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Increased Drug-Metabolizing Activity without Increased Cytochrome P-450 in Rats Treated with Phenolic Antioxidants

By D. GILBERT. (*Biophysics and Biochemistry Laboratory, Wellcome Research Laboratories, Beckenham, Kent*)

The endoplasmic reticulum of the liver contains a cytochrome (*P*-450) that has been implicated in the enzymic oxidation of drugs and other foreign compounds (Cooper, Levine, Narasimhulu, Rosenthal & Estabrook, 1965). Since the activities of several of these oxidative enzymes are increased in rats that have been treated with various phenolic compounds (Gilbert, Martin & Golberg, 1967), the cytochrome *P*-450 content of the livers of similarly treated rats was measured. Most of the compounds that increased the enzyme activities also increased the cytochrome *P*-450 content of the liver, but two compounds caused several-fold increases in the drug-metabolizing enzymes with little or no change in cytochrome *P*-450 content. These two compounds were 2,6-di-*tert*-butyl-4-methylphenol (BHT; I) and 2,6-di-*tert*-butyl-4-methoxymethylphenol (II).

The livers of female rats (100g.) were removed 24 hr. after an oral dose (1.5 m-moles/kg.) of these test compounds. Activities of the microsomal enzyme BHT oxidase (Gilbert & Golberg, 1965) were (expressed as means $\pm$ s.e.m., with numbers of values in parentheses): controls, 0.26 ± 0.04 (4), compound I, 0.78 ± 0.07 (3), and compound II, 1.15 ± 0.02 (4) μ moles/hr./g. of liver. The cytochrome *P*-450 contents of the same livers were: controls, 9.2 ± 0.7 , compound I, 10.5 ± 0.8 , and compound II, 12.4 ± 0.7 nmoles/g. of liver.

A similar result was obtained by using the duration of action of hexobarbitone to measure the rate of drug metabolism *in vivo*. At 48 hr. after doses of 0.2, 0.5 or 1.5 m-moles of compound II/kg. the hexobarbitone sleeping times of female rats (100g.) were significantly decreased (75, 65 and 48% of control respectively; groups of four to six), indicating accelerated hexobarbitone oxidation. The cytochrome *P*-450 contents of the livers of identically treated rats were not significantly changed (107, 121 and 107% of control respectively; groups of six). At 48 hr. after a dose of phenobarbitone (100 mg./kg.) both the hexobarbitone

sleeping time and the cytochrome *P*-450 content were significantly altered (27% and 232% of control respectively; groups of six).

It appears from these experiments that cytochrome *P*-450 may not be rate-limiting for oxidative drug metabolism *in vivo* or *in vitro*.

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The Haem-Protein Interaction in Cytochrome P-450

By H. A. O. HILL, A. RÖDER and R. J. P. WILLIAMS. (*Inorganic Chemistry Laboratory, University of Oxford*)

In order to understand the many spectroscopic peculiarities associated with cytochrome *P*-450 we have made a series of model studies on protohaem complexes, especially those in which the iron is further bound to a thiol. The ferrihaem complexes with thiols give rise to absorption and electron-proton-resonance spectra that closely resemble those of cytochrome *P*-450 and are compatible with the ferric iron being largely in a low-spin state (Bayer, Hill, Roder & Williams, 1969). However, there is always some high-spin ferric iron present and the amount of this form of the iron is dependent on the substrate added (Williams, 1968). As these substrates can not bind the iron in general it follows that they must modify the iron-protein bonding.

The spectroscopic properties of ferrocytochrome *P*-450 indicate that the iron is high-spin. Such ferrohaem complexes have very little affinity for thiols. We conclude that the curious difference spectra of cytochrome *P*-450, oxidized minus reduced, are due to the unusual spin states of the iron, ferric iron low-spin and ferrous iron high-spin, and to the weakening or breaking of the iron-sulphur bond on reduction. On addition of carbon monoxide, nitric oxide or isonitriles ferrocytochrome *P*-450 has absorption bands at 450 and 550 nm. but with no α -band. Model studies show that this type of spectrum is most closely approached when metal atoms in porphyrin complexes become five-co-ordinate and the metal lies out of the plane of the porphyrin by some considerable distance. This state of the metal, should it also arise on reaction with oxygen, would lead to a very open complex that is similar to the five-co-ordinate forms of vitamin B₁₂ derivatives. Vallee & Williams (1968) have referred to such unusual co-ordination conditions as entatic states.