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 riboflavin. On addition of substrates to the enzyme the flavine spectrum was partly bleached, with an increase in extinction between 500 and 650 m μ , suggesting that a semi-quinone 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.

This work was supported by a grant from the Science Research Council.

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

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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 30 min. 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 inter-conversions.

This work was supported by a grant from the Science Research Council.

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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 *b₆*. 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