At commencement of an experiment the gas reservoir of 2.5 litres capacity is filled with $O_2 + CO_2$ (95:5). This provides adequate O_2 for liver tissue for several hours, since O_2 consumption is reported to be $2.2 \mu\text{mol/min per g}$ (Schimassek, 1963). On this assumption 25 ml of CO_2 will be produced in 1 h in an 8 g liver. Therefore in a closed-circuit system at the end of 4 h the gas composition will be $O_2 + CO_2$ (91:9). This increase in CO_2 will affect the bicarbonate-buffered medium, but the minor change in pH is still within the physiological range.

The apparatus described has been found to maintain a liver in a metabolically active condition as shown by gross appearance, bile flow rate, perfusate flow rate, O_2 consumption, perfusate K^+ concentration, bromsulphthalein clearance rate, liver water content and the linear utilization and production of metabolites.

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Evidence for an Atypical Binding of Haem in a Protozoan Cytochrome

By G. PETTIGREW (*Department of Biochemistry, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG, U.K.*) and T. MEYER. (*Department of Molecular Biology, University of Edinburgh, Edinburgh EH8 9AG, U.K.*)

A haemoprotein isolated and purified from *Crithidia oncopelti* was found to have a pyridine haemochrome peak at 553 nm (2.1 M-pyridine-0.08 M-sodium hydroxide). This lies between those of protohaem-containing proteins and cytochromes c.

The haem could not be removed by acid-acetone treatment, but was readily removed by mercuric chloride in acid (Sletten, Dus, Klerk & Kamen, 1968). The pyridine haemochrome from the isolated haemin had its maximum at 552 nm, between that of protohaemin IX and haematohaemin. The test of Fischer & Gibian (1941) for free vinyl functions gave a positive result for both the *Crithidia* haemin and the haemoprotein.

The R_F values in the chromatography system 1 of Chu & Chu (1955) of haemin from *Crithidia* cytochrome, haematohaemin and protohaemin IX were 0.70, 0.87 and 0.40 respectively.

The amino acid composition of the protein per iron atom was: Asp₁₀, Thr₄, Ser₄, Glu₉, Pro₉, Gly₁₄, Ala₁₃, Val₇, Met₂, Ile₄, Leu₇, Tyr₃, Phe₅, His₂, Lys₁₀, Arg₄, Me₃Lys₁₋₂.

Three separate determinations of cysteic acid after oxidation of the haem-free protein gave

consistent values of 1.0–1.1 mol/mol. After carb-oxy-methylation 0.85 mol of carboxymethyl-cysteine/mol was found.

In the initial stages of sequence work a tryptic peptide has been isolated whose partially determined amino acid sequence is:

Ala-Ala-Gln-Cys-His(Thr,Gly,Ala)Lys

The sequence of the haem-binding region of eukaryotic cytochromes c follows the pattern:

Cys-X-Y-Cys-His

T. Meyer & M. A. Cusanovich (in preparation) have commented on the anomalous pyridine haemochromes of *Euglena* cytochrome 558 and *C. fasciculata* cytochrome 555 (Hill & White, 1968; Kusel, Suriano & Weber, 1969), which both have the 553 nm peak. They suggest the possibility of a 2(4)-vinyl-4(2)-ethyl-substituted haem and the substitution of a non-binding amino acid for one of the two haem-binding cysteine residues.

The results presented here suggest that the *C. oncopelti* cytochrome is of the same type and call into question the invariance of the covalent haem-binding in c-type cytochromes and parts of the definition of a cytochrome c.

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The Metabolism *in vitro* of [ureyl-¹⁴C]-Tolbutamide by Microsomal Preparations from Rat and Human Liver

By F. J. DARBY and D. A. PRICE EVANS. (*Nuffield Unit of Medical Genetics, Department of Medicine, University of Liverpool, Liverpool L69 3BX, U.K.*)

The colorimetric methods commonly used to follow the rates of metabolism *in vitro* of various drugs are subject to error from high blanks, which arise from the presence in tissue fractions of interfering natural substances. For example, the assay of formaldehyde (commonly used to follow rates of metabolism of *N*-ethylmorphine, codeine, morphine, aminopyrine and many other drugs) is affected in this way. The use of radioactive drugs eliminates this problem, and also the problem of the lack of

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.

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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.

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