out and the enzyme shows properties similar to that already obtained from several other plant tissues (McCrae, 1971), suggesting that the two pathways for malate oxidation may be a general property of higher-plant mitochondria. The operation of these two pathways may be of importance in controlling the redox equilibrium of NAD in the mitochondrial matrix and in the cytosol.

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The Role of Component C₁ in Cellulolytic Systems

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Over the years, biological attack on cellulose has been variously attributed to a single enzyme, CM-cellulase (Whitaker, 1953), or to a prime component called C₁ thought to produce shorter chains for a more conventional hydrolysis by CM-cellulase (Reese *et al.*, 1950). Other workers support a modified version of the last scheme in which the combined action of both CM-cellulase and component C₁ is believed to be essential, since no specific function has so far been assigned to component C₁ (Selby, 1968).

Recent evidence from this laboratory showed that component C₁ hydrolysed native cellulose to cellobiose in a solubilizing reaction promoted more effectively by cellobiase than by CM-cellulase (Halliwell & Riaz, 1971). Later preparations of component C₁ indicate that the stimulation of cellulolysis so achieved can be increased even further by up to 80%. Prolonging the incubation beyond the usual period of 7 days had little effect on cellulolysis by component C₁ alone. The additional presence of cellobiase, however, proved beneficial in enabling up to 70% solubilization of cotton to be attained in 21 days. The only products recognized chromatographically in the two systems were cellobiose and glucose respectively. The ability of cellobiase, which itself had no action on cellulose, to promote saccharification of the substrate by component C₁ suggested that this component was inhibited by its product cellobiose. Inhibition was competitive, being dependent on the relative concentrations of cellulose

and cellobiose and overcome completely by increasing the substrate concentration. CM-cellulase behaved similarly. Component C₁ exhibits maximum activity and stability at pH 5 and 37°C with K_m approx. 0.05 mg/ml. With cellulose and its derivatives component C₁ apparently acts as a cellobiohydrolase with a preference for the ends of chains and in the vicinity of terminal non-reducing groups.

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The Kinetics of Respiratory Degeneration and Regeneration During Drug Treatment of Two *petite*-Negative Yeasts

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We have studied the respiration rates of the *petite*-negative yeasts (Bulder, 1963) *Kluyveromyces (Saccharomyces) lactis* and *Saccharomyces fragilis* during treatment with drugs known to interfere selectively with mitochondrial macromolecular synthesis (Kellerman *et al.*, 1969), and similarly during respiratory adaptation after pre-growth of the cells in a drug-containing medium. Methods for the measurement of respiration rates and selection of suitable drug concentrations have been described (Luha *et al.*, 1971). The mitochondrial protein-synthesis inhibitors chloramphenicol and erythromycin and the mitochondrial nucleic acid-synthesis inhibitors acriflavine and ethidium bromide appear to have similar effects on the respiration rates of *K. lactis*. No significant inhibition of respiration rate occurs until between 60 and 90 min after addition of the drug. Thereafter the respiration rate falls off exponentially while the cells continue to grow in exponential phase.

Regeneration of respiratory activity has been studied in *K. lactis* after growth in the presence of chloramphenicol at 2 and 4 mg/ml and of ethidium bromide at 5 and 10 µg/ml. In contrast with the repression experiments there is a significant difference. De-repression from chloramphenicol proceeds much more rapidly, the Q_{O_2} (µl of O₂ taken up/h per mg dry wt.) rising from 10 to about 150 in 2½ h. A similar rise after ethidium bromide treatment takes between 6 and 7 h. Ethidium bromide exerts a similar effect on repression of the respiratory system of *S. fragilis*. De-repression from this drug is also observed in this system. In this case, however, a distinct lag

phase of about 2h occurs before the development of respiratory activity. During the next phase of development, lasting about 2h, the equation below suggests the effective mitochondrial mass is doubling every 20min.

The results have been described in terms of a simple model that introduces the concept of mitochondrial mean generation time. For particular conditions it has been possible to derive the equation:

$$\mu_m = \frac{\mu_q \cdot \mu_b}{\mu_q + \mu_b}$$

where μ represents mean generation time and the suffixes m, b and q relate respectively to mitochondrial mass, cell mass and Q_{O_2} .

The results suggest that the systems may offer a useful approach to the study of the synthesis of mitochondrial components, particularly after removal from the inhibitors of nucleic acid synthesis. Such an approach is not possible with *petite*-positive yeasts. The high rate of synthesis of respiratory components suggests it may be possible to label macromolecular species to a high specific activity.

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Metabolism of Pyridine by Soil Bacteria

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Although degradation of pyridine by micro-organisms has been known for over 50 years (Funchess, 1917) and the formation of ammonia from pyridine by an actinomycete has been reported (Moore, 1949), other investigators (Ensign & Rittenberg, 1963; Houghton, 1969; Stafford & Callely, 1970) have not identified other products or metabolic intermediates.

Enrichment techniques were used to isolate two bacteria, *Bacillus* strain 4 and *Nocardia* strain Z1, from soil, and these were found to grow on a mineral salts medium containing pyridine (0.1%, v/v) as carbon, nitrogen and energy source. Cell-free extracts of these organisms would not attack pyridine under

any conditions. Washed cells rapidly oxidized pyridine and released 70% of the pyridine N as ammonia. 2-Fluoropyridine was not oxidized, suggesting involvement of the C-2 position in an early metabolic step. None of the isomeric monohydroxy- or dihydroxy-pyridines was metabolized by washed cells or cell-free extracts.

Nocardia cells, oxidizing pyridine at pH 6.0 in the presence of 10mM-semicarbazide, showed a diminished O_2 uptake and formed a semicarbazone. After conversion into the 2,4-dinitrophenylhydrazone the product was identified by mixed melting point, mass spectrometry, paper chromatography, t.l.c. and catalytic hydrogenation to 5-aminovaleate as the glutaric semialdehyde derivative. Extracts contained high activities of a glutaric semialdehyde dehydrogenase, which were repressed 50-fold after growth on succinate. This enzyme, when partially purified, required thiol groups for activity, utilized both nicotinamide nucleotides, but was specific for glutaric semialdehyde. The product of the dehydrogenase was identified by paper chromatography and g.l.c. of the dimethyl ester as glutaric acid. The formation of a C_5 saturated intermediate indicates that pyridine metabolism in *Nocardia* strain Z1 occurs by a ring fission adjacent to the nitrogen atom together with ring reduction.

Cells of *Bacillus* strain 4 produced successively formamide and then formate when pyridine oxidation was inhibited by 0.2mM-cyanide. Extracts contained high activities of a formamide-specific amidase. The remainder of the carbon skeleton was tentatively identified as succinic semialdehyde, suggesting ring cleavage between C-2 and C-3 (Nyns *et al.*, 1969; Cain *et al.*, 1970). Succinic semialdehyde dehydrogenase activity was elevated 50-fold over that found in succinate-grown cells or in certain mutants that were unable to metabolize pyridine.

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