DMD Fast Forward. Published on May 3, 2022 as DOI: 10.1124/dmd.122.000891
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## Commentary

# Perspective of the Induction of Liver Microsomal P450s by Chemical Compounds

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**Running title**

The Future of P450 CYP Induction

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## Abstract

The concept of hepatic induction of drug metabolizing P450 CYPs by xenobiotics including therapeutic drugs was proposed in the early1960s. A polycyclic aromatic hydrocarbon and phenobarbital have been the two major inducers used to investigate this induction mechanism. Currently, the nuclear receptors AhR and CAR-mediated mechanisms are established. In addition to mammals, insects and fungi also express P450 CYPs and induce them following exposures to insecticides. These inductions may cause environmental consequences. Finding the molecular mechanism regulating these inductions will be of major interest in the future.

**Significance statement:** Timely summarizes the present and future of investigations into induction of drug metabolizing enzymes, by one of the founders of cytochrome P450 CYP research who led this research field for the past 50 years.

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## Introduction

Induction of the drug oxidation activities in the liver of experimental animals by the administration of certain chemical compounds was discovered in the middle of the 1950s. At that time, it was found (Conney et al., 1956) that the intraperitoneal injection of 3-methylcholanthrene stimulated the oxidative N-demethylation of 3-methyl-4-methyl-aminoazobenzene by rat liver homogenates. Soon later, the induction of liver microsomal NADPH-dependent benzopyrene hydroxylation was discovered by intraperitoneal injection of benzopyrene (Conney et al., 1957). Barbiturates were then found (Remmer, 1959) to increase the microsomal drug-metabolizing activity in rat liver.

## History of research on P450 induction

Cytochrome P450 (CYP) was discovered in liver microsomes (Omura and Sato 1962), and its role in the oxidation of various drugs by liver microsomes was confirmed (Cooper et al., 1965). Purification and characterization of various forms of CYPs in the following years confirmed the presence of several molecular species of CYPs in the microsomes of human liver. Further studies showed that some chemical compounds induced specific forms of CYPs in the liver. Induction of CYP1A1 by polycyclic aromatic hydrocarbons attracted particular attention because CYP1A1 catalyzed the conversion of chemically inert polycyclic aromatic hydrocarbons to reactive carcinogenic compounds (Nebert and Gelboin, 1968). Intensive studies in the 1970s to the 1980s elucidated the role of a transcription factor aryl hydrocarbon receptor (AhR) (Poland and Glover, 1976) and its translocation to the nucleus by hydrocarbon receptor nuclear translocator (ARNT) in the induction of CYP1A1 by polycyclic aromatic hydrocarbons. Further studies confirmed the roles of a few other nuclear receptors, including pregnane X receptor

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(PXR) and peroxisome proliferator-activated receptor (PPAR) in the induction of the drug oxidation activities in the liver.

Phenobarbital was found to induce CYP2B1 in rat liver and CYP2B6 in human liver. Since the family CYP2Bs catalyze the metabolism of barbiturates and some other drugs, the molecular mechanism of the induction of CYP2B1 in rat liver and CYP2B2 in mouse liver by phenobarbital was actively studied by many investigators. However, their search for a nuclear receptor that specifically binds phenobarbital to induce the expression of *Cyp2b* genes gave negative results. Negishi and his collaborators continued tenaciously their study on the induction of liver microsomal CYP2B10 in mouse liver by phenobarbital, and discovered the presence of a phenobarbital-responsive enhancer module (PBREM) that activates the expression of *Cyp2b10* gene (Honkakoski et al., 1998). They further clarified the role of an orphan transcription factor constitutive androstane receptor (CAR) in the induction of CYP2B10 in mouse liver (Kawamoto et al., 1999). Phenobarbital does not bind to CAR, but it induces the import of cytosolic CAR into the nucleus. The CAR imported into the nucleus binds to the regulatory region of *Cyp2b10* gene, and induces the expression of the gene. The long-standing problem of the induction of liver microsomal drug-metabolizing activity by barbiturates was thus clarified. It was a grand finale of the long intensive studies by many investigators on the induction of the drug-metabolizing activities of liver microsomes by a variety of chemical compounds.

However, it was not the finale of the study on the induction of drug-metabolizing enzyme activity by chemical compounds in the biological world. Our studies have been focused on human, rat, and mouse species. The elucidated mechanisms of the induction of the drug-metabolizing enzyme activities are valid for mammals and possibly for vertebrate animals, but not for lower forms of animals including *Drosophila melanogaster* and *Caenorhabditis elegans*.

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Invertebrates have AhR gene (Hahn, 2002), but they do not respond to polycyclic aromatic hydrocarbons because their AhR has very low affinity for polycyclic aromatic hydrocarbons. The physiological function of their AhR is not related with drug metabolism. The situation may be true for phenobarbital.

**Future directions**

World-wide extensive use of insecticides, herbicides, and fungicides in recent years has resulted in the emergence of many "resistant" insects, weeds, and fungi. Their induced resistance to various drugs was achieved by the target mutation or by the detoxification of the drugs. Target mutation has often been found in herbicide-resistant weeds and fungicide-resistant fungi, whereas the induction of the detoxification activities was common in insecticide-resistant insects and their larvae. Since the appearance and spread of drug-resistant insects were serious problems in agriculture and hygiene, intensive studies were carried out in the past years, and, in most cases, the resistance was found to be due to the induction of specific forms of P450s in the midgut of the insects. However, the molecular mechanism of the induction of the P450 genes in the insect tissues is not yet well elucidated. The situation seems to be the same for the detoxification of herbicides and fungicides in the tissues of resistant weeds and fungi. Further investigation on the molecular mechanisms of the drug-oxidation activities of invertebrate animals, plants, and fungi is needed, and will be an important research area in drug metabolism study.

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## Authorship contributions

*Wrote or contributed to the writing of the manuscript:* T. Omura

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## References

Conney AH, Miller EC and Miller JA (1956) The metabolism of methylated aminoazo dyes V. Evidence for induction of enzyme synthesis in the rat by 3-methylcholanthrene. *Cancer Res* **16**:450-459.

Conney AH, Miller EC and Miller JA (1957) Substrate-induced Synthesis and other properties of brnzopyrene hydroxylase in rat liver. *J Biol Chem*, **228**:753~766.

Cooper DY Levin SS Narasimhulu S Rosenthal O and Estabrook RW. (1965) Photochemical action spectrum of the terminal oxidase of mixed function systems. *Science* **147**: 400-402

Hahn H. (2002) Ary hydrocarbon receptor; diversity and evolution. *Chemico -Biological Interaction* **141**:131-160.

Honkakoski P Moore R Washburn KA and Negishi M. (1998) Activation by diverse xenochemicals of the 51-base pare phenobarbital-responsive enhancer module in the *CYP2B10* gene. *Mol Pharmacol* **53**:597~601.

Kawamoto T Sueyoshi T Zelko I Moore R, Washburn K and Negishi M (1999) Phenobarbital-responsive nuclear translocation of the receptorCAR in induction of the *CYP2B* gene. *Mol Cell Biol.* **19:** 6318~6322.

Nebert DW and.Gelboin HV (1968) Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture. I. Assay and properties of induced enzyme. *J Biol Chem* **243**: 6242~6249

Omura T and Sato R (1962) A New Cytochrome in Liver Microsomes. *J Biol Chem* **237**:PC1375-1376

Poland A and Glover E(1976) Stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin by hepatic cytosol. Evidence that the binding species is receptor for induction of aryl hydrocarbon hydroxylase. *J Biol Chem*, **251**:4936-4946

Remmer H (1959) Die Beschleunigung der Evipan Oxydation und Methylierung von Methylaminoantipyrin durch Barbiturate. *Arch Exp Pathol Pharmakol* **237**:296~307.

## Footnotes

The Commentary has been edited by Dr. Lee Pedersen, Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27517, pedersen@ad.unc.edu.

No external funding provided.

No author has an actual or perceived conflict of interest with the contents of this article.