[CANCER RESEARCH 45, 4946–4949, October 1985]

Sex Differences in Indomethacin-sensitive 3α-Hydroxysteroid Dehydrogenase of Rat Liver Cytosol[1]

Thomas E. Smithgall[2] and Trevor M. Penning[3]

Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

ABSTRACT

The 3α-hydroxysteroid:nicotinamide adenine dinucleotide (phosphate) oxidoreductase (EC 1.1.1.50) of rat liver cytosol is indistinguishable from *trans*-1,2-dihydrobenzene-1,2-diol dehydrogenase (EC 1.3.1.20) (T. M. Penning, I. Mukharji, S. Barrows, and P. Talalay, Biochem. J., *222:* 601–611, 1984) and has been implicated in the detoxification of ultimate carcinogens (H. R. Glatt *et al.*, Science (Wash. DC), *215:* 1507–1509, 1982). Using *trans*-1,2-dihydroxy-3,5-cyclohexadiene as a model substrate for *trans*-dihydrodiol proximate carcinogens, this study shows that the specific activity of 3α-hydroxysteroid:nicotinamide adenine dinucleotide (phosphate) oxidoreductase is 2-fold higher in the 40–75% ammonium sulfate fraction prepared from female rat liver cytosol than in similar fractions prepared from males. Comparable differences were also observed for the nicotinamide adenine dinucleotide-dependent oxidation of 5α-androstan-3α-ol-17-one. Chromatofocusing of these cytosolic fractions separated the bulk of the protein from the dehydrogenase, which eluted as a single peak at pH 5.4. Examination of the protein profiles indicates that twice as much protein coeluted with the enzyme from female rat liver cytosol, suggesting that induction is responsible for the sex difference in enzyme activity. These differences were abolished by ovariectomy, while administration of a single dose of estradiol 3-sulfate (100 μg) to ovariectomized rats restored enzyme activity to within 90% of normal female levels. These findings suggest that ovarian estrogen is a natural inducer of rat liver 3α-hydroxysteroid:nicotinamide adenine dinucleotide (phosphate) oxidase reductase/*trans*-1,2-dihydrobenzene-1,2-diol dehydrogenase.

INTRODUCTION

The homogeneous 3α-hydroxysteroid dehydrogenase (EC 1.1.1.50)[4] of rat liver cytosol is a versatile NAD(P)-linked oxidoreductase that catalyzes the oxidation of 3α-hydroxysteroids, simple secondary alcohols, and benzenedihydrodiol (1, 2). The properties of this enzyme are indistinguishable from those of the dihydrodiol dehydrogenase originally purified from this source by Vogel *et al.* (3). Addition of the purified dehydrogenase to the Ames test (mouse liver microsomes + NADPH + *Salmonella typhimurium his*$^-$ mutant) for benzo(*a*)pyrene significantly reduced the mutagenicity of this compound (4), suggesting that the enzyme may detoxify the *trans*-dihydrodiols formed *in situ* by oxidizing them to the less reactive catechols. Similar experiments have shown that purified dihydrodiol dehydrogenase can reduce the mutagenicity of a diol-epoxide of benz(*a*)anthracene (5). These data suggest that dihydrodiol dehydrogenase may detoxify both proximate and ultimate carcinogens.

The nonsteroidal antiinflammatory drugs are remarkably potent inhibitors of 3α-hydroxysteroid oxidation catalyzed by the purified dehydrogenase (1, 2). In addition we have recently shown that benzenedihydrodiol and *trans*-1,2-dihydroxy-1,2-dihydronaphthalene oxidation, catalyzed by the same purified enzyme, are potently inhibited by representatives of all of the major classes of the nonsteroidal antiinflammatory drugs at physiological pH.[5] Inhibition of this potentially important detoxification pathway by these widely used compounds may have implications for the initiation phase of chemical carcinogenesis.

A common feature of many xenobiotic-metabolizing enzymes is inducibility, and identification of inducers of enzymes involved in the detoxification of carcinogens may have utility in the chemoprevention of cancer. For example induction of glutathione-*S*-transferase (6), microsomal epoxide hydratase (7), and a NAD(P)H-linked quinone reductase (8) by the common food additive 2(3)-*tert*-butyl-4-hydroxyanisole have all been proposed as mechanisms for the protective effects of this compound against carcinogenesis (9). Steroid hormones can also act as natural inducers of enzymes involved in drug metabolism. For example alcohol dehydrogenase of mouse kidney has been shown to be under androgenic control (10). The present report demonstrates significant sex differences in the specific activity of the 3α-hydroxysteroid/dihydrodiol dehydrogenase from rat liver cytosol and shows that this difference is due to induction by ovarian estrogen.

MATERIALS AND METHODS

Chemicals. Androsterone and estradiol 3-sulfate were purchased from Steraloids (Wilton, NH). β-NAD and NADP (monosodium salts) were obtained from Pharmacia PL Biochemicals (Piscataway, NJ). Indomethacin was purchased from Sigma Chemical Co. (St. Louis, MO). Enzyme grade ammonium sulfate and sucrose were obtained from Schwarz/Mann, Inc. (Spring Valley, NY). Polybuffer exchanger PBE-94 and Polybuffer 74 (for chromatofocusing) were products of Pharmacia P-L Biochemicals. Benzenedihydrodiol was synthesized by treating benzene oxide (1,2-epoxy-3,5-cyclohexadiene) with hydrogen peroxide in

[1] These studies were supported by NIH Grant GM33464, a Biomedical Research Support Grant (2-307-RR-07083-18) awarded to the University of Pennsylvania by the Division of Research Resources, NIH, and by an American Cancer Society Institutional Grant (IN-135D). Statistical analyses and figures were prepared at a PROPHET computer terminal.

[2] Recipient of a Pharmaceutical Manufacturers Association Foundation Advanced Predoctoral Fellowship.

[3] To whom requests for reprints should be addressed, at University of Pennsylvania School of Medicine, Department of Pharmacology/G3, 37th and Hamilton Walk, Phildelphia, PA 19140.

[4] The abbreviations and trivial names used are: 3α-hydroxysteroid dehydrogenase, 3α-hydroxysteroid:NAD(P) oxidoreductase (EC 1.1.1.50); dihydrodiol dehydrogenase, *trans*-1,2-dihydrobenzene-1,2-diol: dehydrogenase (EC 1.3.1.20); benzenedihydrodiol, (±)-*trans*-1,2-dihydroxy-3,5-cyclohexadiene; androsterone, 5α-androstan-3α-ol-17-one; 5α-dihydrotestosterone, 5α-androstan-3-one-17β-ol; 3α-androstanediol, 5α-androstane-3α,17β-diol; estradiol 3-sulfate, 1,3,5(10)-estratriene-3,17β-diol 3-sodium sulfate; DTT, dithiothreitol.

Received 1/23/25; revised 6/25/85; accepted 6/27/85.

[5] T. E. Smithgall and T. M. Penning, unpublished observations.

aqueous base, followed by sodium borohydride reduction of the resulting hydroperoxide (11). Benzene oxide was synthesized from 1,4-cyclohexadiene (Aldrich Chemical Co., Milwaukee, WI) by the method of Vogel (12). IR and UV data for the final product agree with published data (13).

Animals. Male, female, and ovariectomized Sprague-Dawley rats (180–200 g) were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA). Female rats were ovariectomized during the fourth week of life, and 2 weeks later they were either sacrificed or given injections of estradiol 3-sulfate (see below).

Enzyme Preparation. Rats were killed by cervical dislocation and livers were removed, frozen in liquid nitrogen, and stored at −80°C. Thawed livers were homogenized in 3 volumes (w/v) of 50 mM Tris-HCl, pH 8.6, containing 250 mM sucrose, 1 mM EDTA, and 1 mM DTT, and centrifuged at 10,000 × *g* for 30 min. The supernatant was filtered through nonabsorbent cotton and microsomes were sedimented by centrifugation at 100,000 × *g* for 1 h. Cytosolic proteins were precipitated from the resulting supernatant with ammonium sulfate, yielding fractions of 0–40%, 40–75%, and >75% saturation. The protein precipitates were collected by centrifugation (15,000 × *g* for 30 min) and redissolved in a minimal volume of dialysis buffer containing 10 mM Tris-HCl, 1 mM EDTA, and 0.5 mM DTT (pH 8.6 at 4°C). All three ammonium sulfate fractions were dialyzed overnight against three 1-liter changes of this buffer. The dialyzed material was then assayed for enzyme activity.

Spectrophotometric Assay for 3α-Hydroxysteroid and Dihydrodiol Dehydrogenase Activities. Since 3α-hydroxysteroid dehydrogenase catalyzes the oxidation of 3α-hydroxysteroids and *trans*-dihydrodiols, both androsterone and benzenedihydrodiol were used as substrates for the enzyme. Oxidation of androsterone was measured in a 1.0-ml system containing 100 mM potassium phosphate buffer (pH 7.0), 2.3 mM NAD^+, and 75 μM androsterone; benzenedihydrodiol oxidation was assayed in 1.0 ml of 100 mM potassium phosphate buffer (pH 7.0) containing 2.3 mM NADP and 1.0 mM benzenedihydrodiol. Androsterone and benzenedihydrodiol were dissolved in acetonitrile and methanol, respectively, and the final concentration of organic solvent in the assay was 4%. Both reactions were monitored by following the increase in absorbance of the pyridine nucleotide at 340 nm. Reactions were initiated by the addition of the enzyme preparation. Activities were expressed as nmol substrate oxidized per min per mg protein, except for the chromatofocusing experiments where they were expressed as μmol substrate oxidized per min per fraction. Protein determinations were made using the method of Lowry *et al.* (14), using crystalline bovine serum albumin (Armour Pharmaceutical Co., Kankakee, IL) as standard.

Inhibition of 3α-Hydroxysteroid Dehydrogenase by Indomethacin. Using the spectrophotometric assay the concentration of indomethacin required to produce 50% inhibition of the initial rate of both benzenedihydrodiol and androsterone oxidation was determined. Indomethacin was dissolved in the same organic solvent as the substrate (acetonitrile or methanol), and the final organic solvent concentration was kept constant at 4%.

Chromatofocusing of the 40–75% Ammonium Sulfate Fractions of Male and Female Rat Liver Cytosol. Representative 40–75% ammonium sulfate fractions prepared from male or female rat livers were dialyzed against 25 mM imidazole-HCl, pH 7.4, containing 1 mM EDTA and 0.5 mM DTT (three 1-liter changes). After determination of the initial specific activity, the sample was applied to a column (1 x 15 cm) packed with polybuffer exchanger PBE-94 and equilibrated to pH 7.4 with the dialysis buffer. A linear pH gradient from 7 to 5 was generated on the polybuffer exchanger by elution with 10 bed volumes of Polybuffer 74-HCl, pH 5.0, containing 1 mM EDTA and 0.5 mM DTT. Protein elution was monitored at 280 nm. Fractions (2.0 ml) were collected at 10 ml/h and assayed spectrophotometrically as described.

RESULTS

Comparison of the Specific Activity of 3α-Hydroxysteroid Dehydrogenase Present in Liver Cytosol of Male, Female, and Ovariectomized Rats. The oxidation of benzenedihydrodiol in the presence of NADP and of androsterone in the presence of NAD, catalyzed by ammonium sulfate fractions prepared from male, female, and ovariectomized rat liver cytosols, was measured. In all groups of animals the majority of the activity precipitated in the 40–75% ammonium sulfate fraction. Specific activities for the oxidation of both substrates were approximately 2-fold higher in females than in males, and ovariectomy abolished this difference, suggesting that the enzyme is under the control of ovarian hormones (Chart 1). The oxidation of androsterone and benzenedihydrodiol, catalyzed by both male and female rat liver cytosols, was potently inhibited by indomethacin, yielding 50% inhibitory concentrations in the low micromolar range (Chart 2). The apparent differences in the potency of inhibition are most probably due to differences in the K_m values of these two substrates for the enzyme [K_m values for benzenedihydrodiol and androsterone for the purified rat liver 3α-hydroxysteroid dehydrogenase are 1.0 mM and 30 μM, respectively (1)]. At higher indomethacin concentrations the oxidation of both substrates was nearly abolished (greater than 90% inhibition). This result is consistent with the view that the indomethacin-sensitive enzyme is the major activity responsible for the oxidation of benzenedihydrodiol and androsterone in both male and female rat liver cytosols.

Chromatofocusing of the 40–75% Ammonium Sulfate Fractions of Male and Female Rat Liver Cytosols. When the 40–75% ammonium sulfate fraction prepared from either male or female rat liver cytosol was subjected to chromatofocusing, the 3α-hydroxysteroid dehydrogenase (pI 5.4) was separated from the bulk of the protein (Chart 3). In both sexes all of the activity responsible for the oxidation of benzenedihydrodiol and androsterone eluted under this peak. In females activity in the peak fractions was twice as high as that found in males. Examination of the A_{280} profiles indicates that approximately twice as much protein eluted with the activity present in female cytosol, suggesting that enzyme induction may be responsible for sex differences in enzyme activity. Resolution of these differences is possible using this single chromatographic step, since the 3α-hydroxysteroid dehydrogenase comprises almost 1% of the total protein present in male rat liver cytosol (1). These results are consistent with the presence of a single dehydrogenase existing at higher (induced) levels in female rat liver cytosol.

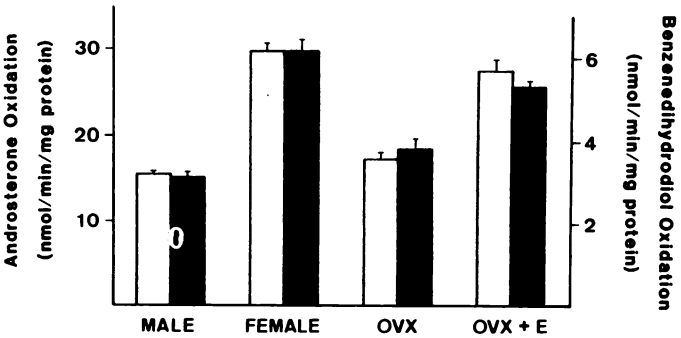

Chart 1. Comparison of 3α-hydroxysteroid dehydrogenase and dihydrodiol dehydrogenase activities in the 40–75% ammonium sulfate fractions of rat liver cytosol prepared from male, female, ovariectomized (*OVX*), and ovariectomized plus estrogen-treated (*OVX+E*) animals. Oxidation of androsterone in the presence of NAD (□) and oxidation of benzenedihydrodiol in the presence of NADP (■) were monitored spectrophotometrically at 340 nm. Each *column* represents the mean of specific activities measured in four animals; *bars*, SE. The statistical significance of these differences was confirmed using Student's *t* test.

Identification of the Natural Inducer of 3α-Hydroxysteroid Dehydrogenase. In order to identify the natural ovarian inducer of 3α-hydroxysteroid dehydrogenase, 12 ovariectomized rats were given a single i.p. dose of estradiol 3-sulfate (100 μg in water via i.p. injection), and groups of 4 animals were sacrificed 2, 4, and 6 days after injection. After the livers were processed, the 40–75% ammonium sulfate fractions were prepared and assayed for the oxidation of benzenedihydrodiol and androsterone. The results shown in Chart 1 clearly indicate that, following the administration of estrogen, both activities were restored to within 90% of those observed in intact females. The maximum increase in enzyme activity was observed 2 days after hormone injection. Oxidation of benzenedihydrodiol and androsterone by the estrogen-treated group was almost completely abolished by 30 μM indomethacin. Chromatofocusing experiments (not shown) indicate that the enzyme from the estrogen-treated group focused as a single peak with a pI of 5.4. These findings suggest that estrogen acts as an endogenous inducer of the indomethacin-sensitive 3α-hydroxysteroid dehydrogenase of rat liver cytosol.

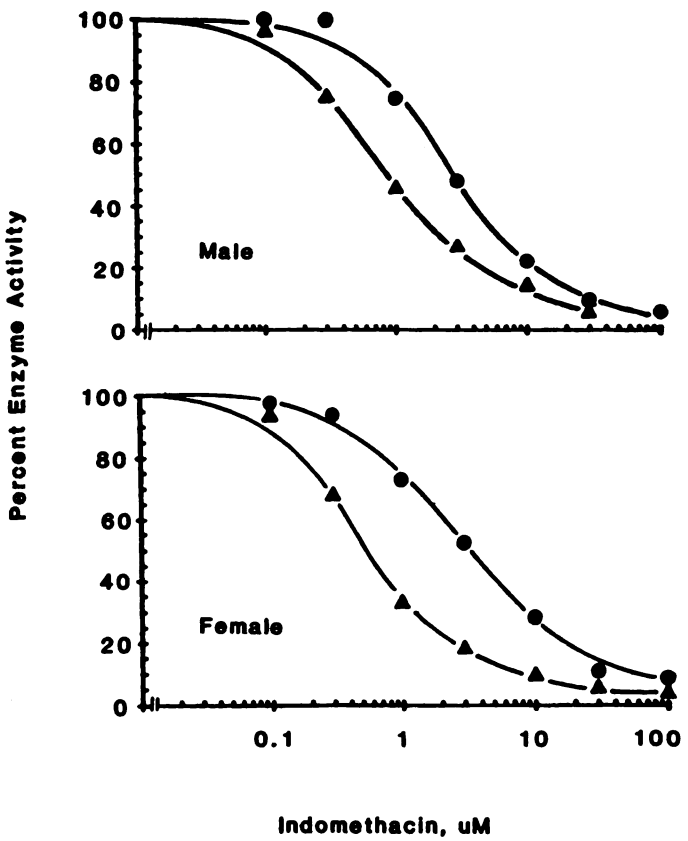

Chart 2. Inhibition of 3α-hydroxysteroid and dihydrodiol dehydrogenase activities by indomethacin in the 40–75% ammonium sulfate fractions prepared from male and female rat liver cytosols. Androsterone oxidation (●) and benzenedihydrodiol oxidation (▲) were assayed by following the absorbance of the pyridine nucleotide at 340 nm in the presence of 1–100 μM indomethacin. Results are expressed as percentage of specific activity measured in the absence of drug.

DISCUSSION

This report demonstrates that the 3α-hydroxysteroid dehydrogenase of female rat liver cytosol is induced by estrogen. Briefly a 2-fold sex difference was observed in the ability of rat liver cytosol to oxidize benzenedihydrodiol and androsterone. This difference was abolished by ovariectomy and restored by the administration of estradiol 3-sulfate. The effect of estrogen appears to be one of true induction rather than activation or decreased degradation of the enzyme, because twice as much protein is associated with the elevated enzyme activities present in females. The induced dehydrogenase appears to be identical to the uninduced enyzme, since both proteins have the same isoelectric point and are equally sensitive to inhibition by low micromolar concentrations of indomethacin.

The oxidation of *trans*-dihydrodiols, catalyzed by 3α-hydroxysteroid dehydrogenase, has been implicated in the detoxification of polycyclic aromatic hydrocarbons (3–5). Inhibition of this enzyme by indomethacin or other "aspirin-like" drugs may interfere with the detoxification of proximate carcinogens *in vivo*. Since estrogens enhance 3α-hydroxysteroid/dihydrodiol dehydrogenase activity, we speculate that they may protect against this possible adverse effect of the nonsteroidal antiinflammatory drugs.

The finding that estrogen acts as a natural inducer of the hepatic 3α-hydroxysteroid dehydrogenase is consistent with earlier reports of elevated 3α-hydroxysteroid dehydrogenase activ-

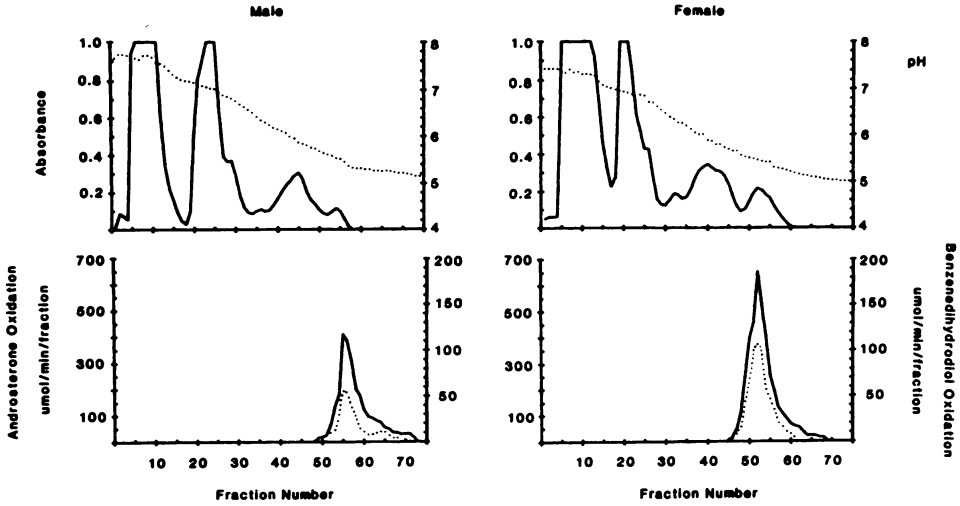

Chart 3. Chromatofocusing of cytosolic proteins from male and female rat livers. Protein (160 mg) from the 40–75% ammonium sulfate fraction prepared from either male or female rat liver cytosol was dialyzed and applied to a chromatofocusing column (1.0 x 15.0 cm). The column was eluted with Polybuffer 74 (pH 5.0), and fractions (2.0 ml) were collected at 10 ml/h. *Top:* ——, protein measured as absorbance at 280 nm; · · · · ·, pH of each fraction. *Bottom:* ——, androsterone oxidation with NAD; · · · · ·, benzenedihydrodiol oxidation with NADP.

ities in cytosols prepared from female rat liver and kidney. Specifically Hoff and Schreifers (15) have demonstrated sex differences in the rate of 5α-androstan-3,17-dione reduction catalyzed by rat liver cytosol, while Verhoeven *et al.* (16) have shown that the reduction of 5α-dihydrotestosterone to 3α-androstanediol catalyzed by rat kidney cytosol is 2-fold greater in females. This latter difference has been attributed to regulation of the enzyme by estrogen (17).

In addition to steroid hormones polycyclic aromatic hydrocarbons such as benz(*a*)anthracene and 3-methylcholanthrene are also potent enzyme inducers, acting primarily on the microsomal mixed function oxidases responsible for their metabolism (18, 19). This inductive effect may represent an important mechanism for increasing the level of polycyclic aromatic hydrocarbon metabolism but may also lead to enhanced formation of toxic intermediates (20). It will be of interest to determine whether polycyclic aromatic hydrocarbons also induce 3α-hydroxysteroid/dihydrodiol dehydrogenase activity. Under such circumstances these compounds may also enhance the detoxification of their own *trans*-dihydrodiol metabolites. Glatt *et al.* (21) have recently shown that dihydrodiol dehydrogenase activity of male C3H mouse liver cytosol is not sensitive to induction by either 3-methylcholanthrene or phenobarbital. However, these studies may not predict the sensitivity of the rat liver enzyme to these classical inducers because significant species variation in hepatic dihydrodiol dehydrogenase has been reported. For example Swiss-Webster mouse liver cytosol has been shown to contain multiple forms of dihydrodiol dehydrogenase, which also display 17β-hydroxysteroid dehydrogenase activity (22).

REFERENCES

1. Penning, T. M., Mukharji, I., Barrows, S., and Talalay, P. Purification and properties of a 3α-hydroxysteroid dehydrogenase of rat liver cytosol and its inhibition by anti-inflammatory drugs. Biochem. J., *222:* 601–611, 1984.
2. Penning, T. M., and Talalay, P. Inhibition of a major NAD(P)-linked oxidoreductase from rat liver cytosol by steroidal and nonsteroidal anti-inflammatory agents and by prostaglandins. Proc. Natl. Acad. Sci. USA, *80:* 4504–4508, 1983.
3. Vogel, K., Bentley, P., Platt, K-L., and Oesch, F. Rat liver cytoplasmic dihydrodiol dehydrogenase: purification to apparent homogeneity and properties. J. Biol. Chem., *255:* 9621–9625, 1980.
4. Glatt, H. R., Vogel, K., Bentley, P., and Oesch, F. Reduction of benzo[*a*]pyrene mutagenicity by dihydrodiol dehydrogenase. Nature (Lond.), *277:* 319–320, 1979.
5. Glatt, H. R., Cooper, C. S., Grover, P. L., Sims, P., Bentley, P., Merdes, M., Waechter, F., Vogel, K., Guenther, T. M., and Oesch, F. Inactivation of a diol-epoxide by dihydrodiol dehydrogenase but not by two epoxide hydrolases. Science (Wash. DC), *215:* 1507–1509, 1982.
6. Benson, A. M., Batzinger, R. P., Ou, S. L., Bueding, E., Cha, Y., and Talalay, P. Elevation of hepatic glutathione-*S*-transferase activities and protection against mutagenic metabolites of benzo(*a*)pyrene by dietary antioxidants. Cancer Res., *38:* 4486–4495, 1978.
7. Cha, Y., Martz, F., and Bueding, F. Enhancement of liver microsome epoxide hydratase activity in rodents by treatment with 2(3)-*tert*-butyl-4-hydroxyanisole. Cancer Res., *38:* 4496–4498, 1978.
8. Benson, A. M., Hunkeler, M. J., and Talalay, P. Increase of NAD(P)H:quinone reductase by dietary antioxidants: possible role in protection against carcinogenesis and toxicity. Proc. Natl. Acad. Sci. USA, *77:* 5216–5220, 1980.
9. Wattenberg, L. W. Inhibition of carcinogenic and toxic effects of polycyclic hydrocarbons by phenolic antioxidants and ethoxyquin. J. Natl. Cancer Inst., *48:* 1425–1430, 1972.
10. Ohno, S., Stenius, C., and Christian, L. C. Sex difference in alcohol metabolism; androgenic steroid as an inducer of kidney alcohol dehydrogenase. Clin. Genet., *1:* 35–44, 1970.
11. Jeffrey, A. M., Yeh, H. J., Jerina, D. M., DeMarinis, R. M., Foster, C. H., Piccolo, D. E., and Berchtold, G. A. Stereochemical course in reactions between nucleophiles and arene oxides. J. Am. Chem. Soc., *96:* 6929–6937, 1974.
12. Vogel, E., and Gunther, H. Benzene oxide-oxepin valence tautomerism. Angew. Chem. Int. Ed. Eng., *6:* 385–401, 1967.
13. Nakajima, M., Tomida, I., Hashizume, A., and Takei, S. Darstellung und Eigenschaften des *trans*-5.6-Dihydroxy Cyclohexadiens-1.3. Chem. Ber., *89:* 2224–2229, 1956.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem., *193:* 265–275, 1951.
15. Hoff, H-G., and Schreifers, H. Sexuell differenzierte und sexuell Undifferenzierte 3α und 3β-Hydroxysteroid Dehydrogenase-Aktivitaten und ihre Intrazellulore Lokalisation in der Rattenleber. Hoppe-Seyler's Z. Physiol. Chem., *354:* 507–513, 1973.
16. Verhoeven, G., Heyns, W., and DeMoor, P. Interconversion between 17β-hydroxy-5α-androstan-3-one (5α-dihydrotestosterone) and 5α-androstane-3α,17β-diol in rat kidney: heterogeneity of 3α-hydroxysteroid oxidoreductases. Eur. J. Biochem., *65:* 565–576, 1976.
17. Ghraf, R., Lax, E. R., Hoff, H., and Schriefers, H. The role of the gonads and the hypophysis in the regulation of hydroxysteroid dehydrogenase activities in rat kidney. Hoppe-Seyler's Z. Physiol. Chem., *356:* 135–142, 1975.
18. Nebert, D. W., and Gelboin, H. V. Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture I. Assay and properties of induced enzyme. J. Biol. Chem., *243:* 6242–6249, 1968.
19. Nebert, D. W., and Gelboin, H. V. Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture II. Cellular responses during enzyme induction. J. Biol. Chem., *243:* 6250–6261, 1968.
20. Gelboin, H. V., Kinoshita, N., and Wiebel, F. J. Microsomal hydroxylases: induction and role in polycyclic hydrocarbon carcinogenesis and toxicity. Fed. Proc., *31:* 1298–1309, 1972.
21. Glatt, H., Vogel, K., Bentley, P., Sims, P., and Oesch, F. Large differences in metabolic activation of chemically closely related compounds: effects of pure enzymes and enzyme induction on the mutagenicity of the twelve monomethylated benz[*a*]anthracenes, 7,12-dimethylbenz[*a*]anthracene and benz[*a*]-anthracene in the Ames test. Carcinogenesis (Lond.), *2:* 813–821, 1981.
22. Bolcsak, L. E., and Nerland, D. E. Purification of mouse liver benzenedihydrodiol dehydrogenases. J. Biol. Chem., *258:* 7252–7255, 1983.