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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These and other findings indicate that cytochrome P-450 is the terminal oxidase functioning in the ω -oxidation of fatty acids and also suggest that fatty acids, like steroid hormones, may be physiological substrates for the hydroxylating enzyme system of the liver microsomes.

This work was supported by grants from the Swedish Medical Research Council and the Swedish Cancer Society.

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Induction of Drug-Metabolizing Enzymes and Cytochrome P-450

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As a result of treatment with certain drugs, animals acquire an increased capacity to metabolize a wide range of drugs administered later. This phenomenon, first observed some 15 years ago, has aroused much interest and a considerable literature exists (Conney, 1967). Several hundred compounds are known to stimulate drug-metabolizing enzymes. They fit into one of two general classes typified by 3-methylcholanthrene and phenobarbital respectively, the former class being restricted almost entirely to polycyclic hydrocarbons, the latter containing many chemically diverse drugs such as barbiturates, phenothiazines, chlorinated insecticides and phenylhydantoin.

The polycyclic hydrocarbons produce a sharp rise in the rate of metabolism of a restricted range of drugs; drugs of the barbiturate type produce a slower increase in the metabolism of a much wider range of drugs. Biosynthesis of protein is involved and inhibitors such as cycloheximide and puromycin prevent induction. Morphological changes in the liver cell, principally a considerable increase in the quantity of smooth endoplasmic reticulum, accompany induction of enzyme activity by barbiturate, but are less noticeable after 3-methylcholanthrene induction. There are marked species, strain and individual variations in the responses of animals to inducing chemicals. All increase in drug-metabolizing capacity is accompanied by increases in the cytochrome P-450 content of the microsomal fraction from liver homogenates (Remmer & Merker, 1965). There is evidence that

the cytochrome P-450 produced after induction has different properties from that present in unstimulated animals, and that that found after 3-methylcholanthrene differs from that found after barbiturate treatment. The spectrophotometric and enzymological evidence advanced to support this view (Sladek & Mannering, 1969b; Alvarez, Schilling, Levin & Kunzman, 1967; Hildebrandt, Remmer & Estabrook, 1968; Daly, Jerina, Farnsworth & Guroff, 1969) will be reviewed and implications for assessing the number of distinct drug-metabolizing enzymes present in liver will be considered.

Although increases in cytochrome P-450 accompany increased rates of drug metabolism after induction, there is not always a quantitative relationship between the two (Guarino, Gram, Gigon, Greene & Gillette, 1969). Evidence on the extent to which changes in enzyme activity are mirrored by changes in cytochrome P-450 concentrations will be reviewed and discussed.

The mechanisms by which drug-metabolizing activity is induced are still uncertain. Gene depression seems to be involved (Wortham, Wilson, Falk & Geboin, 1967), but there is also evidence that, after induction, catabolism of cytochrome P-450 is slowed down (Greim & Remmer, 1969).

The two types of inducer seem to work through different mechanisms, since effects of a compound from one group are additive with those of a compound from the other. Recently more direct evidence for different mechanisms has been provided by the finding that thioacetamide prevents the effects of phenobarbital but not those of 3-methylcholanthrene (Sladek & Mannering, 1969a). Evidence bearing on the mechanisms involved, including that for the participation of endogenous intermediates in a de-repression process, will be considered.

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