showing short plasma half-lives for antipyrine also had short half-lives for phenylbutazone and oxphenbutazone.

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Cytochrome P-450 in Steroid Biogenesis

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Mason, Fowls & Peterson (1955) showed that in certain enzymic hydroxylation reactions the oxygen atom incorporated into the substrate was obtained from molecular oxygen. Mason (1957) introduced the term 'mixed-function oxidase' for oxygenase reactions in which the substrate, oxygen and an electron donor interacted to produce the hydroxylated substrate, the oxidized form of the electron donor and metabolic water. In many mammalian steroid hydroxylase systems the electron donor is NADPH (cf. Grant, 1956).

These hydroxylase enzymes are key catalysts in the control of sterol metabolism because the hydroxylation reaction is virtually irreversible and in certain situations the enzymic reaction results in the conversion of a ubiquitous sterol substrate into a metabolite with selective and potent properties. The multienzyme nature of certain sterol-hydroxylating systems was shown by Tomkins, Michael & Curran (1957) and Tomkins, Curran & Michael (1958). A more detailed separation of the enzymes involved in one such sterol-hydroxylation reaction was achieved by Omura, Sato, Cooper, Rosenthal & Estabrook (1965), whose studies emphasized the importance of cytochrome P-450 as the possible reaction centre for the substrate, oxygen and electrons.

Cytochrome P-450 is considered to be the site of 'activation of oxygen' in the reactions involved in biological hydroxylations. This haemoprotein is present in liver microsomes and adrenal microsomes, adrenal, ovarian and placental mitochondria, and in certain other tissues. Evidence for the

presence of this haemoprotein in a subcellular fraction can be obtained by observing the effect of carbon monoxide on the reduced haemoprotein because cytochrome P-450 exhibits an intense absorption band under these conditions with a maximum near 450nm. Furthermore, enzymic reactions in which cytochrome P-450 is involved are inhibited by carbon monoxide and the inhibition (*in vitro*) can be reversed by illumination with light at 450nm.

The biosynthesis of cholesterol occurs in the microsomal fraction of certain cells, and the reactions between acetate and squalene have been shown to be anaerobic whereas the events between squalene and cholesterol require the participation of oxygen and NADPH. It has been shown that squalene 2,3-oxide is an intermediate in the conversion of squalene into lanosterol (Corey & Russey, 1966; van Tamelen, Willett, Clayton & Lord, 1966), but the overall conversion of squalene into cholesterol was inhibited by carbon monoxide and hence this reaction is considered to be dependent on cytochrome P-450 (Wada, Hirata & Sakamoto, 1969). These results conflict with the observation by Yamamoto & Bloch (1969), who found that the epoxidation was not inhibited by carbon monoxide. As a result of studies on the oxidative demethylation of lanosterol to cholesterol, it has been shown that the reactions involve hydroxylation of certain methyl groups to hydroxymethyl groups. These enzymic reactions are not inhibited by carbon monoxide and hence cytochrome P-450 is possibly not involved (Gaylor & Mason, 1968).

There is evidence that one route for the conversion of cholesterol into bile acids involves the hydroxylation of cholesterol to 7 α -hydroxycholesterol (Bergstrom, 1959). This enzymic reaction occurs in the liver microsomal fraction and the hydroxylation reaction has an absolute requirement for NADPH and oxygen. This enzyme is inhibited by carbon monoxide and the inhibition is reversed by light at 450nm. (Scholan & Boyd, 1968).

It is well established that cholesterol is the precursor of the steroid hormones produced by the adrenals, ovaries, testes and placenta. It has been shown that one of the metabolites of cholesterol that occupies a key position in these tissues is pregnenolone. The mechanism of conversion of cholesterol into pregnenolone is thought to involve hydroxylation of cholesterol at positions 20 and 22 with resultant cleavage of the cholesterol side chain to pregnenolone and isocaproic aldehyde (4-methylpentan-1-al). The enzymes involved in this reaction are located in the mitochondria of the steroid-hormone-producing tissues (Constantopoulos & Tchen, 1961) and the reaction requires the

presence of oxygen and NADPH. This enzyme system is inhibited by carbon monoxide and the inhibition can be released by light at 450nm. (Simpson & Boyd, 1967). These mitochondrial preparations can be shown to contain cytochrome *P*-450 and the cholesterol-side-chain-cleavage system has been shown to be similar to certain other steroid-hydroxylating systems in that it consists of an enzymic complex of a flavoprotein, a non-haem iron protein and cytochrome *P*-450.

In the adrenal gland progesterone and 17 α -hydroxyprogesterone can be hydroxylated by adrenal microsomes to the corresponding 21-hydroxylated derivatives. This reaction requires oxygen and NADPH and is inhibited by carbon monoxide (Ryan & Engel, 1957). Adrenal microsomes contain cytochrome *P*-450 and the carbon monoxide inhibition of the 21-hydroxylase system is released by light at 450nm. (Omura *et al.* 1965).

Deoxycorticosterone is hydroxylated by adrenal mitochondria to corticosterone and this 11 β -hydroxylation reaction has been shown to be cytochrome *P*-450-dependent. This has been the most extensively studied steroid hydroxylase enzyme system (Omura *et al.* 1965). Adrenal mitochondria can also hydroxylate certain substrates at position 18 and it has been shown that this reaction is cytochrome *P*-450-dependent (Greengard *et al.* 1967).

Hydroxylation reactions are involved in the conversion of C₂₁ steroids into androgens, and similarly hydroxylation reactions are involved in the conversion of androgens into oestrogens through a C₁₉-steroid-hydroxylation reaction. None of the steroid hydroxylases has been purified, and until the components of the multienzyme hydroxylase systems have been rigorously purified the exact mechanism of steroid-hydroxylation reactions and the mode of action of cytochrome *P*-450 will not be clarified.

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Cytochrome *P*-450 in the ω -Oxidation of Fatty Acids

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The principal fatty acids of normal tissue lipids are readily oxidized by liver microsomes to the corresponding ω -hydroxy acids (Preiss & Bloch, 1964). Like other mixed-function oxidation reactions in the liver microsomes this process requires NADPH and molecular oxygen (Wakabayashi & Shimazono, 1963). Cytochrome *P*-450, the terminal oxidase functioning in drug and steroid hydroxylation, has more recently been shown to be involved in the ω -oxidation reaction (Das, Orrenius & Ernster, 1968; Lu & Coon, 1968; Wada, Shibata, Goto & Sakamoto, 1968). Accumulated evidence from several laboratories further suggests that the NADPH-cytochrome *c* reductase is another component of the catalytic system participating in the ω -oxidation of fatty acids (Das *et al.* 1968; Lu & Coon, 1968, Wada *et al.* 1968).

As previously shown for various drugs and steroids, addition of fatty acids capable of undergoing ω -oxidation to a suspension of liver microsomes gives rise to a 'type I' spectral change (Orrenius & Thor, 1969), presumably due to the formation of an enzyme-substrate complex between the fatty acid and the mixed-function oxidase (Schenkman, 1968). These observations indicate a common binding site on cytochrome *P*-450 for various substrates, such as drugs, steroids and fatty acids. Further, drugs undergoing oxidative demethylation and fatty acids undergoing ω -oxidation inhibit each other's metabolism in a competitive manner. In the presence of a high fatty acid (laurate) concentration, however, both the oxidative demethylation and ω -oxidation activities are almost completely extinguished, owing to a solubilizing effect of the fatty acid on the microsomes, associated with a conversion of cytochrome *P*-450 into the enzymically inactive cytochrome *P*-420 form.

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