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A Mutant of *Candida utilis* with a Novel Terminal Oxidase

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During continuous culture of *Candida utilis* under conditions of copper limitation with glycerol as the carbon and energy source, as described by Light (1972), we observed that the culture spontaneously changed to give an approximately fourfold increase of cell concentration. At the same time the concentration of unused glycerol in the culture fell to almost zero. In view of the highly selective environment of a chemostat, we tentatively concluded that a mutant had established itself in the culture through its ability to by-pass a copper-requiring process that was previously rate-limiting for growth. Studies of the mutant cells and their isolated mitochondria showed that (i) the content of cytochrome *aa*₃ was decreased to less than 1% of that for the wild-type grown under conditions of glycerol limitation, (ii) respiration rates with natural substrates were unaffected, (iii) mitochondrial respiration was insensitive to cyanide or antimycin A but not to rotenone or piericidin A, (iv) the P/O ratio for isolated mitochondria was 1.0 for NAD-linked substrates and zero for others, (v) growth parameters (Clegg & Light, 1971) indicated a single site of mitochondrial ATP synthesis, rather than three, (vi) the normal antimycin A-sensitive respiratory chain from cytochrome *b* to cytochrome *c* was present but non-functional owing to the absence of cytochrome *c* oxidase, and (vii) the apparent *K_m* of the mitochondrial oxidase for O₂ was less than 10⁻⁶M. From these results we conclude that an alternative oxidase was present and communicated with the respiratory chain at about the level of cytochrome *b*.

Aerobic but non-growing incubation of the cells with or without copper salts did not restore cytochrome *aa*₃, in contrast with the behaviour of the copper-limited wild-type (Light, 1972). Growth of the mutant for 20 generations with added copper resulted in the return of cytochrome *aa*₃ but not the loss of the alternative oxidase. It seems likely that an alternative oxidase, previously shown to be inducible in *C. utilis* by Haddock & Garland (1971), had become constitutive. The copper-limited cells possessed giant mitochondria (Davison *et al.*, 1972).

We are grateful to the Medical Research Council for support, to the Science Research Council for a studentship (J. A. D.) and to Dr. P. Ann Light for advice on copper-limited growth.

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Proton Translocation by Rat Liver Mitochondria with Quinones as Terminal Acceptors

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In attempting to provide a sufficient number (three) of hydrogen carriers in the respiratory chain to meet the demands of the chemiosmotic hypothesis, Mitchell (1966) placed ubiquinone as the donor for cytochrome *c*. A consequence of this arrangement is that the predicted ratio of protons translocated per pair of electrons ($\rightarrow H^+/2e$) is 4.0 for the oxidation of NADH by ubiquinone. We have made direct measurements of this ratio by using ubiquinone analogues as acceptors for the oxidation of L-3-hydroxybutyrate by rat liver mitochondria, the underlying assumption being that the behaviour of the analogues of ubiquinone (duroquinone, ubiquinone-0, ubiquinone-1 and ubiquinone-2) is representative of that of endogenous ubiquinone (ubiquinone-10).

Measurements of the pH of mitochondrial suspensions were made essentially as described by Mitchell & Moyle (1967), except that the incubation medium included antimycin A and had a total volume of 1.0 ml. Parallel investigations of the rate of NADH oxidation by the ubiquinone analogues with sub-mitochondrial particles, and of the kinetics of oxidation of intramitochondrial NADH, showed that the ubiquinone analogues reacted rapidly at low concentration ($<5 \mu M$) with the respiratory chain. These analogues were therefore suitable for use as electron acceptors in pulse experiments of the type performed by Mitchell & Moyle (1967) with O₂.

With pulses of duroquinone, ubiquinone-0, ubiquinone-1 and ubiquinone-2 over the range 0.5–5.0 nmol of quinone/mg of mitochondrial protein, $\rightarrow H^+/2e$ ratios 1.0–1.5 were observed in the presence of antimycin and valinomycin. The lower values were obtained at the higher concentrations of quinone. These pH changes were abolished by

rotenone or carbonyl cyanide *m*-chlorophenylhydrazone. In the latter case a temporary alkalinization occurred, owing to NADH oxidation, with a $\Delta\text{pH}/2e$ value of -1.0 . Correction of the $\rightarrow\text{H}^+/2e$ values for the non-proton-translocating rotenone-insensitive pathway of quinone reduction by NADH gave calculated values for the proton-translocating rotenone-sensitive pathway of 1.7 – 2.0 . It is concluded that, if the respiratory chain is organized into three proton-translocating loops, then only one of these loops exists between NADH and ubiquinone. The identity of the postulated hydrogen carrier preceding cytochrome *c* remains unknown, unless it be one of the multiple forms of cytochrome *b*.

The authors thank the Medical Research Council of Canada (Fellowship for H. G. L.) and the Medical Research Council for support.

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Ornithine Decarboxylase Activity during Kidney Growth

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It has been suggested that increased rates of polyamine biosynthesis are associated with rapid tissue growth (Jänne, 1967; Russell & Snyder, 1968; Raina & Jänne, 1970; Snyder & Russell, 1970). The rise in ornithine decarboxylase activity occurring during liver regeneration is in fact one of the earliest and most dramatic responses to partial hepatectomy (Schrock *et al.*, 1970). We have therefore examined ornithine decarboxylase activity (measured by a modification of the $^{14}\text{CO}_2$ absorption method of Russell & Snyder, 1968) in rat kidney and tried to relate it to the hypertrophy that takes place in the remaining kidney after unilateral nephrectomy. Normal rat kidney has a variable content of ornithine decarboxylase about 10–100-fold higher than that of rat liver, spleen, pancreas, heart, brain or uterus. Prostate is the only other tissue with comparable activity. The enzyme activity in the kidney is equally high in male and female rats and is not significantly affected by orchidectomy, ovariectomy or adrenalectomy, or (in the male) by injection of testosterone (25 mg/kg) or oestradiol (2.5 mg/kg). Unilateral nephrectomy produces a sixfold increase in enzyme activity in the remaining kidney 4 h after the operation, but a comparable though slightly smaller increase can be produced by sham operation or by partial hepatectomy. Thereafter the activity decreases, until 12 h after the operation it is at the normal pre-operative value (in unilaterally nephrect-

omized rats) or below it (in sham-operated rats). This increase can be prevented if the animal is maintained under continual Nembutal anaesthesia throughout the 4 h post-operative period or is treated with metyrapone (125 mg/kg) (a drug that inhibits 11β -hydroxylation of steroids). If normal rats are injected with dexamethasone phosphate (0.25 mg/kg) (a synthetic glucocorticoid) their kidneys 4 h later show an increase in the enzyme comparable with that produced by unilateral nephrectomy. Deoxycorticosterone acetate, a mineralocorticoid, has no effect at doses up to 2.5 mg/kg body wt. It seems likely therefore that the increased enzyme activity in the remaining kidney after unilateral nephrectomy is partially a non-specific response to the stress of the operation.

W. T. M. is grateful to the Science Research Council for a studentship.

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Changes in the Pyrimidine Deoxynucleoside Triphosphate Pools of L5178Y Cells after their Exposure to Methotrexate and 5-Fluorouracil

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The pools of deoxynucleoside triphosphate in mouse L5178Y lymphoma cells in culture have been measured with a highly sensitive assay utilizing DNA polymerase (Solter & Handschumacher, 1969; Lindberg & Skoog, 1970). Contrary to the finding by Baumunk & Friedman (1971) in HeLa cells, we have observed a fall in the dTTP content of L5178Y cells after treatment with either methotrexate or 5-fluorouracil (at concentrations that inhibit the uptake of $[^3\text{H}]\text{dUMP}$ into DNA). This finding supports the hypothesis that methotrexate and 5-fluorouracil inhibit dTMP syntheses. An elevation in the concentration of the other pyrimidine deoxynucleoside triphosphate, dCTP, occurs subsequent to the fall in that of dTTP. No changes were seen in the concentrations of the purine deoxyribonucleoside triphosphates, dATP and dGTP. The extent of these changes in a heterogeneous population of cells was