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

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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

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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