Greville, 1961; Gregolin, Singer, Kearney & Boeri, 1961). *In vivo*, it is probably linked directly to the respiratory chain. The yeast enzyme is known to contain FAD, but little was known about the chemical nature of the enzyme in animals.

This communication reports the properties of the enzyme purified approximately 2500-fold from rabbit kidney mitochondria. The purified preparation appears to be free from major protein contaminants, as shown by disc electrophoresis on polyacrylamide gel and the analytical ultracentrifuge. Its molecular weight has been estimated as approximately 102000 by gel filtration on Sephadex G-200. The enzyme is a flavoprotein. Flavine has been removed by treatment with acid ammonium sulphate solution, and activity was restored to the inactive apoenzyme on addition of FAD but not of FMN or riboflavine. On addition of substrates to the enzyme the flavine spectrum was partly bleached, with an increase in extinction between 500 and 650mμ, suggesting that a semiquinone form is produced. The spectrum of the oxidized enzyme was also slightly modified on addition of the inhibitors oxalate and oxaloacetate, indicating a change in the environment of the flavine.

The enzyme is assayed with D-lactate as substrate and the dye 2,6-dichlorophenol-indophenol or ferricyanide as acceptor. It has been confirmed that the purified enzyme uses other artificial acceptors including cytochrome *c*, phenazine methosulphate and methylene blue, but has no activity with NAD^+ or $NADP^+$. It has also been found that the enzyme reduces ubiquinone derivatives and oxygen, though the rate with the latter is only 10–15% of the rate with artificial acceptors.

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Gregolin, C., Singer, T. P., Kearney, E. B. & Boeri, E. (1961). *Ann. N.Y. Acad. Sci.* **94**, 780.
Tubbs, P. K. & Greville, G. D. (1961). *Biochem. J.* **81**, 104.

Spontaneous Activation of D-2-Hydroxyacid Dehydrogenase of Rabbit Kidney

By R. CAMMACK (*Department of Biochemistry, University of Cambridge*)

Tubbs & Greville (1959) showed that the activity of D-2-hydroxyacid dehydrogenase (EC 1.1.99.6) in extracts of acetone-dried powders of rabbit kidney mitochondria was very low at first, and increased up to 20-fold on standing. These results have been confirmed, using both acetone-dried powder extracts and ultrasonically treated mitochondria as sources of the soluble enzyme.

The activation effect, which can be considered as the conversion of an inactive form, B, into an active form, A, proved to be strongly dependent on pH. At 0° and pH 8·0 activation of extracts containing form B took several days, but at 0° and pH 6·5 it was completed in 30min. For a short time the activation at low pH was reversible; if the pH was raised to 8·0 the enzyme reverted partly to form B. If purified enzyme (form A) was added during this process, it too was partly converted to form B. However, after several days all the enzyme reverted to form A.

Thus it appears that the conversion of form A into form B needs some other, labile, factor present in the extract. On the other hand, the conversion of form B into form A does not appear to require other factors, as it occurred just as rapidly after extracts containing B had been put through various protein fractionation procedures. It was slow above about pH 7·5, so that in fresh extracts the conversion of form A into form B was dominant and the enzyme was mainly inactive; and it was rapid below this pH, so that the enzyme was mainly in the active form. Gradually the factor converting form A into form B lost its activity so that after several days the enzyme was fully active at all pH values.

These processes were unaffected by passage of the enzyme through a column of Sephadex G-25 to remove small molecules. The lack of activity of form B is not due to a contaminant that interferes with the assay, since the addition of form B does not decrease the rate in assays of purified form A. Forms A and B have the same molecular weight as estimated by gel filtration on a column of Sephadex G-200, which rules out monomer–polymer interconversions.

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Tubbs, P. K. & Greville, G. D. (1959). *Biochim. biophys. Acta*, **34**, 290.

Oxidation–Reduction Potentials of Cytochromes in Chloroplasts from Higher Plants

By D. S. BENDALL (*Department of Biochemistry, University of Cambridge*)

Chloroplasts from leaves of higher plants are known to contain three cytochrome components: cytochrome *f*, cytochrome-559 (these two can be reduced by ascorbate) and cytochrome b_6. The function of cytochromes in electron transport and phosphorylation is related to their characteristic oxidation–reduction potentials, yet the potentials of the above components as they occur in the chloroplast have never been determined.

Measurements on cytochrome *f* and cytochrome-559 in chloroplasts have been made with the use of a

sensitive split-beam spectrophotometer and of ferricyanide–ferrocyanide mixtures as redox buffers. For cytochrome-559 in pea chloroplasts a value of E'_0 +0·37v (25°) was obtained at both pH 6·5 and 7·5. The characteristic potential of potassium ferricyanide was assumed to be +0·43v. For cytochrome *f* a value for E'_0 of +0·35v was obtained with both pea chloroplasts (pH 7·5) and etiolated barley plastids (pH 6·5). This value for bound cytochrome *f* is in good agreement with that obtained by Davenport & Hill (1952) for the soluble component (E'_0 +0·365v).

The remarkably high potential of cytochrome-559 suggests its possible role as a donor to P-700. It is incompatible with the suggestion that its position in the electron transport chain is close to photosystem 2 (Bendall & Hill, 1968).

Work is in progress on the measurement of the potential of cytochrome b_6 in chloroplasts. Observations on the kinetics of reduction of cytochrome b_6 by dithionite have shown that it is a complex of at least two components with α-bands at 559 and 563mμ respectively. Neither of these components can be reduced by ascorbate. Thus chloroplasts contain two cytochromes with α-peaks at 559mμ, but these differ in potential by about 350mv.

I am indebted to the Science Research Council for purchase of the split-beam spectrophotometer.

Bendall, D. S. & Hill, R. (1968). *Annu. Rev. Plant Physiol.* (in the Press).
Davenport, H. E. & Hill, R. (1952). *Proc. Roy. Soc.* B, **139**, 327.

Reversed Electron Transport in Mitochondria from the Spadix of *Arum maculatum*

By W. D. Bonner and D. S. Bendall (*Department of Biochemistry, University of Cambridge*)

A dramatic feature of the flowering process in *Arum maculatum* is a sharp increase in the rate of respiration of the sterile portion of the spadix as the spathe opens. The rapid respiration, which is completely resistant to inhibition by mM-cyanide (James & Beevers, 1950), leads to an increase in temperature of the tissue of the order of 10° above ambient. Mitochondria isolated from the tissue are similarly resistant to cyanide, and contain, in addition to a normal cytochrome oxidase system, a cyanide-resistant oxidase that is as yet unidentified (Bendall & Hill, 1956; Bendall, 1958; D. S. Bendall, B. T. Storey & W. D. Bonner, unpublished work). It has generally been supposed that the cyanide-resistant pathway represents a non-phosphorylating bypass to the normal respiratory chain and therefore is of special importance in the generation of heat. We have been unable to detect phosphorylation during the oxidation of either succinate or malate.

Recent experiments with double-beam and split-beam spectrophotometers in which we studied the kinetics of reduction of cytochromes have shown that these mitochondria do retain some energy-linked functions. In the aerobic steady state with cyanide present cytochromes *a* and *c* were only partially reduced by succinate. Addition of uncoupler allowed complete reduction, which suggested that reversed electron transport occurs rather than inhibited forward transport. This idea was confirmed by the following type of experiment. Addition of ascorbate and *NNN'N'*-tetramethyl-*p*-phenylenediamine in the presence of cyanide caused complete reduction of cytochrome *a* and *c* components. Subsequent addition of succinate or malate caused partial oxidation of cytochromes *a* and *c* and partial reduction of cytochrome *b*, a state that persisted until the suspension went anaerobic. The reoxidation was prevented by uncoupling concentrations of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone or 2,4 - dinitrophenol added immediately before succinate. A high concentration of ADP was not inhibitory, and reoxidation could not be obtained with ATP replacing succinate or malate.

These observations suggest that the cyanide-resistant pathway is capable of driving electron transport against the thermochemical gradient from cytochrome *c*, although the mitochondria have lost the normal ability to interact with ADP and ATP.

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Bendall, D. S. (1958). *Biochem. J.* **70**, 381.
Bendall, D. S. & Hill, R. (1956). *New Phytol.* **55**, 206.
James, W. O. & Beevers, H. (1950). *New Phytol.* **49**, 353.

The Amino Acid Sequence of Cytochrome c_3 from *Desulfovibrio vulgaris* (N.C.I.B. 8303)

By R. P. Ambler (*Department of Molecular Biology, University of Edinburgh*)

Cytochrome c_3, discovered and purified by Postgate (1956), is found in *Desulfovibrio vulgaris* (Postgate & Campbell, 1966) and other species of sulphate-reducing bacteria. It is similar to the cytochromes *c* of higher organisms in spectrum and molecular weight, but has a very low redox potential (−0·205v) and has two haem groups per molecule. The amino acid composition of the protein has been determined (Coval, Horio & Kamen, 1961) and the terminal groups have been identified (Bruschi-Heriaud & Le Gall, 1967).

The amino acid sequence of the protein from