CC/NUMBER 38
SEPTEMBER 20, 1993

This Week's Citation Classic®

Alvares A P, Schilling G, Levin W & Kuntzman R. Studies on the induction of CO-binding pigments in liver microsomes by phenobarbital and 3-methylcholanthrene. *Biochem. Biophys. Res. Commun.* 29:521-6, 1967. [Wellcome Research Laboratories, Burroughs Wellcome & Co. (U.S.A.) Inc., Tuckahoe, NY]

This study was the first to show that an inducer of rat liver microsomal enzymes, the polycyclic aromatic hydrocarbon, 3-methylcholanthrene, caused the synthesis of a new hemoprotein. The hemoprotein was termed cytochrome P-448. The study showed that cytochrome P-448 had spectral characteristics different from the cytochrome P450 present in microsomes obtained from untreated or phenobarbital-pretreated rats. [The *SCI*® indicates that this paper has been cited in more than 375 publications.]

Hepatic Cytochrome "P-448"

Alvito P. Alvares
Department of Pharmacology
Uniformed Services University
School of Medicine
Bethesda, MD 20814-4799

In 1964, T. Omura and R. Sato demonstrated the presence and properties of the rat hepatic hemoprotein which they termed cytochrome P450. Direct evidence for the role of this hemoprotein in steroid hydroxylation and the oxidation of drugs soon followed. Several studies by Allan Conney and the Millers at Wisconsin using microsomal enzyme inducers suggested that multiple forms of cytochrome P450 might exist in rat liver microsomes.

in 1965, I joined Gilbert Mannering's laboratory at the University of Minnesota as a postdoctoral fellow. By employing two-substrate kinetics I was able to demonstrate the presence of more than one oxidative enzyme in the dealkylation of drugs. While I was doing my postdoctorate work, Norm Sladek was working on his PhD in Gil's laboratory. Using ethyl isocyanide as a ligand, Sladek demonstrated the presence of a different species of cytochrome P450 in 3-methylcholanthrene (3-MC)-pretreated rats. Sladek and Mannering termed this hemoprotein "cytochrome P_1-450"[1] and postulated the presence of two forms of the native cytochrome P450.

In 1967,I joined Ronald Kuntzman's laboratory at the Wellcome Research Laboratories. By then, Ronnie and Wayne Levin had observed that, unlike microsomal preparations from control or phenobarbital-pretreated rats, the CO difference spectral maximum of microsomes from 3-MC-pretreated rats was not exactly at 450 nm. Because of my prior work with spectral determinations and enzyme kinetics, I immediately became involved in this project. With the use first of a Cary spectrophotometer and subsequently of an Aminco-Chance split beam spectrophotometer it was conclusively shown that an absorbance maximum occurred at 448 nm in the CO difference spectrum of liver microsomes from 3-MC-pretreated rats, in contrast to 450 nm that was observed with microsomes from untreated or phenobarbital-pretreated rats. This *Citation Classic*^ study, published in 1967, coined the term "cytochrome P-448." We also showed a shift in the spectral maximum from 455 nm to 453 nm of the ethyl isocyanide difference spectrum, and a marked change in the apparent K_m for aryl hydrocarbon hydroxylase activity of liver microsomes from 3-MC-pretreated rats, when compared to microsomes from controls or phenobarbital-pretreated rats.[2]

These data were presented at the First International Symposium on Microsomes and Drug Oxidations that was held in 1968 in Bethesda, Maryland. Eminent scientists in the field attributed the altered spectral and catalytic properties of cytochrome P-448 to biophysical changes of a single form of cytochrome P450 caused by the chemicals used as inducers ortheir metabolites. It was very clear to us that the only way to resolve this issue was to go back to the lab.

Using inhibitors of protein and RNA synthesis,[3] administration of ^{3}H-3-MC, and incubation in vitro of microsomes from untreated rats[4] with ^{3}H-3-MC, we were able to prove conclusively that the spectral changes in the CO and ethyl isocyanide difference spectra were not due to the binding of 3-MC or its metabolites to cytochrome P450. Ronald Estabrook, who had previously maintained that cytochrome P448 was not a different P450 hemoprotein, chaired the FASEB session at which I presented these data. I was elated when he stopped the ensuing discussion period by stating that he now agreed that there was, indeed, more than one cytochrome P450.

Levin et al. have subsequently purified and characterized more than a dozen rat cytochrome P450 isozymes.[5] The nomenclature of the P450s has changed, and "cytochrome P-448" is now known in rat liver microsomes as cytochrome P4501A1.

1. **Sladek N E & Mannering** G **J.** Evidence for a new P-450 hemoprotein in hepatic microsomes from melhylcholanlhrene-treatcd rats. *Biochem. Biophys. Res. Commun.* 24:668-74, 1966. (Cited 445 times.)

2. **Alvares A P, Schilling G R & Kuntzman R.** Differences in the kinetics of benzopyrene hydroxylation by hepatic drug-metabolizing enzymes from phenobarbital and 3-methylcholanthrene-trealed rats. *Biochem. Biophys. Res. Commun.* 30:588-93. 1968. (Cited 120 times.)

3. **Alvares A P, Schilling** G, **Levin W** & **Kuntzman R.** Alteration of the microsomal hemoprotein by 3-methylcholanthrene: effects of elhionine and actinomycin D. *J. Pharmacol. Exp. Ther.* 163:417-24, 1968.

4. ---------- . Inability of substrates to alter the carbon monoxide and ethyl isocyanide difference spectra of microsomal hemoprotein. *J. Pharmacol. Exp. Ther.* 176:1-10. 1971.

5. **Levin** W. Functional diversity of hepatic cytochromes P-450. *Drug Metab. Disposition* 18:824-30. 1990.

Received July 20. 1993