Tris–HCl buffer, pH 9.5, containing NaCl (100 mM), $MgCl_2$ (5 mM), 5-bromo-4-chloro-3-indoyl phosphate (0.17 g/l), and nitroblue tetrazolium (0.33 g/l). Cover slips were then washed in 10 mM EDTA, followed by distilled water, and viewed under phase contrast and bright field light microscopy. Cells containing AP activity show blue-black precipitates in the cytoplasm, while cells negative for AP activity appear light grey.

HPLC assay for estrogen metabolites in cell culture

Estrogen metabolism was monitored by measuring the conversion of [6,7-^{3}H]estradiol to various [6,7-^{3}H]estrogen metabolites separated on reverse-phase HPLC [25, 26]. Estrogen 2-hydroxylase activity was determined by measuring the conversion of [6,7-^{3}H]estradiol to [6,7-^{3}H]2-hydroxyestradiol and [6,7-^{3}H]2-hydroxyestrone. [6,7-^{3}H]Estradiol (10.0 μM, 2.5 μCi in 10 μl ethanol) was added to cultures that were approximately 90% confluent (approximately 3×10^6 cells) and incubated at 37°C. Blank samples contained [^{3}H]estradiol in media only (no cells). At varying periods from 0 to 48 h, flasks were removed from the incubator. The media was removed from the cells. An aqueous solution (2 ml) containing 5% ascorbic acid was added to the media, followed by the addition of 2-hydroxyestradiol and 2-hydroxyestrone (5 μg in 5 μl of methanol) to the media. The ascorbic acid was added to minimize catechol estrogen degradation, and the catechol estrogens were added as carriers during the isolation procedures. Ammonium sulfate (10 g/10 ml) was added to the media solution to precipitate proteins. After centrifugation at 4°C for 10 min, the supernatant was removed. The pellet was washed with ethyl acetate three times and the washings added to the supernatant fraction. The supernatant layer was then extracted three times with equal volumes of ethyl acetate. The ethyl acetate layers were dried (Na_2SO_4), evaporated under nitrogen, and the residue dissolved in 60% methanol in water (1.0 ml). The isolation of [^{3}H]estrogen metabolites from an aliquot (50 μl) of each sample was accomplished by reverse-phase HPLC on an Ultrasphere ODS column, eluting with a linear gradient of 50% methanol in water to 75% methanol in water at 1.0 ml/min over 50 min [25, 26]. Fractions (0.5 ml) were collected and counted by LSC. The amount of [^{3}H]estrogen metabolites isolated were determined by liquid scintillation counting and the total produced in the cultures calculated.

Radiometric assay for estrogen 2-hydroxylase in cell culture

Estrogen 2-hydroxylase was monitored by measuring the conversion of [2-^{3}H]estradiol to ^3H_2O [27]. [2-^{3}H]Estradiol (10.0 μM, 2.5 μCi in 10 μl ethanol) was added to cultures that were approximately 90% confluent (approximately 3×10^6 cells) and incubated at 37°C. Blank samples contained [^{3}H]estradiol in media only (no cells). At varying periods from 0 to 48 hours, flasks were removed from the incubator. A solution of cold 30% TCA (10 ml) was added to the media to precipitate proteins and the media extracted three times with chloroform (30 ml each time) containing 1% ascorbic acid. An aliquot of the aqueous solution was counted by liquid scintillation.

RESULTS

Initial velocity studies of the conversion of estradiol to 2-hydroxyestradiol, as determined by the ^3H_2O release assay with the substrate [2-^{3}H]estradiol, have been performed on freshly prepared, washed microsomes from both hamster liver and renal tissues. Both tissues demonstrate similar apparent K_ms of estrogen 2-hydroxylase of 2.85 μM (SE 1.4 μM) and 6.25 μM (SE 3.2 μM) for liver and renal microsomes, respectively. The apparent V_{max} for freshly prepared liver microsomes is 0.13 $nmol \cdot mg^{-1} \cdot min^{-1}$ (SE 0.015), while that for renal microsomes was 0.040 $nmol \cdot mg^{-1} \cdot min^{-1}$ (SE 0.016).

Evaluations of estrogen metabolism in primary kidney cell cultures were also initiated. Primary cultures of kidney cells from castrated Syrian hamsters or from castrated Syrian hamsters treated with DES were established following the procedures of Lin *et al.*[16]. γ-Glutamyl transpeptidase (GGT) and alkaline phosphatase (AP) are two enzymes particularly active in the proximal tubule epithelial cells of the kidney [21–24]. Histochemical analysis of the primary kidney cell cultures from Syrian hamsters were performed for GGT and AP. Upon establishment of the primary cell cultures, 74% of the cells stained positively for GGT and 76% stained positively for AP activity. Thus, approximately 75% of the cells in the primary cultures consist of epithelial cells.

[6,7-^{3}H]Estradiol was incubated for 0–48 h with 3×10^6 kidney cells in primary culture. Since the values for apparent K_ms for microsomal estrogen 2-/4-hydroxylase in this study are in the micromolar range and are consistent with literature values [18, 28], an estradiol concentration of 10 μM was utilized in the cell cultures. After incubation, the media was removed, proteins precipitated with ammonium sulfate, and the aqueous solution extracted with ethyl acetate. Approximately 75–80% of the total amount of radioactivity was extracted into the ethyl acetate layer. The remaining activity was distributed between the aqueous layer (10%) and the protein pellet (10–15%). The organic soluble radioactivity was analyzed by reverse-phase HPLC in order to determine the nature and extent of the primary estrogen metabolites formed. This HPLC analysis is identical to procedures developed by Brueggemeier *et al.*[25, 26] for the analysis of estrogen metabolism in isolated hepatocytes. The retentions times for estradiol and the primary metabolites under these HPLC conditions are listed in Table 1. The primary metabolites were identified by both elution with authentic standards and by mass spectrometry on isolated fractions [25, 26]. The steroids eluting very early from the reverse-phase HPLC column (0.5–2.5 min) are a mixture of more polar metabolites and the structures have not been confirmed by mass spectrometry.

Radiochromatograms were constructed from the data (Figs 1 and 2) and data tabulated (Table 2). After 48 h of incubation of estradiol with cells from untreated hamsters, the majority of the radioactivity present was estradiol. Three smaller radioactive peaks were observed, with one of these peaks (6% of total; 6 nmol) being estrone (peak *g*). The 2-hydroxyestrogens (peaks *d* and *f*), 4-hydroxyestrogens (peaks *c* and *e*), and the methoxyestrogens (peaks *i* and *j*) were not detected within the limits of sensitivity of the HPLC assay. Varying the ascorbic acid concentration added to the media from 1 to 10% did not affect the results on the catechol estrogens. The majority of the remaining radioactivity (9% of total; 9 nmol) eluted in two peaks as mixtures of more popular metabolites. These studies in the primary cultures of kidney cells from untreated hamsters demonstrate that primary metabolism of estradiol occurs to a minor degree and that the cells biosynthesize significant amounts of very polar metabolites.

Kidney cells from castrated hamsters treated with DES pellets for 12 months were isolated in the

Table 1. HPLC retention times of authentic estrogen metabolites

Estrogen	Retention time (min)	Peak
2-Hydroxyestriol	7.8–8.2	*a*
Estriol	11.5 ± 12.2	*b*
4-Hydroxyestrone	20.6 ± 22.0	*c*
2-Hydroxyestrone	22.8 ± 24.2	*d*
4-Hydroxyestradiol[a]	23.8 ± 25.8	*e*
2-Hydroxyestradiol	24.8 ± 26.4	*f*
Estrone	29.5 ± 31.0	*g*
Estradiol	30.5 ± 32.5	*h*
2-Methoxyestrone	32.8–33.8	*i*
2-Methoxyestradiol	33.6–35.6	*j*

[a]4-Hydroxyestradiol appears as a broad peak and overlaps with 2-hydroxyestradiol.

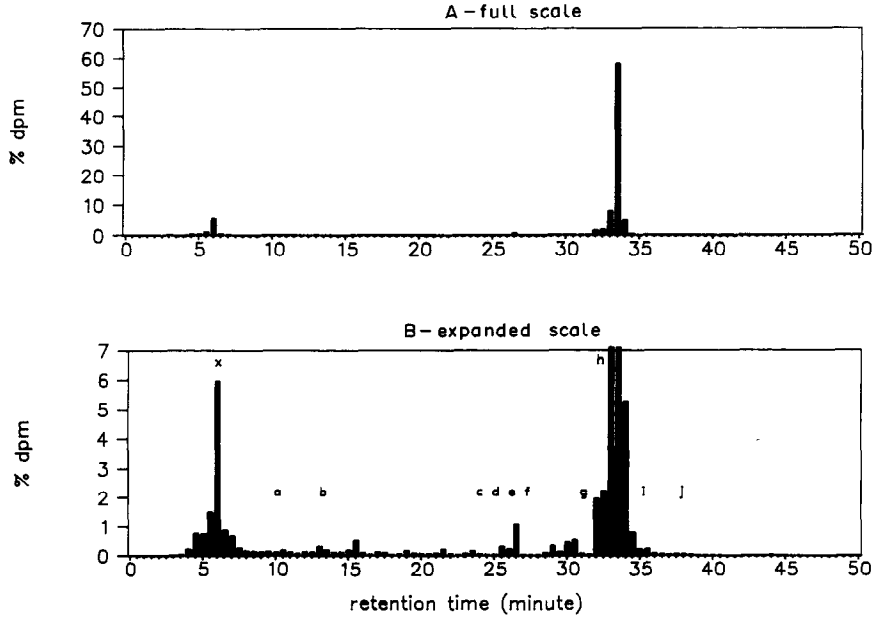

Fig. 1. Reverse-phase HPLC radiochromatograms of estradiol and metabolites at 24 and 48 h by primary kidney cell cultures from untreated hamsters. Organic-soluble radioactivity was separated by HPLC and fractions counted by liquid scintillation as described in the Materials and Methods. Histograms of the data are presented in full scale (A) and in expanded scale (B). The peaks are the heterogenous mixture (peak *x*), 2-hydroxyestriol (peak *a*), estriol (peak *b*), 4-hydroxyestrone (peak *c*), 2-hydroxyestrone (peak *d*), 4-hydroxyestradiol (peak *e*), 2-hydroxyestradiol (peak *f*), estrone (peak *g*), estradiol (peak *h*), 2-methoxyestrone (peak *i*), and 2-methoxyestradiol (peak *j*).

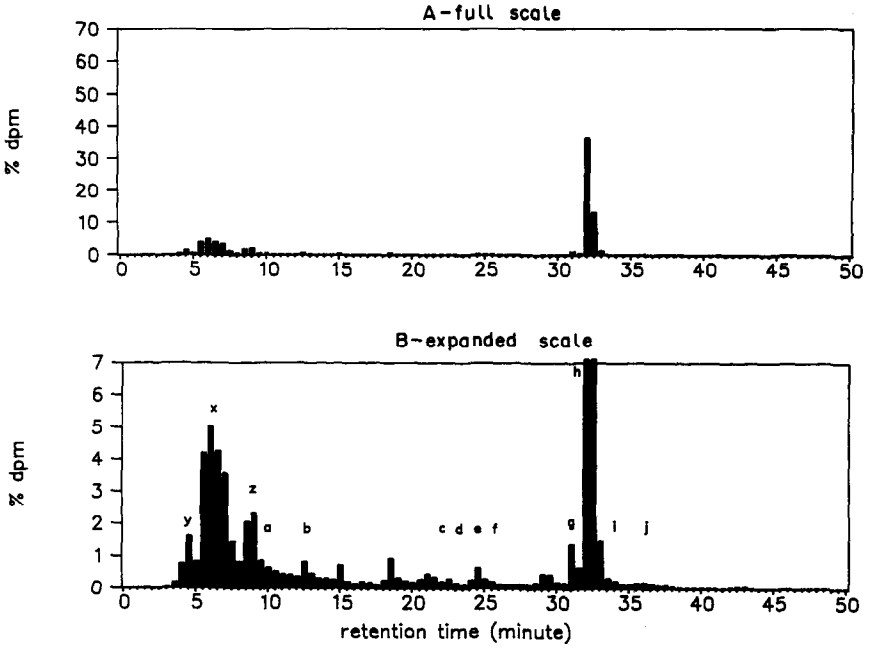

Fig. 2. Reverse-phase HPLC radiochromatograms of estradiol and metabolites at 24 and 48 h by primary kidney cell cultures from DES-treated hamsters. Organic-soluble radioactivity was separated by HPLC and fractions counted by liquid scintillation as described in the Materials and Methods. Histograms of the data are presented in full scale (A) and in expanded scale (B). The peaks are the heterogenous mixtures (peaks *x*, *y*, *z*), 2-hydroxyestriol (peak *a*), estriol (peak *b*), 4-hydroxyestrone (peak *c*), 2-hydroxyestrone (peak *d*), 4-hydroxyestradiol (peak *e*), 2-hydroxyestradiol (peak *f*), estrone (peak *g*), estradiol (peak *h*), 2-methoxyestrone (peak *i*), and 2-methoxyestradiol (peak *j*).

same fashion. Estradiol metabolism studies in these primary cultures demonstrated that the majority of the radioactivity was again present as estradiol and that estrone levels were approximately the same as observed in cells from untreated hamsters. However, three distinct radioactive peaks were observed in the region of the more polar metabolite mixtures. Additionally, the amount of radioactivity present in these HPLC peaks of more polar metabolites is greater (approximately 20%; 20 nmol).

Furthermore, the activity of estrogen 2-hydroxylase in these cultures was determined using the ^3H_2O release assay with the substrate [2-^{3}H]estradiol. [2-^{3}H]Estradiol (1 μM, 1 μCi) was added to 70% confluent, T-25 flasks of hamster kidney cells, incubated at 37°C, and media removed 24 or 48 h later. The steroids were extracted from the media and the amount of radioactivity remaining in the media was determined by LSC. After 24 h, 1.6 nmol (SD $\pm$ 0.25) of product was detected; 4.7 nmol (SD $\pm$ 0.4) of product was detected after 48 h of incubation.

CONCLUSIONS

The biochemical roles of estrogens in the initiation and development of hormone-dependent cancers remains to be fully elucidated. The estrogen-induced, estrogen-dependent kidney tumor in the Syrian hamster provides a unique *in vivo* model for examining these questions. The importance of oxidative metabolism of estrogens to catechol estrogens in the development of this cancer has been suggested by several researchers from *in vivo* studies and correlations with *in vitro* microsomal oxidation studies [9–11].

Table 2. Distribution of estrogen metabolites from hamster kidney cell cultures

	% Radioactivity (±SD)		
Estrogen	0 h	24 h	48 h
Untreated hamsters			
Polar peak *x*	1.66 (±0.04)	5.95 (±0.32)	9.04 (±0.59)
Catechol estrogens	0.86 (±0.14)	1.03 (±0.05)	1.52 (±0.38)
Estradiol	90.88 (±1.47)	83.48 (±0.32)	76.54 (±4.45)
DES-treated hamsters			
Polar peak *x*	1.76 (±0.07)	10.78 (±0.29)	17.60 (±0.42)
Polar peak *z*	0.60 (±0.01)	4.41 (±0.29)	5.61 (±0.34)
Catechol estrogens	0.93 (±0.05)	1.38 (±0.08)	1.87 (±0.21)
Estradiol	88.54 (±0.37)	72.15 (±0.42)	61.22 (±0.45)

Catechol estrogens = total % radioactivity of 2-hydroxyestrone, 2-hydroxyestradiol, 4-hydroxyestrone and 4-hydroxyestradiol.

In the studies described here, freshly isolated hamster liver and kidney microsomes exhibit estrogen 2-hydroxylase activity, as determined by the ^3H_2O assay. The K_ms for this enzymatic activity were similar in both microsomal preparations. On the other hand, the V_{max}, expressed as nmol formed per mg protein per min, for liver microsomes was higher than the V_{max} of the kidney enzyme. Thus, this enzymatic activity is observed in microsomal preparations and confirms previous reports.

A short-term primary kidney cell culture system was utilized in order to investigate if the formation of the catechol estrogens themselves, their own action, or further metabolism of the 2-/4-hydroxyestrogens via oxidative or peroxidative pathways occurs in intact cells. Primary hamster kidney cell cultures from untreated, castrated hamsters and from DES-treated, castrated hamsters were established. The cultures from untreated, castrated hamsters metabolize [^{3}H]estradiol to yield small quantities of estrone and significant amounts of polar metabolites. No catechol estrogens were isolated, even though ascorbic acid and unlabeled carrier catechol estrogens were added prior to extractions and isolation. The major difference between estrogen metabolism by DES-treated hamster kidney cell cultures and untreated hamster kidney cell cultures is the appearance of additional polar metabolites (peaks *y* and *z*) in the cultures from DES-treated hamsters. The structures of these polar metabolites remain to be determined. Again, no catechol estrogens were isolated from the primary cultures from the DES-treated hamsters.

Finally, investigations were initiated to determine if estrogen 2-hydroxylase activity was present in the primary cultures. The radiometric assay measuring the release of ^3H_2O from [2-^{3}H]estradiol was utilized. Primary hamster kidney cell cultures exhibited estrogen 2-hydroxylase activity, producing 1.6 nmol (SD $\pm$ 0.25) of product in 24 h and 4.7 nmol (SD $\pm$ 0.4) of product after 48 h.

An explanation for this difference between the HPLC isolation assay and the radiometric assay is that one or more of the compounds present in the polar metabolite mixture (peaks *x*, *y*, *z*) are derived from further metabolism of the catechol estrogens by the cells. Catechol estrogens can be converted to the semiquinone and quinone derivatives via further oxidative/peroxidative pathways [29]. Such pathways can provide more polar primary metabolites of estrogens as well as secondary, conjugated metabolites. The results of estrogen metabolism in these primary kidney cell cultures suggests that these pathways are operational. Furthermore, the increased amounts of more polar metabolites in the cells from the DES-treated hamsters suggest that this pathway is enhanced in these cells. The mechanism of kidney tumorigenesis in the estrogen-treated Syrian hamster remains unknown, but the increases in oxidative metabolism of estrogens observed in this study with primary kidney cells suggests that such transformations may be relevant in either a direct or indirect manner in tumorigenesis.

Thus, the short-term primary kidney cell cultures from the Syrian hamster are capable of metabolizing estrogens. Furthermore, the enzymatic processes appear to be available for the conversion of any catechol estrogens formed into more polar metabolites. Validation will require complete structural elucidation of the various steroidal components of the mixture of polar metabolites. Investigations in intact cells, capable of performing all biochemical processes, will complement both *in vivo* and subcellular biochemical studies. Such studies are necessary in understanding the roles of estrogens and estrogen metabolism in the initiation and development of the estrogen-induced, estrogen-dependent kidney tumors in the Syrian hamster.

Acknowledgements—This work was supported in part by Grant P30-CA16058 from the National Cancer Institute and a grant from the Research Challenge Investigator Funds, The Ohio State University.

REFERENCES

1. Kirkman H. and Bacon R. L.: Estrogen-induced tumors of the kidneys. I. Incidence of renal tumors in intact and gonadectomized male golden hamsters treated with DES. *J. Natn. Cancer Inst.* **13** (1952) 745–752.
2. Kirkman H. and Algard F. T.: Characteristics of an androgen-estrogen-induced uterine smooth muscle cell tumor of the Syrian hamster. *Cancer Res.* **30** (1970) 794–798.
3. McQuire W. L., Julian J. A. and Chamness G. C.: A dissociation between ovarian-dependent growth and estrogen sensitivity in mammary carcinoma. *Endocrinology* **89** (1971) 969–973.
4. Herbst A. L., Ulfelder H. and Poskanzer D. C.: Adenocarcinoma of the vagina. Association of maternal stilbestrol therapy with tumor appearance in young women. *New Engl. J. Med.* **284** (1971) 878–881.
5. Greenwald P., Barlow J. J. and Nasa P. C.: Vaginal cancer after maternal treatment with synthetic estrogens. *New Engl. J. Med.* **285** (1971) 390–392.
6. Stern E., Clark V. A. and Coffelt C. F.: Contraceptives and dysplasia: higher rate for pill choosers. *Science* **169** (1970) 497–498.
7. Matthews V. S., Kirkman H. and Bacon R. L.: Kidney damage in the golden hamster following chronic administration of DES and sesame oil. *Proc. Soc. Exp. Biol. Med.* **66** (1947) 195–196.
8. Kirkman H. and Algard F. T.: In *The Golden Hamster: Its Biology and Use in Medical Research* (Edited by R. D. Hoffman *et al.*). Iowa State Univ. Press, Ames, Iowa (1968) p. 227.
9. Li J., Li S. A., Klicka J. K., Parsons J. A. and Lam L. K. T.: Relative carcinogenic activity of various synthetic and natural estrogens in the Syrian hamster kidney. *Cancer Res.* **43** (1983) 5200–5204.
10. Li J. J. and Li S. A.: Roles for hormonal and carcinogenic activities. *Arch. Toxic.* **55** (1984) 110–118.
11. Li S. A., Klicka J. K. and Li J. J.: Estrogen 2- and 4-hydroxylase activity, catechol estrogen formation, and implications for estrogen carcinogenesis in the hamster kidney. *Cancer Res.* **45** (1985) 181–185.

12. Li S. A., Klicka J. K. and Li J. J.: Effect of androgen and estrogen treatment on hamster liver and kidney estrogen 2-/4-hydroxylase activity. *Endocrinology* **119** (1986) 1810–1815.
13. Liehr J. G., Hall E. R., Avitts T. A., Randerath E. and Randerath K.: Localization of estrogen-induced DNA adducts and cytochrome P-450 activity at the site of renal carcinogenesis in the hamster kidney. *Cancer Res.* **47** (1987) 2156–2159.
14. Degen G. H., Eling T. E. and MaLachlan J. A.: Oxidative metabolism of DES by prostaglandin synthetase. *Cancer Res.* **42** (1982) 919–923.
15. Degen G. H., Wong A., Eling T. E., Barrett J. C. and McLachan J. A.: Involvement of prostaglandin synthetase in the peroxidative metabolism of DES in Syrian hamster embryo fibroblast cell cultures. *Cancer Res.* **43** (1983) 992–996.
16. Lin Y. C., Talley D. J. and Villee C. A.: Dynamics of progesterone binding in nuclei and cytosol of estrogen-induced adenocarcinoma cells in primary culture. *J. Steroid Biochem.* **13** (1980) 29–37.
17. Eckert R. L. and Katzenellenbogen B. S.: Effects of estrogens and antiestrogens on receptor dynamics and the induction of progesterone receptor in MCF-7 human breast cancer cells. *Cancer Res.* **42** (1982) 139–144.
18. Brueggemeier R. W.: Kinetics of rat liver microsomal estrogen 2-hydroxylase. *J. Biol. Chem.* **256** (1981) 10239–10242.
19. Brueggemeier R. W.: Inhibition of estrogen 2-hydroxylase. *J. Steroid Biochem.* **19** (1983) 1683–1685.
20. Phillips J. J.: In *Tissue Culture* (Edited by P. F. Kruse Jr and M. K. Patterson Jr). Academic Press, New York (1973) p. 406.
21. Glenner G. G. and Folk J. E.: Glutamyl peptidases in rat and guinea pig kidney slices. *Nature* **192** (1961) 338–340.
22. Glenner G. G., Folk J. E. and McMillan P. J.: Histochemical demonstration of gamma-glutamyl transpeptidase-like activity. *J. Histochem. Cytochem.* **10** (1962) 481–490.
23. Glenner G. G.: Alkaline phosphatase of cell nuclei. *J. Lab. Clin. Med.* **37** (1951) 526–531.
24. Gibson-d'Ambrosio R. E., Samuel M., Chang C. C., Trosko J. E. and D'Ambrosio S. M.: Characteristics of long-term human epithelial cell cultures derived from normal human fetal kidney. *In Vitro Cell. Dev. Biol.* **23** (1987) 279–287.
25. Brueggemeier R. W., Kimball J. G. and Kraft F.: Estrogen metabolism in rat liver microsomal and isolated hepatocyte incubations: I. Metabolite formation and irreversible binding to cellular macromolecules. *Biochem. Pharmac.* **33** (1984) 3853–3859.
26. Brueggemeier R. W., Kimball J. G. and Kraft K.: Estrogen metabolism in rat liver microsomal and isolated hepatocyte incubations: II. Inhibitor studies. *J. Steroid Biochem.* **21** (1984) 709–716.
27. Brueggemeier R. W., Katlic N. E., Palmer C. W. and Stevens J. M.: Catechol estrogen formation in MCF-7 cell culture and effects of bromoestrogen inhibitors. *Molec. Cell. Endocr.* **64** (1989) 161–167.
28. Merriam G. R. and Lipsett M. B. (Eds): *Catechol Estrogens.* Raven Press, New York (1983).
29. Kuss E.: Estrogens and glutathione. In *Glutathione: Chemical, Biochemical and Medical Aspects, Part B* (Edited by D. Dolphin, R. Poulson and O. Avramovic). Wiley, New York (1989) pp. 511–549.