The major regulatory gene product of the murine Ah complex appears to be a cytosolic receptor: 2,3,7,8-[1,6,11]Tetrachlorodibenzo-p-dioxin or [1H]3-methylcholanthrene binding to the Ah receptor and other moieties in hepatic cytosol was examined by gel permeation chromatography, velocity sedimentation (sucrose density gradients), dextran-charcoal adsorption, and anion exchange chromatography. In the liver of Ah-responsive C57BL/6N and the Ahb/Ahd heterozygote, both radioligands bind to three major components: peak I, a large aggregate which is eluted in the void volume of Sephacryl S-300 columns and which sediments as a residue to the bottom of sucrose density gradients; peak II, an asymmetric protein (Mr = 245,000) with a Stokes radius of about 75 Å; and peak III, a globular protein (Mr = 87,000) with an estimated Stokes radius of 40 Å. The peak I aggregate is not adsorbed by dextran-coated charcoal and therefore represents the large proportion of nonsaturable radioligand binding measured by dextran-charcoal adsorption. The peak II protein has a size of about 9.0 S in low ionic strength and 7.5 S in high ionic strength, high affinity for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and saturability at TCDD concentrations greater than about 1.0 nM. The peak II protein is not detectable in the liver of Ah-nonresponsive DBA/2N and the Ahb/Ahd homozygote and therefore represents the Ah receptor. The peak III protein has an estimated size of 5.0 S, is not saturable with either TCDD or 3-methylcholanthrene under the conditions of these experiments, and is not associated with the Ahb allele. 3-Methylcholanthrene binds to the peak III protein to a greater extent than TCDD.

These data explain the discrepancies between the dextran-charcoal adsorption and sucrose density gradient assays. Any further studies of the function of the Ah receptor and these other ligand-binding moieties (e.g., nuclear translocation) should include gel permeation chromatography in order to distinguish among the various binding components.

The murine Ah locus controls the induction (by polycyclic aromatic compounds such as 3-methylcholanthrene, benzo[a]pyrene, β-naphthoflavone, and TCDD) of many drug-metabolizing enzyme activities in virtually all tissues examined (reviewed in Ref. 1). The Ah complex is believed to comprise regulatory, structural, and probably temporal genes which may or may not be linked (2). Multiple forms of cytochrome P-450 are believed to be among the many structural gene products "turned on" during the sequence of events following exposure of the animal to the polycyclic aromatic inducer (3, 4).

A cytosolic receptor for these inducers in genetically "responsive" mice was postulated (5, 6) and is now believed to be the major product of the Ah regulatory genes. Experimental evidence in support of this hypothesis has been reported, with the use of a dextran-coated charcoal adsorption assay (7-9), isoelectric focusing following trypsin treatment (10, 11), sucrose density gradient following dextran-charcoal adsorption (12, 13), and a detergent-washing procedure with purified nuclei (14). There is no detectable receptor in "Ah-nonresponsive" strains of mice (8, 12). The cytosolic receptor with specifically bound inducer appears to translocate into the nucleus during cytochrome P-450 induction by polycyclic aromatic compounds in the intact liver of Ah-responsive mice (12), in cytosolic and nuclear preparations in vitro (14), and in cell cultures (13). The cytosolic location of the Ah receptor (7-14) and the temperature-dependent translocation of the inducer-receptor complex (13) are very similar to the properties of the steroid hormone receptors.

Estimates of TCDD-specific binding sites (8, 12, 13) have ranged between about 10 and 90 fmol/mg of cytosolic protein (~900 to 8,100 sites/cell), with an apparent Kd for [1H]TCDD of approximately 0.7 nM (8, 10, 12). The cytosolic location of a 3-methylcholanthrene-specific binding protein and apparent nuclear uptake of this complex were recently reported; estimates of 3-methylcholanthrene-specific binding sites and apparent Kd were 770 fmol/mg of cytosolic protein and 2.8 nM, respectively (15). In the current study we use differences at the Ah locus and four independent separatory methods to demonstrate that mouse liver cytosol contains at least two distinct proteins that bind TCDD and 3-methylcholanthrene. Only one, however, is shown to be the Ah receptor, because this peak is absent in Ah-nonresponsive (Ahb/Ahd) mice.

**EXPERIMENTAL PROCEDURES**

**Chemicals—**[1H]TCDD (55 Ci/mmol) was purchased from KOR Isotopes (Cambridge, MA); gas chromatographic analysis by the manufacturer indicated that the product might contain as much as 20% tri-, penta-, and hexachlorodibenzo-p-dioxins. Nonlabeled TCDD was a generous gift of Dow Chemical Company (Midlands, MI). [1H]3-Methylcholanthrene (generally labeled, 35 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Nonlabeled 3-methylcholanthrene, Hepes, trypsin, dextran 200C, and Triton X-100 were purchased from Sigma; Norit A was from Fischer Scientific Company (Pittsburgh, PA); Sephacryl S-300 (superfine) was from...
Pharmacia (Uppsala, Sweden); DEAE-S2 (preswollen, microgranular) was from Whatman (Irvine, CA); Aquasol was from New England Nuclear; sodium deoxoycholate was from Mann Research Laboratory (New York, NY); blue dextran, thyroglobulin, ferritin, bovine serum albumin, and cytochrome c were from Bio-Rad Laboratory (Rich mond, CA); and [14C]formaldehyde-labeled bovine serum albumin (20 µCi/mg) and [14C]formaldehyde-labeled bovine serum albumin- p-dodecylbenzene sulfonate (1000 µCi/mg) was generously gift from Dr. Leonard M. Hjelmdal, Developmental Pharmacology Branch, National Institute of Child Health and Human Development, Bethesda, MD. The remainder of the chemicals were purchased from the sources cited previously (13).

Assay of Ah—responsive B6 (Ah+/Ah+) and Ah—nonresponsive D2 (Ah−/Ah−) mice were obtained from the Veterinary Resources Branch, National Institutes of Health. Breeding to obtain responsive heterozygotes (Ah+/Ah−) and nonresponsive homozygotes (Ah−/Ah−) from the B6DF2, × D2 backcross was carried out in the Developmental Pharmacology Branch mouse colony, National Institute of Child Health and Human Development. Phenotyping progeny of the B6DF2, × D2 backcross, with respect to the Ah locus, was carried out by the zoxazolamine paralysis test (16) on weanlings (either sex) between 3 and 4 weeks of age. The rapid environment and free access to food and water were described previously (13).

Buffers and Solutions—HEDG consisted of: 25 mM Hepes, 1.5 mM ethylenediaminetetraacetic acid, 1 mM dithiothreitol, and 10% glycerol (v/v), pH 7.6; dextran charcoal consisted of 5 mg of charcoal (Norit A) and 0.5 mg of dextran/ml of HEDG buffer, pH 7.6.

Preparation of Cytosol—Mice were killed by cervical fracture. The postmortem organs with the livers were perfused in situ with HEDG buffer containing 0.1 M NaCl. All subsequent operations were carried out at 4 °C. The livers were removed, minced, and then homogenized in 2 volumes of HEDG buffer (with 0.1 M NaCl) using a Teflon-glass homogenizer. The homogenate was centrifuged at 10,000 × g for 15 min and the supernatant fraction was centrifuged at 100,000 × g for 1 h. The supernatant (cytosolic) fraction were quickly frozen in 2-ml aliquots and stored in liquid nitrogen. No losses of [3H]TCDD or [3H]-methylcholanthrene binding capacity were noted during storage in liquid nitrogen for 3 months.

Treatment with Radioligand In Vitro—Usually 1 ml of cytosol was treated with the desired concentration of [3H]TCDD or [3H]-methylcholanthrene for 1 h at 0–4 °C. Unless otherwise specified, 15 mg of cytosolic protein/ml were used in all experiments, except sucrose density gradient centrifugation, in which case 5 mg of protein/ml was used. In some samples, nonlabeled TCDD or 3-methylcholanthrene (in 100-fold excess) was added to the radioligand before treating the cytosol. As noted before (13), p-dioxane was the solvent for TCDD. A minimal amount of acetone was the solvent for 3-methylcholanthrene. After treatment at 4 °C for 1 h, the cytosol was examined by one of four independent techniques.

Chromatography—The radioligand-treated cytosol was used directly for Sephacryl S-300 gel filtration. The 3% column (Figs. 1 and 2) therefore represents radiolabel bound to macromolecules. More than 95% of the total [3H]-TCDD eluted from the column was associated with the nonsaturable peak I (Table I). [3H]TCDD associated with peak I was not adsorbed to dextran-coated charcoal (data not illustrated). Comparing the two methods (Table I), we found approximately the same amount of bound radioligand in the dextran-charcoal adsorption assay as by gel permeation chromatography (the sum of peaks I, II, and III).

Comparison of Velocity Sedimentation with Dextran Charcoal Adsorption—The dextran-coated charcoal adsorption assay (7–9) provides a rapid method for separating free from macromolecular-bound [3H]TCDD; however, all three components of binding are included in this measurement. The addition of excess nonlabeled TCDD as a competitor has variable effects on the nonsaturable components. As shown in Figs. 1 and 2, [3H]TCDD binding to peak I is often decreased; however, at higher protein and radioligand concentrations, [3H]TCDD binding to peak I is increased by addition of competitor (data not shown). These effects make it extremely difficult to use dextran-coated charcoal adsorption alone as the basis for measuring [3H]TCDD binding to the peak II moiety. In sucrose density gradients, peak I material sediments to the bottom of the centrifuge tube (Figs. 1B and 2B); hence, peak I does not interfere with the detection of peak II.

Effect of Protein Concentration—There are certain situations (e.g., estimation of “free” TCDD concentrations when
Genetics of TCDD and MeChol Binding To Ah Receptor

**Fig. 1.**[^H]TCDD binding to B6 cytosol. A, gel permeation chromatography of B6 hepatic cytosol treated with [^H]TCDD. Void volume determined with blue dextran, thyroglobulin, ferritin, bovine serum albumin, and cytochrome c, with each of their Stokes radii indicated in Å, were used to calibrate the column. B, analysis of peaks I, II, and III by velocity sedimentation. Cytosol (15 mg protein/ml) was treated with 5 nM [^H]TCDD for 1 h at 4 °C and then chromatographed on a Sephacryl S-300 column. Samples from the peak I (fractions 16 to 22), peak II (fractions 23 to 27), and peak III (fractions 28 to 35) regions were then added to sucrose density gradients (5% to 20%). Because of the low concentration of protein in these samples, the material was not treated with dextran-coated charcoal before application to the sucrose gradients. In addition to the usual gradient fractions, the bottoms of the centrifuge tubes were cut off, soaked in Aquasol, and counted for radioactivity (disintegrations per min shown in boxes). Approximate sedimentation values are shown for bovine serum albumin (4.4 S), bovine liver catalase (11.3 S), and ferritin (17.1 S); these standards were centrifuged in a separate gradient.[^14C]Albumin (2,000 dpm) was also included as an internal standard. Further details are described under “Experimental Procedures.”

**Table 1**

| In vitro treatment | Gel permeation chromatography | Dextran-charcoal adsorption assay |
|--------------------|------------------------------|----------------------------------|
|                     | Peak I | Peak II | Peak III | Total | dpm x 10^-3 |
| 1.0 nM [^H]TCDD    |        |        |          |       |             |
| plus 100 nM TCDD   | 53     | 35     | 26       | 114   | 129         |
| 1.0 nM [^H]TCDD    | 42     | 15     | 24       | 81    | 110         |

Fig. 2. [^H]TCDD binding to D2 cytosol. A, gel permeation chromatography of D2 hepatic cytosol treated with [^H]TCDD. B, analysis of peaks I, II, and III by velocity sedimentation. These experiments with D2 mice were identical to those with B6 mice illustrated in Fig. 1.
attempting to perform Scatchard analysis) in which it becomes essential to know the amount of $[^3H]TCDD$ associated with peak I. Peak I represents approximately one-fourth of the total $[^3H]TCDD$ added in vitro to cytosol concentrations of 5 and 10 mg of protein/ml (Fig. 3A). At 15 mg of protein/ml, however, peak I comprised almost 70% of the total $[^3H]TCDD$ added in vitro. Although peak II increased in proportion to protein concentration, peak III was markedly diminished at 15 mg/ml. Under conditions when peak I is greatest (i.e., high protein concentration) and therefore “free” $[^3H]TCDD$ would be lowest, relatively little binding to the peak III moiety occurred. It is thus concluded that peak III material has lower affinity for TCDD than peak II material. These data illustrate why there has been so much difficulty with Scatchard analysis by either the dextran-charcoal adsorption method (8, 9) or the sucrose density gradient method (12). $[^3H]TCDD$ binding in the 5.0 S region of sucrose density gradients (peak III material) was quite low at both high and low cytosolic protein concentrations (Fig. 3B), in contrast to what was seen for peak III with gel permeation chromatography. Both the gel permeation chromatography and velocity sedimentation methods measure ligand binding under nonequilibrium conditions. The time required for analysis on sucrose density gradients (about 18 h) is considerably longer than that for gel permeation chromatography (about 6 h). The quantitative differences in peak III in Fig. 3 between $A$ and $B$ are therefore probably related to dissociation of ligand (“TCDD off-rate”) from the peak III macromolecule(s) as a function of time after the in vitro $[^3H]TCDD$ treatment. The large proportion of radioligand associated with peak I has made it impossible for us to study accurately the kinetics of $[^3H]TCDD$ binding to the peak II and peak III moieties. The partial saturability of peak III may reflect heterogeneity, as well as a large number of binding sites.

Estimation of Size—The approximate molecular weights of the peak II and peak III moieties were calculated from their respective Stokes radii and sedimentation coefficients (Table II). The peak II component, believed to represent the Ah receptor, appears to be an asymmetric molecule ($M_r \approx 245,000$; $f/f_0 = 1.6$). The peak III material is presumably more symmetric ($M_r \approx 87,000$; $f/f_0 \approx 1.2$).

Comparison of Gel Permeation Chromatography with Velocity Sedimentation: $[^3H]3$-Methylcholanthrene Binding to B6 and D2 Cytosol—Various properties of the peak III material in mouse liver caused us to wonder if this were analogous to the 3-methylcholanthrene-binding protein in rat liver recently reported by Fuller et al. (18) and Tierney et al. (15). Three major peaks were detected in B6 cytosol by gel permeation chromatography (Fig. 4A), and the positions of elution of the $[^3H]3$-methylcholanthrene-binding peaks were essentially the same as those of the $[^3H]TCDD$-binding peaks. Peak III was proportionately larger with $[^3H]3$-methylcholanthrene than with $[^3H]TCDD$. Peak II $[^3H]3$-methylcholanthrene binding was not detectable in the Ah-nonresponsive D2 cytosol (Fig. 4B).

With sucrose density gradient analysis (Fig. 5) $[^3H]3$-methylcholanthrene eluted in the positions of the peak II and peak III material, as had been seen for $[^3H]TCDD$ (Fig. 1B). At either 1 or 10 nM $[^3H]3$-methylcholanthrene, 100-fold excess concentrations of nonlabeled 3-methylcholanthrene decreased peak II almost completely while decreasing peak III less than 50%. These data suggest that the saturable binding for $[^3H]3$-methylcholanthrene is principally in peak II but that some saturable binding sites may exist in the peak III material.

Effect of Trypsin and Detergents—Peak II was entirely destroyed by trypsin treatment under conditions that did not affect peak I or peak III to any substantial degree (Fig. 6).

The detergents sodium deoxycholate and NP-40 abolished binding to peak II, but the $[^3H]TCDD$ was then eluted in the void fractions of the Sephacryl S-300 column after detergent treatment, i.e., as an increase in peak I (data not illustrated). TCDD and other polycyclic aromatic inducers are extremely hydrophobic and insoluble, and it seems plausible that cyto-

![Figure 3. Distribution of $[^3H]TCDD$ binding to peaks I, II, and III as a function of protein concentration. A, gel permeation chromatography. B6 liver cytosolic preparations (1 ml of three different protein concentrations) were treated with 2 nM $[^3H]TCDD$ for 1 h at 4 °C and then chromatographed on Sephacryl S-300 columns. B, velocity sedimentation analysis. Following treatment with 2 nM $[^3H]TCDD$ for 1 h at 4 °C and then dextran-coated charcoal adsorption, B6 cytosolic preparations were examined with the use of 5 to 20% sucrose density gradients, as detailed under "Experimental Procedures."](image)

### Table II

| Parameter          | Peak II | Peak III |
|--------------------|---------|----------|
| Stokes radius (nm) | 7.5     | 4.0      |
| Sedimentation coeff. (s$_{20, w}$) | 7.5 | 5.0 |
| $M_r$ estimation   | 245,000 | 87,000   |
| $f/f_0$ estimation | 1.6     | 1.2      |
Genetics of TCDD and MeChol Binding To Ah Receptor

Genetic Analysis of [\(^{3}\text{H}\)]3-Methylcholanthrene and [\(^{3}\text{H}\)]TCDD Binding to Liver Cytosol—Ah-responsive B6 (Ah\(^{b}/\)Ah\(^{b}\)) and the heterozygote (Ah\(^{b}/\)Ah\(^{a}\)) possess levels of receptor that are sufficient for the induction of cytochrome P-450 and its associated aryl hydrocarbon hydroxylase activity, whereas Ah-nonresponsive D2 (Ah\(^{d}/\)Ah\(^{d}\)) and the Ah\(^{d}/\)Ah\(^{d}\) homozygotes as children of the B6D2F1 × D2 backcross do not exhibit detectable levels of cytosolic receptor (1, 12). Because B6 and D2 mice represent very different inbred strains, we chose to study the relationship of peak II and peak III [\(^{3}\text{H}\)]3-methylcholanthrene binding as a function of the Ah\(^{b}\) allele.

Individual offspring from the B6D2F1 × D2 backcross were phenotyped 10 days earlier by the zoxazolamine paralysis test (16) and therefore classified as Ah-responsive Ah\(^{b}/\)Ah\(^{d}\) or Ah-nonresponsive Ah\(^{d}/\)Ah\(^{d}\) individuals (Fig. 7). The responsive heterozygote exhibited both peak II and peak III [\(^{3}\text{H}\)]3-methylcholanthrene binding, whereas the nonresponsive heterozygote demonstrated large peak III [\(^{3}\text{H}\)]3-methylcholanthrene binding only. With [\(^{3}\text{H}\)]TCDD, peak II was prominent in the Ah\(^{b}/\)Ah\(^{d}\) mouse and absent in the Ah\(^{d}/\)Ah\(^{d}\) mouse, and peak III was negligible in both; this result is consistent with that previously reported (12). We therefore conclude that this [\(^{3}\text{H}\)]3-methylcholanthrene binding moiety of peak III is not associated with the Ah receptor, whereas the peak II binding correlates well.

Analysis of Peak II and Peak III [\(^{3}\text{H}\)]3-Methylcholanthrene Binding by Anion-Exchange Chromatography—In all the above experiments, peak II and peak III have been separated on the basis of their hydrodynamic properties. Anion-exchange chromatography also can resolve [\(^{3}\text{H}\)]TCDD and

Fig. 4. Gel permeation chromatography of B6 liver cytosol (A) and D2 liver cytosol (B) treated with [\(^{3}\text{H}\)]3-methylcholanthrene ([\(^{3}\text{H}\)]MeChol). Following treatment of 1 ml of cytosol (15 mg of protein) with 10 nM [\(^{3}\text{H}\)]3-methylcholanthrene for 1 h at 4°C, the samples were chromatographed on Sephacryl S-300 columns, as described under “Experimental Procedures.”

Fig. 5. Velocity sedimentation analysis of B6 hepatic cytosol treated with [\(^{3}\text{H}\)]3-methylcholanthrene ([\(^{3}\text{H}\)]MeChol). Treatment for 1 h at 4°C included 10 nM [\(^{3}\text{H}\)]3-methylcholanthrene alone and with 1.0 \(µ\)M nonlabeled 3-methylcholanthrene (A— ), or 1 nM [\(^{3}\text{H}\)]3-methylcholanthrene (MeChol) alone (C— ) and with 100 nM nonlabeled 3-methylcholanthrene (M — M). Following treatment and then dextran-charcoal adsorption, the samples were examined with the use of 5 to 20% sucrose density gradients, as detailed under “Experimental Procedures.”

Fig. 6. Effect of trypsin on the [\(^{3}\text{H}\)]TCDD-binding moieties in B6 liver cytosol, examined by gel permeation chromatography. Following incubation of the cytosol (15 mg of protein in 1 ml) at 22°C for 1 h in the presence or absence of 100 µg of trypsin, the cytosolic samples were cooled to 4°C and treated for 1 h with 10 nM [\(^{3}\text{H}\)]TCDD. The material was then applied to Sephacryl S-300 columns, as described under “Experimental Procedures.”
After the column was washed with 10 mM [3H]3-methylcholanthrene for 100 ml of cytosol (10 mg of protein) from individual mice was treated with 10 nM [3H]TCDD or [3H]MeChol. Mice were from the same litter: A, responsive Ah⁺/Ah⁺ mouse; B, nonresponsive Ah⁺/Ah⁻ mouse. The backcross mice had been phenotyped by the roxazolamine paralysis test (16) 10 days earlier. One ml of cytosol (10 mg of protein) from individual mice was treated with 1 mM [3H]TCDD or 10 mM [3H]3-methylcholanthrene for 1 h at 4 °C; following dextran-coated charcoal adsorption, the samples were then examined on sucrose density gradients, as detailed under “Experimental Procedures.”

With the use of gel permeation chromatography, we have resolved the apparent discrepancies between the dextran-charcoal adsorption assay (7–9) and the sucrose density gradient assay (12, 13) for the Ah receptor. If saturating concentrations of [3H]TCDD are used, the sucrose density gradient assay provides an accurate measure of [3H]TCDD binding to the peak II moiety. Using this assay, we have shown that binding is saturable and proportional to protein concentration over a wide range of experimental conditions. The aggregated material (peak I) does not correlate with the Ah locus. With the sucrose density gradient assay, this material sediments to the bottom of the tube and therefore does not interfere with determination of Ah receptor levels (peak II). We have also shown in this report that the major 3-methylcholanthrene binding moiety (peak III in this report) described in rat liver cytosol (15, 18) is not associated with the Ah receptor and the cytochrome P-450 induction process.

Polycyclic aromatic inducers are able to enhance pyrene N-demethylase (19), aniline hydroxylase, d-benzphetamine
Genetics of TCDD and MeChol Binding To Ah Receptor

amine N-demethylase, chlorcyclene N-demethylase, ethylmorphine N-demethylase, and pentobarbital hydroxylase (20) activities in Ah-nonresponsive mice (usually 40% to 2-fold enhancement). Small increases in electrophoretic bands are detectable by sodium dodecyl sulfate-polyacrylamide gels of liver microsomes from Ah-nonresponsive mice. Hepatic glutathione transferase induction (with 1-chloro-2,4-dinitrobenzene dinitrobenzene as substrate) by 3-methylcholanthrene is also not associated with the Ah complex and occurs in some nonresponsive mice having no detectable Ah receptor (21). It is therefore possible that we are technically unable to measure the presence of some Ah receptor in these nonresponsive mice. Alternatively, some other receptor or a "nonreceptor" mechanism might be responsible for this induction process by polycyclic aromatic compounds in Ah-nonresponsive mice. Also, peak III could be heterogeneous and might contain a component responsible for this process in Ah nonresponsive mice.

Although Ah-nonresponsive mice have no detectable Ah receptor, there is significant aryl hydrocarbon hydroxylase induction in mouse fetal cell cultures exposed to benzo[a]anthracene (23) and in numerous tissues in vivo when nonresponsive mice receive sufficient doses of TCDD (5). This difference in sensitivity (Ah-nonresponsive mice requiring 12 to 18 times the dose of inducer given to Ah-responsive mice in order to attain the same response (6)) implies that the Ah structural genes are intact and that some defect lies in the Ah regulatory genes, presumably the Ah receptor. This 12- to 18-fold difference in sensitivity cannot be readily explained by the binding data in this report, however, which show a complete absence of peak II in liver cytosol from Ah-nonresponsive mice. It is possible that a peak II- to peak I aggregate.

Several recent studies have dealt with the interesting possibility that the TCDD- or 3-methylcholanthrene-binding moieties undergo translocation to the cell nucleus during the process of induction. Okey et al. (12) have shown that the 7.5 S moiety moves to the nucleus after in vitro injection of [3H]TCDD. Okey et al. (13) more recently have demonstrated an apparent temperature-dependent step during the nuclear translocation of the [3H]TCDD-receptor complex with the use of cells in culture. Using a detergent-washing procedure with isolated purified liver cell nuclei (14), Greenle and Poland showed that the [3H]TCDD-receptor complex moved presumably from the cytosol to the nucleus in vitro. Okey et al. (12),

2 James S. Felton and Daniel W. Nebert, unpublished data.

REFERENCES

1. Nebert, D. W., and Jensen, N. M. (1979) in CRC Crit. Rev. Biochem. 6, 401-437
2. Nebert, D. W., Negishi, M., Lang, M. A., Hjelmeland, L. M., and Eisen, H. J. (1981) Adv. Genet., in press
3. Negishi, M., and Nebert, D. W. (1979) J. Biol. Chem. 254, 11015-11023
4. Lang, M. A., Nebert, D. W., and Negishi, M. (1980) in Biochemistry, Biophysics and Regulation of Cytochrome P-450 (Gustafsson, J.-Å., Carlstedt-Duke, J., Mode, A., and Rafter, J., eds) pp. 415-422, Elsevier/North-Holland Biomedical Press, Amsterdam
5. Poland, A. P., Glover, E., Robinson, J. R., and Neibert, D. W. (1974) J. Biol. Chem. 249, 5599-5606
6. Nebert, D. W., Robinson, J. R., Niwa, A., Kamaki, K., and Poland, A. P. (1975) J. Cell. Physiol. 85, 393-414
7. Guenther, T. M., Poland, A. P., and Nebert, D. W. (1976) Fed. Proc. 35, 282 Abstr.
8. Poland, A. P., Glover, E., and Kende, A. S. (1976) J. Biol. Chem. 251, 4936-4946
9. Guenther, T. M., and Nebert, D. W. (1977) J. Biol. Chem. 252, 8981-8989
10. Carlstedt-Duke, J., Ellström, G., Snoochowz, M., Högbreg, B., and Gustafsson, J.-Å. (1978) Toxicol. Lett. 2, 367-373
11. Carlstedt-Duke, J. M. B. (1979) Cancer Res. 39, 3172-3176
12. Okey, A. B., Bondy, G. P., Mason, M. E., Kahl, G. F., Eisen, H. J., Guenther, T. M., and Nebert, D. W. (1979) J. Biol. Chem. 254, 11636-11648
13. Okey, A. B., Bondy, G. P., Mason, M. E., Neibert, D. W., Forster-Gibson, C. J., Muncan, J., and Dufresne, M. J. (1980) J. Biol. Chem. 255, 11418-11422
14. Greengale, W. F., and Poland, A. (1979) J. Biol. Chem. 254, 9814-9821
15. Tierney, B., Weaver, D., Heitz, N. H., Schaeffer, W. L., and Brenchik, E. (1980) Arch. Biochem. Biophys. 200, 513-523
16. Robinson, J. R., and Nebert, D. W. (1974) Mol. Pharmacol. 10, 484-493
17. Sherman, M. R., Corvall, P. L., and O'Malley, B. W. (1970) J. Biol. Chem. 245, 6085-6096
18. Fuller, R., Morey, K. S., and Litwack, G. (1974) Biochem. Biophys. Res. Commun. 60, 431-438
19. Nebert, D. W., Considine, N., and Owene, I. S. (1973) Arch. Biochem. Biophys. 157, 158-159
20. Atlas, S. A., Taylor, B. A., Diwan, B. A., and Nebert, D. W. (1976) Genetics 83, 537-550
21. Felton, J. S., Ketley, J. N., Jakoby, W. B., Atto, A., Bend, J. R., and Nebert, D. W. (1980) Mol. Pharmacol. 18, 550-564
22. Nebert, D. W., and Gieken, J. E. (1971) J. Biol. Chem. 246, 5990-5996
23. Nebert, D. W., and Bausserman, L. L. (1970) J. Biol. Chem. 245, 6373-6382