CC/NUMBER 5
JANUARY 30, 1984

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Nebert D W & Gelboin H V. Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture. I. Assay and properties of induced enzyme. *J. Biol. Chem.* **243**:6242-9, 1968. [Chemistry Branch. National Cancer Institute. National Institutes of Health. Bethesda. MD]

The aryl hydrocarbon (benzo[a]pyrene) hydroxylase (EC 1.14.14.1) assay is detailed. This rapid and sensitive method offers great promise for studying metabolism of the environmental carcinogen not only in laboratory animal liver, but also in extrahepatic tissues and in cell culture systems. [The *SCI*® indicates that this paper has been cited in over 890 publications since 1968.]

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October 28, 1983

"As a graduate student with Howard S. Mason (University of Oregon Medical School, Portland), I had already developed a fascination with carcinogenesis and drug metabolism.[1,2] When a foreign chemical enters the body, is it broken down by constitutive enzymes or are new enzymes mobilized to challenge this foreign substance? Is the resulting metabolism of carcinogens a cause, or a prevention, of tumorigenesis? These same questions are being asked today, although much more is understood.

"During my postdoctoral fellowship at the National Cancer Institute, NIH, several important events converged. I had gained experience in Mason's lab with the drug-metabolizing enzyme 'cytochrome P-450' (previously also called 'microsomal Fe_x'). Polycyclic hydrocarbon 'transformation' experiments in the tissue culture laboratory of Larry J. Alfred and Joseph A. Dipaolo,[3] across the hall from my mentor, Harry V. Gelboin, could best be explained on the basis of induction of polycyclic hydrocarbon metabolism in fetal hamster secondary cultures. This idea was heresy at the time. Further, there was a published spectrophotofluorometric method[4] for detecting the conversion of benzo[a]pyrene to hydroxylated products. It thus seemed ideal for me to develop a rapid and sensitive assay for this enzyme of carcinogen metabolism and to study its induction properties in a well-controlled cell culture system instead of the intact animal.

"Our first two publications[5] were accepted with relative ease and enthusiasm. I believe the first paper has been so highly cited because it described all the enzyme assay conditions. The second paper dealt with the hydroxylase induction kinetics and demonstrated, for the first time, inducible P-450 in cultured cells. I feel the second paper was more exciting than the first. A third paper[6] demonstrated the sensitivity of this induction process to actinomycin D and cycloheximide in cell culture.

"The two 1968 papers represented a 'shot heard 'round the world' in the combined fields of cell culture and pharmacology. Dozens of laboratories began studying, and presently continue to study, the induction of drug-metabolizing enzymes in cell culture. Hundreds of laboratories are characterizing the induction of P-450-mediated enzyme activities in the intact animal. Hardly an issue of any pharmacology or toxicology journal now can be found without the term 'aryl hydrocarbon hydroxylase' or the magic abbreviation 'AHH' appearing somewhere.

"The paper I regard as my major breakthrough, however, is the one which reported our discovery of Mendelian inheritance among inbred strains of mice lacking, or not lacking, the normal AHH induction response by polycyclic hydrocarbons.[7] This key finding led to the thorough characterization of the *Ah* receptor and more recently the cloning of the entire mouse P_1-450 gene.[8] Dozens of other labs are now in the process of cloning various rat and rabbit P-450 genes. Not necessarily from the 'most-cited' 1968 paper, but rather from my persistence in this line of work during the past two decades, I have received several recognitions, including Annual Pfizer Lectureship Awards, a scholarship from the Japanese Society for the Promotion of Science, and the US Public Health Service Meritorious Service Medal.

"The next ten years of research in this field should tell us: (i) the evolution of this P-450 superfamily (from *Pseudomonads*, yeast, and plants to the human); (ii) the mechanism of control of P-450 gene expression by one or more unique receptors that bind avidly to foreign chemicals; and (iii) perhaps development of an assay to determine within the human population individuals at increased risk for certain types of environmentally caused cancers and drug toxicities."

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2. ..... A microsomal difference between normal liver and "minimal deviation" hepatoma 5123 detectable by electron spin resonance. *Biochim. Biophys. Acta* **86**:415-17, 1964.
3. **Dipaolo J A & Donovan P J.** Properties of Syrian hamster cells transformed in the presence of carcinogenic hydrocarbons. *Exp. Cell Res.* **48**:361-77, 1967. (Cited 115 times.)
4. **Wattenberg L W, Leong J L & Strand P J.** Benzpyrene hydroxylase activity in the gastrointestinal tract. *Cancer Res.* **22**:1120-5, 1962. (Cited 345 times.)
5. **Nebert D W & Gelboin H V.** Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture. II. Cellular responses during enzyme induction. *J. Biol. Chem.* **243**:6250-61, 1968. (Cited 195 times.)
6. .....The role of ribonucleic acid and protein synthesis in microsomal aryl hydrocarbon hydroxylase induction in cell culture: the independence of transcription and translation. *J. Biol. Chem.* **245**:160-8, 1970. (Cited 80 times.)
7. **Gielen J E, Goujon F M & Nebert D W.** Genetic regulation of aryl hydrocarbon hydroxylase induction. II. Simple Mendelian expression in mouse tissues *in vivo*. *J. Biol. Chem.* **247**:1125-37. 1972. (Cited 225 times.)
8. **Nakamura M, Negishi M, Altieri M, Chen Y T, Ikeda T, Tukey R H & Nebert D W.** Structure of the mouse cytochrome P_1-450 genomic gene. *Eur. J. Biochem.* **134**:19-25, 1983.