Significantly, a water-soluble analogue of cholesterol, 25-hydroxycholesterol, was converted into pregnenolone at the same rate by adrenal mitochondria from both stressed and cycloheximide-treated rats. Thus the ether stress, presumably mediated through adrenocorticotrophin, ultimately produces an increase in the fraction of mitochondrial cholesterol bound to the 'side-chain-cleavage' cytochrome *P*-450 even although the total mitochondrial cholesterol is depleted. These changes in the steady-state distribution of cholesterol may reflect activation of specific steps in cholesterol transport. The inhibition of the stress effect by cycloheximide suggests that a labile protein could be involved in this adrenocorticotrophin-induced redistribution process.

The rate-limiting step in the degradation of cholesterol to bile acids in liver appears to be the insertion of a 7α -hydroxyl group into the sterol molecule (Lindstedt, 1957). This reaction, in common with many other hydroxylase systems, is present in the liver 18000g supernatant. This enzyme system requires an external electron donor (NADPH) and oxygen, and is therefore a mixed-function oxidase. The reaction is inhibited by carbon monoxide, and this inhibition can be reversed most efficiently by illumination of the reaction mixture with light of wavelength 450nm. The enzyme system is not very sensitive to cyanide, and hence there appears to be good evidence that cytochrome *P*-450 is involved as a terminal oxidase in this reaction.

Studies on the cholesterol 7α -hydroxylase enzyme system is complicated because, when cholesterol is incubated with liver microsomal fraction, NADPH and oxygen-aberrant (autoxidative) reactions also occur. These reactions produce a range of cholesterol oxidation products, of which few appear to be physiological intermediates in cholesterol metabolism, the remainder being artifacts related to lipoperoxidation events triggered off as a result of the cell homogenization and microsomal isolation procedures. These autoxidation products include 7β -hydroxycholesterol, cholestan- $3\beta,5\alpha,6\beta$ -triol, 7-oxocholesterol and another, as yet unidentified, product (Mitton, Scholan & Boyd, 1971). In the presence of the cell supernatant, or the thermostable components of the cell supernatant, the lipoperoxidation reactions can be almost eliminated. However, these reactions are not influenced by carbon monoxide in the gas phase. A range of thiol compounds related to 2-mercaptoethylamine can also diminish the lipoperoxidation reactions and stimulate the cholesterol 7α -hydroxylase enzyme (Scholan & Boyd, 1968). It has been found that GSH, together with another component in the liver supernatant thermostable fraction, can inhibit lipoperoxidation and stimulate the chole-

sterol 7α -hydroxylase system (Grimwade, Lawson & Boyd, 1971).

Attempts have been made to solubilize and resolve the cholesterol 7α -hydroxylase enzyme system by using various detergents coupled to gel filtration and ion-exchange chromatographic methods. The components of liver microsomal fraction have been separated into several flavoproteins, cytochrome *b*₅ and cytochrome *P*-450. Although certain combinations of these protein fractions can catalyse an oxidative attack on the sterol molecule, it has not been possible so far to reconstitute the physiological cholesterol 7α -hydroxylase enzyme complex. The cholesterol 7α -hydroxylase enzyme system is inducible by biliary drainage (Danielsson, Einarsson & Johansson, 1967), cholestyramine feeding (Boyd, Mitton, Simpson & Sulimovici, 1967) or other procedures that affect the feedback of bile salts on the liver, but is unaffected by phenobarbitone. The mechanism of the enzyme induction and control is under study.

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Functional Components of the Liver Microsomal Enzyme System Catalysing Fatty Acid, Hydrocarbon and Drug Hydroxylation

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Cytochrome *P*-450 is known to play a central role in the hydroxylation of a variety of substrates in

liver microsomal fraction. Fatty acids, alkanes, steroids, polycyclic carcinogens, aniline and a variety of drugs and other foreign compounds serve as substrates for this versatile catalyst. Although previous attempts to separate cytochrome *P*-450 from microsomal membranes yielded an altered haemoprotein called cytochrome *P*-420 (see Omura & Sato, 1962), more recent studies have led to the resolution of the enzyme system into three functional components: cytochrome *P*-450, NADPH-cytochrome *P*-450 reductase and a heat-stable lipid fraction (Lu & Coon, 1968). The reconstituted enzyme system was found to catalyse the hydroxylation of fatty acids and alkanes as well as the demethylation of a variety of drugs containing *N*- and *O*-methyl groups (Lu, Strobel & Coon, 1970).

The solubilized cytochrome *P*-450 remains in the supernatant fraction on centrifugation at 100 000*g*, but otherwise retains the properties of the membrane-bound form (Lu, Junk & Coon, 1969). Thus the reduced pigment gives the expected carbon monoxide difference spectrum, and the oxidized form gives a typical type I difference spectrum in the presence of laurate, hexobarbital or benzphetamine and a type II difference spectrum with aniline. The electron-paramagnetic-resonance spectrum is that of a low-spin ferric haemoprotein with *g* values of 1.92, 2.25 and 2.42, which correspond to the spectrum described for the microsome-bound pigment (Mason, Yamano, North, Hashimoto & Sakagishi, 1965). Of greater importance, the solubilized cytochrome *P*-450 retains catalytic activity towards a variety of substrates. The sedimentation coefficient, *s*_{20,w}, is about 12 S.

Our detergent-solubilized reductase preparation, which transfers electrons to cytochrome *P*-450 as well as to cytochrome *c*, differs from the lipase-solubilized NADPH-cytochrome *c* reductase described by Williams & Kamin (1962). The latter preparation, which was kindly furnished by Dr Kamin, is inactive towards cytochrome *P*-450 and therefore is not functional in the hydroxylation system. Possibly the lipase treatment alters the reductase so that it retains activity only towards cytochrome *c*, which is an artificial electron acceptor since it does not occur in liver microsomal fraction.

The active component in the lipid fraction has been identified as phosphatidylcholine (Strobel, Lu, Heidema & Coon, 1970). Synthetically prepared dioleoylglycerol-3-phosphorylcholine was fully active when substituted for the microsomal lipid, as judged by the rate of laurate, hexane, octane, ethylmorphine or benzphetamine hydroxylation in the presence of the other components of the enzyme system. Phosphatidylcholine neither causes spectral changes when added to the solubilized cytochrome *P*-450 nor alters the binding of

substrates to the cytochrome. Evidence has recently been obtained that the lipid plays a critical role in electron transfer from NADPH to cytochrome *P*-450. Thus, under anaerobic conditions and with carbon monoxide as the gas phase, a mixture of NADPH, cytochrome *P*-450 and the reductase gives the expected absorption maximum at 450 nm only when the lipid is also present. Since the reaction was too rapid to be measured in a conventional spectrophotometer, stopped-flow measurements were carried out. Biphasic kinetics was observed, with a fast phase having a first-order rate constant of about 100 min⁻¹. Under similar conditions the overall hydroxylation rate, calculated as mol of benzphetamine hydroxylated/mol of cytochrome *P*-450, is about 30 min⁻¹. These results indicate that the lipid-dependent rate of electron transfer is more than adequate to account for the turnover rate of cytochrome *P*-450 during hydroxylation.

The role of the lipid in cytochrome *P*-450 reduction will be discussed with emphasis on related studies on the stoichiometry and the mechanism of the hydroxylation reaction.

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Oxygenated Cytochrome *P*-450 as an Intermediate in Hydroxylation Reactions

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The transfer of reducing equivalents from reduced nicotinamide nucleotides to cytochrome *P*-450 enables it to function in a series of reduction and oxidation reactions resulting in the introduction of one atom of molecular oxygen into an organic molecule. The sequence of reactions associated with the cyclic alterations of cytochrome *P*-450 are proposed to be as follows. (a) The interaction of ferric cytochrome *P*-450 (Fe³⁺) with the substrate