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This Week's Citation Classic™

Schenkman J B, Remmer H & Estabrook R W. Spectral studies of drug interaction with hepatic microsomal cytochrome. *Mol. Pharmacol.* **3**:113-23, 1967. [Dept. Biophys. and Phys. Biochem., Johnson Res. Foundation, Univ. Pennsylvania, Philadelphia, PA]

The addition of some two dozen substrates of the hepatic monooxygenase to liver microsomes was shown to evoke one of two types of spectral changes. The spectral changes were shown to be reversible and to be dependent upon both substrate and microsomal protein concentrations. The dissociation constants for substrates causing one type of spectral change (type I) were shown to be similar to the K_m values for some of those substrates, and it was suggested that the spectral changes were indicative of enzyme substrate complexes. [The *SCI*® indicates that this paper has been cited in over 1,025 publications since 1967.]

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"Shortly after I joined Ron Estabrook's lab at the Johnson Foundation as a postdoctoral fellow in 1964, Ron suggested I study the hepatic microsomal mixed function oxidase. It was a good suggestion. Tsuneo Omura from Ryo Sato's department had joined Ron and David Y. Cooper at the University of Pennsylvania in a study of the adrenal cortex steroid hydroxylase and was a ready source of knowledge of P-450. Herbert Remmer would soon be joining the lab from Germany and was a source of knowledge of drug metabolism. Shakunthala Narasimhulu, who worked with Dave, had noted steroids affected the absorption spectrum of adrenal cortex microsomes,[1] so Ron suggested I examine the liver microsomes spectrophotometrically. With great trepidation I approached the Yang machine, a Johnson Foundation creation that seemed, at my level of sophistication, to be a monochromator and photomultiplier caught in a spaghetti factory explosion. Work went well and by the spring of 1965 it appeared that we had a method conducive to the spectral examination of the monooxygenase. The excitement level ran high and Ron invited Jim Gillette to join the fun. Jim arrived with Henry Sasame. Dave joined us and soon the lab was full, with Herbert and me preparing microsomes, Henry and Jim making solutions, and Dave and Ron cranking the Yang machine. By 2 or 3 a.m. it was clear –the results were not artifact; substrates did perturb the absorption spectrum of the microsomes, probably by binding to cytochrome P-450.[2]

"I completed these studies over the next year with great enjoyment and we published our findings in the 1967 *Molecular Pharmacology* paper in which we described and named the interaction of cytochrome P-450 with substrates. We suggested that substrates affect the binding of a ligand to cytochrome P-450 and also showed substrate to perturb the EPR spectrum[3] of the cytochrome. Within a few years, it became clear that cytochrome P-450 undergoes substrate-dependent spin state changes.

"Perhaps the reason for the large number of citations to the 1967 paper lies in the ease with which this method measures the binding of compounds to the cytochrome P-450 monooxygenase, and in the recognition that most of the perturbants are also substrates. Many studies have since been reported on the spectral titration of the enzyme with substrates, and many investigators have examined the nature of the spectral changes in an attempt to understand the role of the spin shift in the mechanism of this enzyme action.[4] As we indicated in a recent report,[5] more and more studies will make use of physical/chemical approaches for analysis of the cytochrome P-450 system. To date, such studies have aided greatly in unraveling the mechanism of this enzyme system."

1. **Narasimhulu S, Cooper D Y & Rosentbal O.** Spcctrophotometric properties of a triton clarified steroid 21-hydroxylase system of adrenocortical microsomes. *Life Sci.* **4**:2101-7, 1965. (Cited 95 times.)
2. **Remmer H, Schenkman J, Estabrook R W, Sasame H, Gillette I, Narasimhulu S, Cooper D Y & Roseathal O.** Drug interaction with hepatic microsomal cytochrome. *Mol. Pharmacol.* **2**:187-90, 1966. (Cited 405 times.)
3. **Cammer W, Schenkman J B & Estabrook R W.** EPR measurements of substrate interaction with cytochrome P-450. *Biochem. Biophys. Res. Commun.* **23**:264-8, 1966.
4. **Scbenkman J B, Sligar S G & Cinti D L.** Substrate interaction with cytochrome P-450. *Pharmacol. Ther.* **2**:43-71, 1981.
5. **Schenkman J B & Gibson G G.** Status of the cytochrome P-450 cycle. *Trends Pharmacol. Sci.* **2**:150-2, 1981.