PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **517th Meeting** *of the Society was held at the University of Edinburgh on Thursday and Friday,* 29 *and* 30 *July* 1971, *when the following papers were presented:*

SYMPOSIUM ON 'BIOLOGICAL HYDROXYLATION MECHANISMS'

Cholesterol Hydroxylation in the Adrenal Cortex and Liver

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Cholesterol is widely distributed in mammalian cells and body fluids. This sterol is oxidatively metabolized to steroid hormones in the adrenals, gonads and placenta; it is also oxidatively metabolized to bile salts in the liver.

The rate-limiting step in the degradation of cholesterol to adrenal steroid hormones involves oxidative attack at C-20 and C-22 with a subsequent desmolase reaction yielding pregnenolone, the overall reaction being termed cholesterol 'side-chain cleavage'. These reactions, in common with certain other hydroxylase reactions in the steroid-hormone-producing tissues, such as the 11β- and 18-hydroxylases, occur in the mitochondria (Halkerston, Eichhorn & Hechter, 1961). The reaction requires an external electron donor (NADPH) and oxygen, and has other characteristics to show that cytochrome *P*-450 is involved as a terminal oxidase in the reaction (Simpson & Boyd, 1967).

In this laboratory the adrenal mitochondrial cytochrome *P*-450 has been resolved into two components, one being associated with the side-chain-cleavage reaction and the remainder having a higher 11β-hydroxylase activity (Jefcoate, Hume & Boyd, 1970). This 'side-chain-cleavage' cytochrome *P*-450 is in a predominantly high-spin state, which is converted into a low-spin form by certain steroids such as pregnenolone (Whysner, Ramseyer & Harding, 1970). The resulting type II spectral change has been used to estimate the proportions of 'side-chain-cleavage' cytochrome *P*-450 in the high-spin state. The transformation from a high-spin state into a low-spin state is also effected by pH changes from 6 to 8; these spin-state changes are reversible (Jefcoate & Boyd, 1971).

A flow of electrons via the reductase system to 'side-chain-cleavage' cytochrome *P*-450 induces a rapid change from high-spin to low-spin states (Simpson, Jefcoate & Boyd, 1971). This spin-state change has been attributed to the rate of oxidation of cholesterol in the high-spin complex exceeding the low rate of cholesterol transport to the 'side-chain-cleavage' cytochrome *P*-450. This conclusion is supported by the kinetics of pregnenolone formation, oxygen consumption and the reversible nature of the spin-state changes.

Cholesterol side-chain-cleavage activity has been examined in intact adrenal mitochondria from rats that have been stressed with ether and rats treated with cycloheximide to inhibit adrenocorticotrophin action (Garren, Ney & Davis, 1965). In both cases addition of isocitrate as an electron donor to the mitochondria effected a rapid phase of pregnenolone formation from endogenous cholesterol lasting 3–5 min, followed by a slower phase. The effect of stress was to increase by two- to three-fold the initial rate of pregnenolone formation. These effects could also be seen when adrenal mitochondria from stressed rats were compared with adrenal mitochondria from quiescent rats.

The initial cholesterol content of adrenal mitochondria from stressed rats was appreciably lower than that of adrenal mitochondria from rats treated with cycloheximide. Further, more cholesterol was metabolized in the isolated adrenal mitochondria from the stressed rats. There was also a lower steady-state content of reduced nicotinamide nucleotides in adrenal mitochondria of stressed rats, consistent with a higher rate of utilization of NADPH reducing equivalents for the cholesterol side-chain-cleavage events.

The total cytochrome *P*-450 content and the 11β-hydroxylase activity in rat adrenal mitochondria were found to be unchanged by stress. However, the rat adrenal mitochondrial content of high-spin 'side-chain-cleavage' cytochrome *P*-450 (measured by the pregnenolone difference spectrum) varied according to the treatment of the rats, that for ether-stressed rats being greater than that for the quiescent rats, this in turn being greater than that for the cycloheximide-inhibited rats. The two- to three-fold increase in the content of the high-spin complex in the adrenal mitochondria from stressed rats as compared with cycloheximide-treated rats was maintained throughout the pH range 6–8, and is in agreement with the increase in initial rate of cholesterol side-chain-cleavage activity.

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-3β,5α,6β-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 cholesterol 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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Boyd, G. S., Mitton, J. R., Simpson, E. R. & Sulimovici, S. (1967). *Abstr. 7th int. Cong. Biochemistry, Tokyo*, vol. 3, p. 461.

Danielsson, H., Einarsson, K. & Johansson, G. (1967). *Eur. J. Biochem.* **2**, 44.

Garren, L. D., Ney, R. L. & Davis, W. W. (1965). *Proc. natn. Acad. Sci. U.S.A.* **53**, 1443.

Grimwade, A. M., Lawson, M. E. & Boyd, G. S. (1971). *Biochem. J.* **126**, 14P.

Halkerston, I. D. K., Eichhorn, J. & Hechter, O. (1961). *J. biol. Chem.* **236**, 374.

Jefcoate, C. R. & Boyd, G. S. (1971). *FEBS Lett.* **12**, 279.

Jefcoate, C. R., Hume, R. & Boyd, G. S. (1970). *FEBS Lett.* **9**, 41.

Lindstedt, S. (1957). *Acta chem. scand.* **11**, 417.

Mitton, J. R., Scholan, N. A. & Boyd, G. S. (1971). *Eur. J. Biochem.* (in the Press).

Scholan, N. A. & Boyd, G. S. (1968). *Hoppe-Seyler's Z. physiol. Chem.* **349**, 40.

Simpson, E. R. & Boyd, G. S. (1967). *Eur. J. Biochem.* **2**, 275.

Simpson, E. R., Jefcoate, C. R. & Boyd, G. S. (1971). *FEBS Lett.* (in the Press).

Whysner, J. A., Ramseyer, J. & Harding, B. W. (1970). *J. biol. Chem.* **245**, 5441.

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