PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 498th Meeting of the Society was held in the Department of Biochemistry, University College, Cardiff, on Thursday and Friday, 25 and 26 September 1969, when the following papers were presented:

PHARMACOLOGICAL BIOCHEMISTRY GROUP COLLOQUIUM ON 'CYTOCHROME P-450'

Cytochrome P-450 and Drug Oxidation

By DONALD S. DAVIES. (*Medical Research Council Clinical Pharmacology Research Group, Department of Medicine, Royal Postgraduate Medical School, London W.12*)

Studies in the early 1950s established that the liver endoplasmic reticulum or its fragments, the microsomes, contain an enzyme system that can convert drugs and other foreign chemicals into more polar compounds (Axelrod, 1955; Brodie *et al.* 1955; Mitoma, Posner, Reitz & Udenfriend, 1956). At present many reactions are known to be catalysed by liver microsomal enzymes and to require NADPH and molecular oxygen. The reactions include *N*-, *O*- and *S*-dealkylations as well as aliphatic and aromatic hydroxylations (Gillette, 1966). However, the problem of whether the various reactions are catalysed by different enzymes remains unresolved.

Klingenberg (1958) and Garfinkel (1958) reported the presence of a carbon monoxide-binding pigment in liver microsomes. Omura & Sato (1964) showed the pigment to be a cytochrome and named it cytochrome P-450 from the position of the absorption peak of the carbon monoxide complex of the reduced pigment. Photochemical activation spectra for a number of microsomal mixed-function oxidases that are inhibited by carbon monoxide have demonstrated the role of cytochrome P-450 as the oxygen-activating enzyme for many microsomal oxidations. For example, the inhibition by carbon monoxide of the demethylation of codeine can be maximally reversed by light of 450nm. (Omura, Sato, Cooper, Rosenthal & Estabrook, 1965).

Drugs and other compounds that are substrates or inhibitors for the liver microsomal mixed-function oxidases interact with cytochrome P-450 even in the absence of NADPH to give two types of spectral change. The 'type I' spectral change is characterized by the appearance of a trough at 420nm. in the difference spectrum of microsomal suspensions when compounds such as hexobarbitone or aminopyrine are added to the sample cuvette. The 'type II' spectral change is produced by compounds such as aniline and pyridine and is charac-

terized by the appearance of a peak at about 430nm. The intensity of the spectral change is related to the concentration of the substrate. Indeed, for some substrates there is a close similarity between the spectral dissociation constant and the Michaelis constant for the metabolism of the substrate, suggesting that the spectral change indicates the formation of an enzyme-substrate complex (Schenkman, Remmer & Estabrook, 1967).

Thus at the present time the general mechanism for the oxidation of drugs is thought to involve the following reactions. Oxidized cytochrome P-450 reacts with drug to form a drug-cytochrome complex, which is reduced by NADPH-cytochrome *c* reductase, either directly or indirectly through an unidentified carrier. The reduced cytochrome then combines molecular oxygen to form an 'active' oxygen complex, which breaks down to give oxidized cytochrome P-450 and oxidized drug.

Recent studies have established that a rate-limiting step in the overall oxidation of a drug by liver microsomal enzymes is the reduction of the cytochrome P-450-drug complex. Accordingly, species differences in the *N*-demethylation of ethylmorphine by liver microsomes are more closely related to NADPH-cytochrome P-450 reductase activity than to the amount of cytochrome P-450, the magnitude of the 'type I' spectral change, or the activity of NADPH-cytochrome *c* reductase (Davies, Gigon & Gillette, 1969). In addition, the lack of correlation between NADPH-cytochrome *c* reductase and NADPH-cytochrome P-450 reductase activities lends support to the view that some other component is involved in the transfer of electrons from NADPH-cytochrome *c* reductase to cytochrome P-450.

Recently there has been a good deal of interest in the large individual difference in rates of drug oxidation in man. If these differences are due to variations in a rate limiting component such as cytochrome P-450 reductase, then it might be expected that subjects could be classified as either fast or slow 'oxidizers' and that this classification would hold for a number of drugs. In our preliminary studies with antipyrine, phenylbutazone and oxyphenbutazone this appears to be true. Subjects

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

By G. S. BOYD. (*Department of Biochemistry, The Medical School, University of Edinburgh*)

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