sensitivity associated with the measurement of substrate disappearance. Kuntzman, Ikeda, Jacobson & Conney (1967) and Daly (1970) have respectively described the use of [2-¹⁴C]pento-barbitone and [4-³H]acetanilide for the determination of apparent Michaelis constants. By extensively modifying the method of Tagg, Yasuda, Tanabe & Mitoma (1967), we have found that [ureyl-¹⁴C]-tolbutamide can be used successfully to determine the apparent Michaelis constants (K_m and V_{max}) for the metabolism of tolbutamide by microsomal preparations from rat and human liver. Pento-barbitone and *N*-ethylmorphine have been found to inhibit [ureyl-¹⁴C]tolbutamide metabolism *in vitro*.

With microsomal preparations prepared as described by Darby, Newnes & Evans (1970) and Darby (1971), K_m values for the hydroxylation of tolbutamide by male human liver microsomal preparations have been found to range from 0.06 to 0.20 mM, compared with K_m values for male rat liver microsomal preparations of 0.50 ± 0.06 mM (mean $\pm$ s.e.m.; $n=8$). Values of V_{max} (nmol of tolbutamide hydroxylated/min per mg of microsomal protein) ranged from 0.15 to 0.29, those for male rats being 1.15 ± 0.16 (mean $\pm$ s.e.m.; $n=8$). Microsomal preparations from human liver frozen after being obtained *post mortem*, from human liver operation biopsy samples and from human liver microsomal pellets frozen for 1 week at -20°C gave results very similar to those from human liver processed immediately *post mortem*.

Pentobarbitone (1.0 mM) inhibits the metabolism *in vitro* of [ureyl-¹⁴C]tolbutamide by human liver microsomal preparations in a 'mixed' manner, i.e. neither fully competitive nor fully non-competitive. Ackermann (1970) reported a similar kind of inhibition with compound SKF-525A as the inhibitor of aminophenazon metabolism. Both pentobarbitone and *N*-ethylmorphine inhibit the metabolism of [ureyl-¹⁴C]tolbutamide by rat liver microsomal preparations in a manner consistent with the 'mixed' type of inhibition, although results indicating a fully competitive inhibition were occasionally found.

The Wellcome Foundation generously provided a grant for this work.

- Ackermann, E. (1970). *Biochem. Pharmac.* **19**, 1955.
 Daly, J. W. (1970). *Analyt. Biochem.* **33**, 286.
 Darby, F. J. (1971). *Biochem. J.* **122**, 41.
 Darby, F. J., Newnes, W. & Evans, D. A. P. (1970). *Biochem. Pharmac.* **19**, 1514.
 Kuntzman, R., Ikeda, M., Jacobson, M. & Conney, A. H. (1967). *J. Pharmac. exp. Ther.* **157**, 220.
 Tagg, J., Yasuda, D. M., Tanabe, M. & Mitoma, C. (1967). *Biochem. Pharmac.* **16**, 143.

Steroid Hydroperoxides as Intermediates in Steroid Biogenesis

By J. E. VAN LIER and G. KAN (introduced by P. J. O'BRIEN). (*Biochemistry Laboratories, Department of Nuclear Medicine and Radiobiology, Centre Hospitalier Universitaire, Sherbrooke, Quebec, Canada*)

As a logical extension of our work that showed that the cholesterol 20 α -hydroperoxide may be regarded as an intermediate in the cleavage of the side chain of cholesterol (van Lier & Smith, 1970a,b,c) we have been prompted to investigate the possible role of the cholesterol 26-hydroperoxide (van Lier & Kan, 1971), cholesterol 25-hydroperoxide and cholesterol 7 β -hydroperoxide as intermediates in the biodegradation of cholesterol and pregnenolone 17 α -hydroperoxide and progesterone 17 α -hydroperoxide as possible intermediates in the biogenesis of steroid hormones.

Incubation mixtures employed consisted of 10 mg of mitochondrial or microsomal protein derived from bovine adrenal cortex and 0.2 mg of steroid hydroperoxide dissolved in 0.02 ml of ethanol in a total volume of 5 ml of 0.1 M-phosphate buffer, pH 7.5, containing 0.1 mM-EDTA. Incubations were conducted for 15 min at 30°C ; steroid products were extracted with 20 ml of methylene chloride. T.l.c. analyses of the extracts revealed that although incubation with mitochondria of the 20 α -hydroperoxide gave both 20 α ,22 R - and 20 α ,21-dihydroxy-cholesterol incubation with microsomal preparations resulted only in the formation of an as yet unidentified polar steroid. The other steroid hydroperoxides on incubation were all converted into the corresponding alcohol as the only product with both enzyme preparations. With progesterone 17 α -hydroperoxide a small amount of 4-androstene-3,17-dione was found.

Our present findings suggest a different mechanism by which the side chain of cholesterol is cleaved from the mechanism by which other positions are hydroxylated.

This work was supported by the Medical Research Council of Canada by Grant MA-4051, and by the Conseil de la Recherche Médicale du Québec.

- van Lier, J. E. & Kan, G. (1971). *Proc. Can. Fedn Biol. Soc.* **14**, 367.
 van Lier, J. E. & Smith, L. L. (1970a). *Biochim. biophys. Acta*, **210**, 153.
 van Lier, J. E. & Smith, L. L. (1970b). *Biochim. biophys. Acta*, **218**, 320.
 van Lier, J. E. & Smith, L. L. (1970c). *Biochem. biophys. Res. Commun.* **40**, 3.