aerobically on several fermentable sugars. Cells grown on glucose, galactose and maltose manifested a decreased respiratory capacity per cell mass (Q_{O_2}) while the original carbon source was being metabolized; however, once fermentation ceased (as monitored by ethanol production) the Q_{O_2} increased markedly. This decrease in respiration (i.e. repression) is proportional to the rate of fermentation (glucose > maltose > galactose). If these respiratory data are plotted semi-logarithmically as total respiration (μmol of O_2 /min per ml of culture) against time the three sets of data fall progressively on the same straight line. This unexpected result suggests that some respiration-limiting substance is being synthesized at an identical rate that is independent of the carbon source in all three cultures.

To exploit this latter observation yeast cells were grown aerobically on 2.7% galactose medium in the presence and absence of D(-)-chloramphenicol, an antibiotic that inhibits mitochondrial protein synthesis (Clark-Walker & Linnane, 1967). Chloramphenicol had no effect on the synthesis of hexokinase, alcohol dehydrogenase, malate dehydrogenase or isocitrate dehydrogenases while galactose was still present in the medium. The effect of this inhibitor on succinate dehydrogenase and pyruvate decarboxylase activities was gradual and cumulative, resulting in a 25% inhibition. In contrast, the synthesis of cytochrome *c* reductase and cytochrome *c* oxidase was immediately affected and curtailed by chloramphenicol. Strikingly the synthesis of these last two enzymes was exponential, insensitive to change of carbon source and paralleled by the total respiratory activity. The fermentative and tricarboxylic acid-cycle enzymes on the other hand were sensitive to change of metabolic carbon source (i.e. from galactose to ethanol) and demonstrated parabolic kinetics during synthesis. Genetic evidence from petite mutation (Sherman, 1965) suggests that mitochondrial DNA is responsible in part for the activities of cytochrome *c* oxidase and cytochrome *c* reductase, and the chloramphenicol data (Clark-Walker & Linnane, 1967) showed that these enzymes are initially synthesized in part at least in the mitochondrion. Thus the 'limiting metabolite' referred to above is most probably a protein that may be assembled from incomplete cytoplasmic protein by mitochondrial DNA, and the latter experiment indicates that the synthesis of this substance is not affected by the external environment of the cell and is insensitive to nuclear cytoplasmic control (cf. Ball & Tustanoff, 1968).

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Subcellular Fractionation of Anaerobically Grown *Saccharomyces carlsbergensis* by Zonal Centrifugation

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Sphaeroplasts were prepared under strictly anaerobic conditions from yeast cells grown anaerobically with 10% (w/v) glucose in the presence of Tween 80 and ergosterol. Cycloheximide (0.1mg/ml) and chloramphenicol (1mg/ml) were added to prevent any possibility of respiratory adaptation during the disruption and centrifugation procedures, which were identical with those described in the following communication (Cartledge, Howells & Lloyd, 1970).

The activities of cytochrome *c* oxidase and succinate-cytochrome *c* oxidoreductase in whole homogenates were not detectable (<0.5nmol/min per mg of protein), nor was that of catalase (<0.2nmol/min per mg of protein).

Equilibrium-density centrifugation in the B XIV zonal rotor yielded two populations of organelles with median buoyant densities of (A) 1.147 and (B) 1.21g/ml, which were detected by their absorption at 520nm. Both these populations contained antimycin A-sensitive NADH-cytochrome *c* oxidoreductase activity; both populations also showed NADPH-cytochrome *c* oxidoreductase. The specific activity of adenosine triphosphatase in population A was ten times that in B; oligomycin-sensitivity was evident in population A and not in population B.

The distribution of NADH- and NADPH-cytochrome *c* oxidoreductase and NADPH-ferricyanide oxidoreductase showed a complex distribution in the region of the gradient ρ 1.10, which indicated that, although a portion of all three activities is non-sedimentable, some of the activities are associated with slowly sedimenting particles. Acid phosphatase showed a sharp distribution in the non-sedimentable region and a broader sedimentable zone at median buoyant density 1.14.

Rate-zonal centrifugation confirmed that NADH- and NADPH-cytochrome *c* oxidoreductase and NADH- and NADPH-ferricyanide oxidoreductase as well as adenosine triphosphatase are associated with rapidly sedimenting particles. The percentage total enzyme units present in this population were as follows: protein, 4.5%; NADH-cytochrome *c* oxidoreductase, 25%; NADPH-cytochrome *c* oxidoreduc-

tase, 40%; NADH-ferricyanide oxidoreductase, 15%; NADPH-ferricyanide oxidoreductase, 27%. Acid phosphatase was 27% sedimentable.

Cartledge, T. G., Howells, L. & Lloyd, D. (1970). *Biochem. J.* **116**, 40 P.

Changes in Subcellular Distribution of Enzymes during Respiratory Adaptation of *Saccharomyces carlsbergensis*

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Anaerobically grown cultures of *Saccharomyces carlsbergensis* [containing glucose (10%, w/v), ergosterol and Tween 80] were allowed to adapt in the growth medium by sparging the fermenters with air. Adaptation was arrested by the addition of cycloheximide and chloramphenicol. Homogenates prepared anaerobically from sphaeroplasts of yeast cells after respiratory adaptation were analysed by isopycnic zonal centrifugation as described by Cartledge, Howells & Lloyd (1970a).

After adaptation for 10 min, both cytochrome *c* oxidase and catalase were detectable (4.6 and 4.9 nmol/min per mg of protein respectively). Cytochrome *c* oxidase was found mainly (66%) at ρ 1.225, whereas catalase was virtually all non-sedimentable (95%) although some enzyme was detected at ρ 1.19. The distribution of NADH- and NADPH-cytochrome *c* oxidoreductase and NADH- and NADPH-ferricyanide oxidoreductase was similar to that found in anaerobically grown non-adapted cells (Cartledge, Howells & Lloyd, 1970b). Thus the distribution of newly synthesized cytochrome *c* oxidase was similar to, but not identical with, that of those enzymes of particles of population B found in anaerobically grown cells.

After 30 min adaptation the specific activities of cytochrome *c* oxidase and catalase had increased (26.3 and 8.6 nmol/min per mg of protein respectively) and a marked change in distribution of cytochrome *c* oxidase, adenosine triphosphatase and NADH-cytochrome *c* oxidoreductase had occurred. Although NADH-cytochrome *c* oxidoreductase and cytochrome *c* oxidase were still detectable (6.3% and 8.3% of the total respectively) at ρ 1.21–1.22, both enzymes also showed a broad distribution up to ρ 1.15. Although a portion of both enzymes corresponded with the peak of soluble protein the heterogeneous density distribution suggested that both enzymes (and also adenosine triphosphatase) were associated with either slowly sedimenting particles or particles of $\rho < 1.15$.

After adaptation for 1 h the specific activities of cytochrome *c* oxidase and catalase were 37.8 and

8.9 nmol/min per mg of protein respectively; succinate-cytochrome *c* oxidoreductase was also now detectable (6.2 nmol/min per mg of protein). Again most of the cytochrome *c* oxidase (83.4%) and NADPH-cytochrome *c* oxidoreductase (92%) showed $\rho < 1.15$; three other peaks of activity of cytochrome *c* oxidase were present at ρ 1.13, 1.18 and 1.225. Succinate-cytochrome *c* oxidoreductase had a broad distribution in the soluble region and at ρ 1.18. The sedimentable oligomycin-sensitive adenosine triphosphatase showed a broad distribution between ρ 1.12 and ρ 1.20 (median ρ 1.16).

'Soluble' adenosine triphosphatase (50% of the total) was not oligomycin-sensitive.

Distribution of acid phosphatase did not alter during adaptation, although the specific activity of whole homogenates showed a threefold increase over the anaerobic level of activity after 1 h adaptation.

Cartledge, T. G., Howells, L. & Lloyd, D. (1970a). *Biochem. J.* **116**, 24 P.

Cartledge, T. G., Howells, L. & Lloyd, D. (1970b). *Biochem. J.* **116**, 40 P.

The Functional Characterization of Mitochondria from Rat Liver and Krebs II Ascites-Tumour-Cell Mitochondria

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Mitochondria synthesize proteins both in intact cells and after isolation (Work, Coote & Ashwell, 1968). Reports have appeared that mitochondria from animal cells contain a 55S ribosome-like particle (O'Brien & Kalf, 1967a,b). We find that washed sterile mitochondria from liver or Krebs cells can be pulse-labelled with [^{14}C]leucine or [^{35}S]methionine. After short pulses (up to 5 min) most of the radioactivity is found attached to a particle sedimenting with *Escherichia coli* ribosome fraction in sucrose gradients containing 10 mM-Mg $^{2+}$. After longer periods of incubation or after a 'chase' of unlabelled leucine there is transfer of radioactivity to membrane proteins.

The incorporation of [^{14}C]leucine into the radioactive peak is not inhibited by the presence of cycloheximide (100 $\mu\text{g/ml}$) or ribonuclease (20 $\mu\text{g/ml}$) during the pulse, but the presence of D-threo-chloramphenicol (100 $\mu\text{g/ml}$) will cause 80% inhibition of incorporation. The radioactivity attached to the ribosomal particles is presumed to be in the form of incomplete peptide chains, since exposure of mitochondria to puromycin for 30 s or longer under conditions favouring protein synthesis results in complete removal of radioactivity from the peak; moreover this effect of puromycin is partially blocked by chloramphenicol. The