Characterization of Dihydrodiol Dehydrogenase in Rat H-4IIe Hepatoma Cells

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ABSTRACT

Dihydrodiol dehydrogenase (EC 1.3.1.20) catalyzes the NADP⁺-dependent oxidation of a variety of trans-dihydrodiol proximate carcinogens, a reaction that may suppress their carcinogenicity. Using benzene-dihydrodiol [(+)-trans-1,2-dihydroxy-3,5-cyclohexadiene] as a substrate, this enzyme can be detected spectrophotometrically in rat H-4IIe hepatoma cells, or in a specific activity similar to that observed in rat liver cytosol. The hepatoma cell enzyme is potently inhibited by 6-methoxyprogestosterone acetate (IC₅₀ = 38 nM) and indomethacin (IC₅₀ = 3.5 μM).

These cells contain 3α-hydroxysteroid dehydrogenase which is also sensitive to inhibition by the same two drugs. Chromatofocusing of hepatoma cell lysates indicates that both dihydrodiol dehydrogenase and 3α-hydroxysteroid dehydrogenase activities coelute with a pl of 5.8. Western blot analysis of hepatoma cell lysates, using rabbit anti-rat 3α-hydroxysteroid/dihydrodiol dehydrogenase serum detects a single immunoreactive species with a Mᵋ of 34,000. Using this antiserum it was possible to immunotitrate both these enzyme activities in H-4IIe lysates. Exposure of confluent cells to either 10 μM benz[e]anthracene or 10 μM dexamethasone, which are known inducers in H-4IIe cells of aryl-hydrocarbon hydroxylase and tyrosine aminotransferase respectively, failed to elevate dihydrodiol dehydrogenase activity. The following agents also failed to induce dihydrodiol dehydrogenase activity: phenobarbital, ethoxyquin, phenolic anti-oxidants, testosterone, estradiol-17β, and growth hormone.

Since the hepatoma cell enzyme has properties in common with the purified dihydrodiol dehydrogenase in the further metabolism of trans-dihydrodiols, interestingly, the enzyme does not appear to be under the control of known inducers of phase I and phase II drug metabolizing enzymes.

INTRODUCTION

Metabolic activation of PAHs is required for expression of their mutagenic and carcinogenic effects. Upon hydration of these epoxides intermediates, trans-dihydrodiols (proximate carcinogens) are formed. Secondary oxidation of the trans-dihydrodiols leads to the formation of reactive diol-epoxides (ultimate carcinogens), which are capable of forming covalent bonds with DNA; an event that can initiate tumor formation.

Earlier studies have implicated dihydrodiol dehydrogenase in the detoxification of PAH. Addition of the purified dihydrodiol dehydrogenase to the Ames test reduces the mutagenicity of both the trans-dihydrodiol and the diol-epoxide metabolites of PAHs (3, 4). Dihydrodiol dehydrogenase (EC 1.3.1.20) has been purified to homogeneity from rat liver cytosol by two independent groups (5, 6). The cytosolic enzyme is identical to 3α-hydroxysteroid dehydrogenase (6). Recently, dihydrodiol dehydrogenase has been shown to catalyze the oxidation of a wide variety of polycyclic aromatic hydrocarbons trans-dihydrodiols including the proximate carcinogens derived from benz[a]pyrene, 5-methylchrysene, and 7,12-dimethylbenz[a]-anthracene (7, 8). The products that result from the enzymatic oxidation of trans-dihydrodiols are highly reactive ortho-quinones which are rapidly scavenged by cellular nucleophiles such as glutathione and cysteine to form water soluble conjugates (9). Although these data suggest that an alternative pathway of trans-dihydrodiol metabolism involves oxidation to ortho-quinones and subsequent formation of glutathionyl conjugates (a pathway that would suppress diol-epoxide formation) there has been no demonstration that this pathway exists in whole cells.

Identification of a continuous cell line which expresses dihydrodiol dehydrogenase would be useful in detecting whether metabolism of trans-dihydrodiols can be diverted to the putative ortho-quinone glutathionyl conjugates. H-4IIe cells derived from an acetylaminofluorene-induced rat hepatoma have been shown previously to possess monooxygenase (10, 11), glutathionyl S-transferase (11) and epoxide hydratase (11); activities which can collectively metabolize polycyclic aromatic hydrocarbons. In this report we demonstrate that these cells contain a dihydrodiol dehydrogenase activity which by several criteria appears to be indistinguishable from the enzyme found in normal rat liver. The substantial amount of enzyme activity in these transformed cells suggest that H-4IIe cells may be a useful intact cell system in which to investigate the contribution of dihydrodiol dehydrogenase to trans-dihydrodiol metabolism.

MATERIALS AND METHODS

Chemicals. Benzenedihydrodiol was synthesized according to published procedures (12). 6-Methoxyprogestosterone acetate, indomethacin, estradiol-17β, testosterone, thyroxine, phenobarbital, ethoxyquin, and butylated hydroxyanisole were purchased from Sigma Chemical Co. (St. Louis, MO). BHT was obtained from the Aldrich Chemical Co. (Milwaukee, WI). Androsterone and dexamethasone were purchased from Steraloids (Wilton, NH). NADP⁺, NAD⁺, Polybuffer Exchanger 94, and Polybuffer 74 were products of Pharmacia-LKB Biotechnology (Piscataway, NJ).

Growth of Hepatoma Cells. The H-4IIe rat hepatoma cell line was obtained from American Type Culture Collection (CRL1548), and grown in 75-cm² culture flasks in Eagle's minimal essential media (Piscataway, NJ).

Detection of Dihydrodiol Dehydrogenase in Hepatoma Cell Lysates. Confluent cells were washed with 3-mл phosphate buffered saline containing 1 mM EDTA and harvested by centrifugation at 3,000 rpm for 10 min. The cells were lysed by hypotonic shock in sterile distilled water. The lysate was homogenized in a ground glass homogenizer and microfuged for 5 min. Aliquots of the lysate were then added to 0.5 ml assay systems containing: 1.0 ml benzenedihydrodiol, 2.3 mM NADP⁺, 50 mM glycine buffer, pH 9.0, and 4% methanol. The enzyme reaction...
was monitored by following the change in pyridine nucleotide absorbance at 340 nm on a Beckman DU-7 spectrophotometer over 30 min at 25°C. Rates of benzenediol oxidation were calculated as nmol NADP⁺-reduced/min/mg protein. The lysate from one plate (75 cm²) gave sufficient material for nine enzyme assays.

Detection of Hydroxysteroid Dehydrogenases in Hepatoma Cell Lysates. Hepatoma cell lysates prepared as described above were assayed for either 3α- or 17β-hydroxysteroid dehydrogenase activity. In the former case, assays were initiated by the addition of lysate to 100 mM potassium phosphate (pH 7.0) containing 75 μM androsterone, 2.3 mM NAD⁺, and 4% acetonitrile. In the latter case assays were initiated by the addition of lysate to 50 mM glycine (pH 9.0) containing 50 μM estradiol-17β, 2.3 mM NAD⁺, and 4% acetonitrile. Enzyme activity was monitored as described in the previous section.

Detection of Other Enzyme Activities in Hepatoma Cell Lysates. Aryl hydrocarbon hydroxylase (10), quinone reductase (13), and glutathione S-transferase activities (14) were measured in H-4IIe lysates by the methods cited.

Immunoblot Analysis of Hepatoma Cell Lysates. Hepatoma lysates or purified rat liver dihydrodiol dehydrogenase were subjected to discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (15). Following electrophoresis, proteins were transferred to nitrocellulose sheets at 60 V for 3 h at room temperature (16). Nitrocellulose sheets were then incubated with rabbit anti-serum raised against purified dihydrodiol dehydrogenase and developed using goat anti-rabbit IgG horseradish peroxidase conjugate. A detailed characterization of rabbit anti-rat dihydrodiol dehydrogenase serum has been previously described (17). This antibody is monospecific and will detect only one band of immunoreactive protein in rat liver cytosol. The antibody is also of high titer since 0.2 μg of purified rat liver dihydrodiol dehydrogenase can be detected at a dilution of 1 in 30,000 on a Western blot.

Protein Determinations. The concentration of protein in hepatoma cell lysates was determined by the method of Lowry et al. (18) using crystalline bovine serum albumin as standard (Armour Pharmaceutical, Kankakee, IL).

RESULTS

Comparison of Dihydrodiol Dehydrogenase and 3α-Hydroxysteroid Dehydrogenase Activities in H-4IIe Cell Lysates and Rat Liver. Basal levels of dihydrodiol dehydrogenase and 3α-hydroxysteroid dehydrogenase were measured in H-4IIe rat hepatoma cell lysates. Both activities were readily detected using standard spectrophotometric assays. The specific activities determined (Table 1) were compared to the corresponding values obtained for the enzyme present in a 40–75% ammonium sulfate fraction of rat liver cytosol. The ratio of enzyme activities (3α-hydroxysteroid dehydrogenase/dihydrodiol dehydrogenase) present in the lysate were similar to that observed for the ammonium sulfate fraction of rat liver. The lower activities present in the lysate may be explained by the 2.5-fold enrichment of activity routinely seen in the preparation of an ammonium sulfate fraction of rat liver.

Table 1: Comparison of specific activities of dihydrodiol dehydrogenase and 3α-hydroxysteroid dehydrogenase in H-4IIe cells with normal rat liver

<table>
<thead>
<tr>
<th>Activity</th>
<th>H-4IIe cell lysate</th>
<th>Rat liver 40–75% ammonium sulfate fraction</th>
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<tbody>
<tr>
<td>Dihydrodiol dehydrogenase</td>
<td>0.915 ± 0.077 (n=4)</td>
<td>2.37 ± 0.36 (n=4)</td>
</tr>
<tr>
<td>3α-Hydroxysteroid dehydrogenase</td>
<td>3.3 ± 0.11 (n=4)</td>
<td>10.5 ± 0.88 (n=4)</td>
</tr>
<tr>
<td>Ratio of activities</td>
<td>3.5</td>
<td>4.4</td>
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Table 2: Comparison of kinetic constants for dihydrodiol dehydrogenase and 3α-hydroxysteroid dehydrogenase of hepatoma cells with those for the rat liver enzyme

Confluent cultures were lyzed by hypotonic shock in sterile distilled water. The lysate from one plate (75 cm²) of cells (1 x 10⁷) gave sufficient material for nine enzyme assays (n, number of measurements performed on cells from separate plates of confluent cultures). Enzyme assays were performed spectrophotometrically as described in "Materials and Methods." Specific activities of the enzyme(s) in hepatoma cell lysates and the corresponding values for a 40–75% ammonium sulfate fraction of rat liver cytosol are given as means ± SD.

<table>
<thead>
<tr>
<th>Activity</th>
<th>H-4IIe cell lysate</th>
<th>Rat liver 40–75% ammonium sulfate fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dihydrodiol dehydrogenase</td>
<td>12.2 ± 0.42</td>
<td>2.37 ± 0.36</td>
</tr>
<tr>
<td>3α-Hydroxysteroid dehydrogenase</td>
<td>12.2 ± 0.42</td>
<td>10.5 ± 0.88</td>
</tr>
<tr>
<td>Ratio of activities</td>
<td>3.0</td>
<td>4.4</td>
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Inhibition of Rat H-4IIe Hepatoma Cell Dihydrodiol Dehydrogenase and 3α-Hydroxysteroid Dehydrogenase Activities by Indomethacin and 6α-Medroxyprogesterone Acetate. A striking feature of rat liver 3α-hydroxysteroid/dihydrodiol dehydrogenase is its sensitivity to inhibition by nonsteroidal antiinflammatory agents such as indomethacin (6, 19) as well as the synthetic progestin 6-MPA (20). To determine whether the hepatoma cell enzyme(s) demonstrated a similar pharmacological profile, inhibition studies were performed using these two drugs (Fig. 1A and 1B). Both 6-MPA and indomethacin were potent inhibitors of 3α-hydroxysteroid dehydrogenase in hepatoma cell lysates yielding IC₅₀ values of 180 nM and 2.4 μM, respectively. In addition, hepatoma cell dihydrodiol dehydrogenase was sensitive to inhibition by the same drugs yielding an IC₅₀ value of 38 nM for 6-MPA and an IC₅₀ value of 3.5 μM for indomethacin. The differences observed in the IC₅₀ values for the inhibition of both activities by 6-MPA may be explained by the mixed inhibition patterns observed with this compound [Kₘ increases and Vₘₐₓ decreases (20)]. Such an inhibitor is expected to have a differential effect if the substrate concentrations used in the respective assays are not the same. In these studies 3α-hydroxysteroid dehydrogenase was assayed using an androsterone concentration set at 1.6 x Kₘ and dihydrodiol dehydrogenase was assayed using a benzenediol concentration set at 0.8 x Kₘ.

Since dihydrodiol dehydrogenase has been shown to copurify with 17β-hydroxysteroid dehydrogenase in mouse (21, 22), and guinea pig (23) liver, the 17β-hydroxysteroid dehydrogenase activity was also monitored in hepatoma cell lysates. Although H-4IIe cells do contain this activity (0.52 ± 0.0604 nmol/min/mg, n = 6), no inhibition could be observed with 30 μM indomethacin or 10 μM 6-MPA (data not shown). These data suggest that 17β-hydroxysteroid dehydrogenase can be distinguished from 3α-hydroxysteroid and dihydrodiol dehydro-
Dihydrodiol dehydrogenase Coelute on Chromatofocusing Columns. In rat liver, corresponding to Mr 34,000 was observed in hepatoma cell lysates. Both these enzyme activities in H-4IIe cell lysates (Fig. 3). The presence of both enzymes in hepatoma cell lysates is immunologically related to the rat liver enzyme. A single immunopositive band corresponding to Mr 34,000 was observed in hepatoma cell lysates (Fig. 2). This immunoreactive band comigrated with the purified 3α-hydroxysteroid/dihydrodiol dehydrogenase of rat liver. Using the rabbit anti-rat 3α-hydroxysteroid/dihydrodiol dehydrogenase serum it was also possible to immunotitrate both these enzyme activities in H-4IIe cell lysates (Fig. 3). The immunotitration curves are essentially superimposable providing further evidence that both activities are catalyzed by the same protein in these cells.

Dihydrodiol dehydrogenase and 3α-Hydroxysteroid Dehydrogenase Coelute on Chromatofocusing Columns. In rat liver, dihydrodiol dehydrogenase and 3α-hydroxysteroid dehydrogenase are physically indistinguishable and copurify to homogeneity. The presence of both enzymes in hepatoma cell lysates and their sensitivity to the same inhibitors, suggested that, as in rat liver, one enzyme may be responsible for the two activities.

To test this hypothesis, hepatoma cell lysates were subjected to chromatofocusing (Fig. 4). Both activities were found to coelute from the column in fractions which had a pH of 5.8, a value similar to that reported for the homogeneous enzyme from rat liver cytosol (6). Estimates of specific activity in the pooled peak fractions indicate that the enzyme has been purified 18-fold from H-4IIe cell lysates.

Lack of Effect of Common Inducers of Xenobiotic Metabolizing Enzymes on Dihydrodiol Dehydrogenase Activity in H-4IIe Cells. A number of enzymes involved in PAH and xenobiotic metabolism are inducible. Enzymes among this group include: cytochrome P450 (24, 25), epoxide hydrolase (26, 27), glutathione S-transferase (28), and quinone reductase (13). Indeed, benz(a)anthracene, 3-methylcholanthrene, and 2,3,7,8-tetrachlorodibenzo-p-dioxin are known inducers of aryl hydrocarbon hydroxylase in H-4IIe cells (10, 29). It was reasonable to hypothesize, that dihydrodiol dehydrogenase, also a xenobiotic

**Fig. 1.** Inhibition of dihydrodiol dehydrogenase (A), and 3α-hydroxysteroid dehydrogenase (B), by indomethacin and 6-methoxyprogesterone acetate in H-4IIe hepatoma cells. Enzyme assays were performed in the presence of the appropriate inhibitor and compared to activity in the absence of the inhibitor. A complete dose-response curve was generated in a single experiment using the lysate prepared from one plate of cells. Each point represents the mean ± SD from four separate experiments. 100% control values for dihydrodiol dehydrogenase activity were 0.985 ± 0.25 nmol/min/mg (indomethacin inhibition experiments) and 1.01 ± 0.25 nmol/min/mg (6-MPA inhibition experiments). 100% control values for 3α-hydroxysteroid dehydrogenase activity were 3.6 ± 0.40 nmol/min/mg (indomethacin inhibition experiments) and 3.3 ± 0.11 nmol/min/mg (6-MPA inhibition experiments). O, indomethacin dose-response curves; •, 6-MPA dose-response curves.

**Fig. 2.** Western blot analysis of hepatoma cell lysates. Hepatoma cell lysate proteins were separated by electrophoresis through 12% sodium dodecyl sulfate-polyacrylamide gels. Proteins were transferred to nitrocellulose membranes and membranes were incubated with rabbit anti-rat dihydrodiol dehydrogenase serum (1 in 100 final dilution). Following a second incubation with goat-anti-rabbit IgG horse radish peroxidase conjugate, immunoreactive bands were visualized by incubation with 4-chloro-1-naphthol and H₂O₂. From left to right; lane 1, molecular weight markers (in kilodaltons) stained with Coomassie blue; lane 2, hepatoma cell lysate proteins (50 μg) stained with Coomassie blue; lane 3, Western blot of 0.2 μg purified rat liver dihydrodiol dehydrogenase; lane 4, Western blot of 12.5 μg hepatoma cell lysate.

**Fig. 3.** Immunotitration of 3α-hydroxysteroid/dihydrodiol dehydrogenase activities in H-4IIe cell lysates. Aliquots (100 μl) of H-4IIe cell lysates (1 × 10⁷ cells lysed/1.0 ml H₂O) were incubated with increasing quantities of either rabbit anti-rat 3α-hydroxysteroid/dihydrodiol dehydrogenase serum (0–20 μl) or preimmune serum (0–20 μl) in a total volume of 200 μl of phosphate buffered saline. Incubations were performed at 4°C for 18 h. The entire sample was then assayed spectrophotometrically for either 3α-hydroxysteroid dehydrogenase or dihydrodiol dehydrogenase activity. Dihydrodiol dehydrogenase activity following incubation with preimmune serum (□); dihydrodiol dehydrogenase activity following incubation with antiserum (O); 3α-hydroxysteroid dehydrogenase activity following incubation with antiserum (扎实); and 3α-hydroxysteroid dehydrogenase activity following incubation with antiserum (扎实). The mean and the range of duplicate measurements are shown on the titration curves.
Table 3 Evaluation of a panel of inducers to elevate dihydrodiol dehydrogenase activity in hepatoma cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ratio of dihydrodiol dehydrogenase activities (T/C)*</th>
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<tbody>
<tr>
<td>Control</td>
<td>1.00</td>
</tr>
<tr>
<td>0.2% dimethyl sulfoxide</td>
<td>1.14</td>
</tr>
<tr>
<td>10 μM benz[a]anthracene</td>
<td>1.18</td>
</tr>
<tr>
<td>10 μM dexamethasone</td>
<td>0.82</td>
</tr>
<tr>
<td>10 μM phenobarbital</td>
<td>1.22</td>
</tr>
<tr>
<td>10 μM BHA</td>
<td>1.03</td>
</tr>
<tr>
<td>10 μM ethoxyquin</td>
<td>0.90</td>
</tr>
<tr>
<td>10 μM estradiol-17β</td>
<td>1.09</td>
</tr>
<tr>
<td>10 μM testosterone</td>
<td>1.18</td>
</tr>
<tr>
<td>10 μM thyroxine</td>
<td>1.77</td>
</tr>
<tr>
<td>0.2 Units growth hormone</td>
<td>0.78</td>
</tr>
</tbody>
</table>

* Ratio of dihydrodiol dehydrogenase activities in treated (T) versus control (C) cells.

metabolizing enzyme, might be inducible. Towards this end, a panel of potential inducers were tested for their ability to elevate dihydrodiol dehydrogenase in rat H-4IIe cell lysates (Table 3). In these experiments cells at 70% confluency were fed media plus or minus fetal calf serum containing 10 μM inducer for 24 h. Of the inducers used, none produced more than a percentage increase in enzyme activity and the highest elevation in activity was observed with 10 μM thyroxine. The lack of effect of both estradiol-17β and growth hormone on dihydrodiol dehydroge

Second, BHT induced quinone reductase twofold in the presence and absence of fetal calf serum.

DISCUSSION

These studies represent the first documentation of dihydrodiol dehydrogenase activity in a continuous cell line. Previous studies from this laboratory have shown that the purified dihydrodiol dehydrogenase from rat liver cytosol oxidizes trans-di-hydrodiols of polycyclic aromatic hydrocarbons to the corresponding ortho-quinones which can be readily scavenged by cysteine and glutathione (7, 9). However, since these studies were performed using purified enzyme, there is as yet no evidence that cysteiny1 or glutathionyl conjugates of ortho-quinones form as a consequence of trans-dihydrodiol metabolism in whole cells. The H-4IIe cell line provides an opportunity to examine this question by studying the metabolism of the [1H]-(-)trans-7,8-dihydrodiol of benz[a]pyrene. Indeed preliminary studies indicate the formation of either a phenolic or catecholate metabolite from the dihydrodiol (30). Since these studies involve the further metabolism of the trans-dihydrodiol the prevalent level of monooxygenase observed in these cells should not adversely effect the detection of the products of the dihydrodiol dehydrogenase reaction.

Some of the advantages of H-4IIe cells for the ongoing metabolism studies include their ability to conduct a variety of phase II reactions which may be pertinent to the further metabolism of enzymatically generated ortho-quinones. For example, they contain high levels of glutathione S-transferase and quinone reductase relative to dihydrodiol dehydrogenase. Thus using CDNB as substrate for glutathione S-transferase, the activity was 0.2 μmol of substrate consumed/min/mg and using dichloroindophenol as a substrate for quinone reductase, the activity was 0.3 μmol of NADPH oxidized/min/mg. Second, these cells are robust in culture, they passage quickly and unlike primary hepatocytes (31) maintain their phenotypic enzyme pattern for at least 40-50 passages.

The H-4IIe cell line also contains 3α-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase activities and the present report provides evidence demonstrating that 3α-hydroxysteroid dehydrogenase and dihydrodiol dehydrogenase activity may be catalyzed by the same protein. Hepatoma 3α-hydroxysteroid dehydrogenase and dihydrodiol dehydrogenase activities are potent ly inhibited by indomethacin and 6-methoxyprogesterone acetate; they coelute on chromatofocusing columns with a pl of 5.8, they are immunoreactive with 3α-progesterone acetate; they coelute on chromatofocusing columns with a pl of 5.8, they are immunoreactive with 3α-hydroxysteroid/dihydrodiol dehydrogenase antisera and both activities can be immunotitrated.

Unlike several other PAH-metabolizing enzymes, dihydrodiol dehydrogenase activity was not elevated by a panel of potential inducers. It should be emphasized that both arylhydrocarbon hydroxylase and tyrosine aminotransferase activities have been shown to be induced by benz[a]anthracene and dexamethasone respectively in H-4IIe cells (10). Our own control experiments indicate that benz[a]anthracene will induce aryl-hydrocarbon hydroxylase activity 5-fold in these cells. The finding that dihydrodiol dehydrogenase is not induced by these agents suggests that it may be regulated independently of the Ah locus and the tyrosine aminotransferase gene.

Dihydrodiol dehydrogenase was not induced in H-4IIe cells by phenobarbital, the phenolic anti-oxidants (BHA and BHT) and ethoxyquin. In addition neither estradiol-17β or growth hormone induced enzyme activity in H-4IIe cells. Estradiol-17β will elevate 3α-hydroxysteroid/dihydrodiol dehydrogenase in
rat liver cytosol if it is administered to intact males, ovariecto-
imized females, and immature hypophysectomized females (32,
33). In addition, growth hormone will elevate 3α-hydroxyster-
oid/dihydrodiol dehydrogenase in rat liver cytosol if it is
administered to immature hypophysectomized male and female
animals (33). These in vivo changes are accompanied by in-
creases in the expression of the mRNA for 3α-hydroxysteroid/
dihydrodiol dehydrogenase (33).

Of the many possible explanations that exist to explain the
failure of estradiol-17β and growth hormone to induce dihy-
drodiol dehydrogenase in H-4IIe hepatoma cells, two are worthy
of mention. First, the H-4IIe cells contain 17β-hydroxyster-
diol dehydrogenase which could catalyze the rapid metabolism of
estradiol-17β. Second during maintenance in culture, H-4IIe
cells may secrete increasing amounts of insulin-like growth
factor into the culture medium. Insulin-like growth factor
in turn induce 3α-hydroxysteroid/dihydrodiol dehydrogenase
activities in an autocrine manner. Insulin-like growth factor
is a well recognized mediator of growth hormone action (34). The
ability of rat hepatocytes in culture to secrete increasing
amounts of insulin-like growth factor has been previously re-
ported (35). The inability to observe induction of 3α-hydrox-
steroi/dihydrodiol dehydrogenase by growth hormone and
estradiol-17β in H-4IIe hepatoma cells implies that these cells
may not be a good system in which to study the regulation of
3α-hydroxysteroid/dihydrodiol dehydrogenase by these
hormones.

The experiments presented here establish the H-4IIe he-
toma cell line as a model system in which to study the role
of dihydrodiol dehydrogenase in cellular PAH metabolism. Future
studies will examine the fate of the trans-7,8-dihydrodiol
of benzo[a]pyrene in H-4IIe cells and the contribution of dihy-
drodiol dehydrogenase to the cellular metabolism of this proximate
carcinogen.

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hydrocarbon hydroxylase assays.

REFERENCES

1. Conney, A. H. Induction of microsomal enzymes by foreign chemicals and
carcinogenesis by polycyclic aromatic hydrocarbons: G.H.A. Clowes Memo-

2. Gelboin, H. V. Benzo[a]pyrene metabolism, activation and carcinogenesis:
role and regulation of mixed function oxides and related enzymes. Physiol.

3. Glatt, H. R., Vogel, K., Bentley, P., and Oesch, F. Reduction of benzo[a]
pyrene mutagenicity by dihydrodiol dehydrogenase. Nature (Lond.), 277:

Waechter, F., Vogel, K., Guenther, T. M., and Oesch, F. Inactivation of a
diol-epoxide by dihydrodiol dehydrogenase but not by two epoxide hy-

5. Vogel, K., Bentley, P., Platt, K.-U., and Oesch, F. Rat liver cytoplasmic
dihydrodiol dehydrogenase. Purification to apparent homogeneity and prop-

6. Penning, T. M., Mukharji, I., Barrows, S., and Talalay, P. Purification and
properties of a 3α-hydroxysteroid dehydrogenase of rat liver and its inhibition

7. Smithgall, T. E., Harvey, R. G., and Penning, T. M. Regio- and stereo-
specificity of homogeneous 3α-hydroxysteroid/dihydrodiol dehydrogenase
for trans-dihydrodiol metabolites of polycyclic aromatic hydrocarbons. J.

8. Smithgall, T. E., Harvey, R. G., and Penning, T. M. Oxidation of the trans-
3,4-dihydrodiol metabolites of the potent carcinogen 7,12-dimethylbenz[a]an-
thracene and other benz[a]anthracenes derivatives by 3α-hydroxysteroid/
dihydrodiol dehydrogenase effects of methyl substitution on the velocity and
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