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This Week's Citation Classic®

Cooper D Y, Levin S, Narasimhulu S, Rosenthal O & Estabrook R W.
Photochemical action spectrum of the terminal oxidase of mixed function oxidase systems. *Science* 147:400-2, 1965. [Harrison Dept. Surgical Res. and Johnson Foundation for Medical Physics, Univ. Pennsylvania Sch. Medicine, Philadelphia, PA]

This paper provided the initial proof that cytochrome P-450 is the oxygen-activating enzyme for hepatic as well as adrenocortical microsomal mixed function oxidases. [The *SCI*® indicates that this paper has been cited in over 405 publications.]

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It may seem paradoxical that the discovery that cytochrome P-450 is the oxygen-activating enzyme for mixed function oxidases (MFOS) originated in the Harrison Department of Surgical Research at the University of Pennsylvania's School of Medicine. However, the discovery evolved logically from a study of steroid formation *in vitro* by human adrenal glands.[1]

When adrenalectomy for treatment of hypertension was abandoned in the late 1950s because hydralazine, reserpine, and methyl dopa were more effective, we began studies on the action of ascorbate and catecholamines on steroid formation by bovine adrenal tissue slices. Otto Rosenthal, my collaborator since the beginning of this work, suggested that more progress could be made by studying a single hydroxylation step: C-21 hydroxylation of 17-hydroxy progesterone. Hj. Staudinger and his coworkers had concluded that cytochrome b_5 and a monohydroascorbate acid radical were involved in C-21 hydroxylation,[2] but Rosenthal did not believe that their mechanism was correct. Mary Jane Spiro and Eric G. Ball of Harvard University created more confusion in the field when they reported in the *Journal of Biological Chemistry* that adrenocortical microsomes did not contain cytochrome b_5.[3]

Our first progress towards elucidating the nature of the C-21 hydroxylase occurred when Shakunthala Narasimhulu found that Triton X-100 clarified adrenocortical microsomal suspensions,[4] permitting their study in the new Cary Model 14 spectrophotometer purchased to equip the I.S. Ravdin Institute at the University of Pennsylvania's School of Medicine. In our initial studies only b_5 was detected in the Triton preparation; however, Narasimhulu soon discovered that addition of substrate caused a spectral change (Type I) and that irreversible inhibition of C-21 hydroxylase activity developed with the addition of sulphydryl reagents. Later, after P-450 was found in the Triton preparations, Narasimhulu discovered that these reagents also converted P-450 to the inactive derivative cytochrome P-420.

In January 1961 at the John Morgan Society, Ronald Estabrook and I discussed whether adrenocortical microsomes contained cytochrome b_5. He felt that this question could be answered using the Yang-Chance spectrophotometer. Spectral studies soon showed that adrenocortical microsomes contained cytochrome b_5. Out of frustration, while studying the stoichiometry of C-21 hydroxylation, I tried something else. I bubbled a Triton preparation with CO and measured its difference spectrum in the Cary 14 and observed a large 450-nm absorption band we had never seen before. We soon demonstrated that the CO compound existed also in adrenal microsomes and that it was the pigment of unknown function that Britton Chance and G.R. Williams had discovered and that David Garfinkel and Martin Klingenberg had independently reported in 1958.[5,6] Remembering that Kenneth J. Ryan and Lewis L. Engel had found that C-21 hydroxylation was inhibited by CO,[7] we repeated and confirmed their findings.

To prove that the microsomal CO-combining pigment was a component of the C-21 hydroxylase, we turned to Otto Warburg's photochemical action spectrum. A Johnson Foundation type makeshift irradiation apparatus was assembled, and we had no difficulty obtaining an action spectrum for C-21 hydroxylation. We immediately sent a short report to the *Journal of Biological Chemistry*. It was rejected. The first publication of the function of the CO pigment was in the *Biochemische Zeitschrift*'s festschrift honoring Warburg's 80th birthday.[8]

To establish cytochrome P-450's role as the terminal oxidase for the hepatic microsomal drug reactions, we improved the irradiating apparatus by adding a 1,600-watt xenon lamp and obtaining additional interference filters. Action spectra for the hepatic MFOS were difficult to measure because, as was subsequently shown,[9] CO inhibition of N-dealkylations is only partially reversed by intense light.

This paper, which described these action spectra, is frequently cited because it established the role of cytochrome P-450 as the oxygen-activating enzyme for MFOS, the step that led to the realization that P-450 is one of nature's fundamental building blocks, a discovery of great importance in the life sciences.

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2. **Staudinger Hj, Krisch K & Leonhäuser S.** Role of ascorbic acid in microsomal electron transport and the possible relationship to hydroxylation reactions. *Ann. NY Acad. Sci.* 92:195-207, 1961. (Cited 85 times.)
3. **Spiro M J & Ball E G.** Studies on the respiratory enzymes of the adrenal gland. II. The cortex. *J. Biol. Chem.* 236:231-5, 1961. (Cited 25 times.)
4. **Narasimhulu S, Cooper D Y & Rosenthal O.** Spectrophotometric properties of a Triton-clarified steroid C-21 hydroxylase system of adrenalcortical microsomes. *Life Sci.* 4:2101-7, 1965. (Cited 105 times.)
5. **Garfinkel D.** Studies on pig microsomes. I. Enzymic and pigment composition of different microsomal fractions. *Arch. Biochem. Biophys.* 77:493-509, 1958. (Cited 280 times.)
6. **Klingenberg M.** Pigments of rat liver microsomes. *Arch. Biochem. Biophys.* 75:376-86, 1958. (Cited 390 times.)
7. **Ryan K J & Engel L L.** Hydroxylation of steroids at carbon 21. *J. Biol. Chem.* 225:103-14, 1957. (Cited 245 times.)
8. **Estabrook R W, Cooper D Y & Rosenthal O.** The light-reversible carbon monoxide inhibition of the steroid C-21 hydroxylase system of the adrenal cortex. *Biochem. Z.* 388:741-5, 1963. (Cited 355 times.)
9. **Hamill S J, Cooper D Y, Schleyer H & Rosenthal O.** A kinetic study comparing the light reversal of CO inhibition of ethyl morphine, benzphetamine, 7-ethoxycoumarin dealkylations with those of the hydroxylation of 17-hydroxy progresterone and testosterone. *Arch. Biochem. Biophys.* 224:625-35, 1985.

IA-16