fication and characterization of the separated isoenzymes in analytical gels have, however, stimulated interest in the possibility of obtaining corresponding resolutions on a preparative scale. Several types of apparatus for preparative gel electrophoresis have been described (e.g. Jovin, Chrambach & Naughton, 1964; Gordon & Louis, 1967) and that used in the present study has certain features in common with them in making use of downward migration of protein zones in a vertical, annular gel column with a central condenser for the cooling water and a stream of elution buffer flowing across the lower face of the gel. The elution chamber is of a new pattern, however, which is designed to provide complete elution of protein zones entering the chamber in a small volume of buffer, thus preserving the high resolution of the separation process during collection. The performance of this apparatus has been assessed by the fractionation of alkaline phosphatases from several human tissues and of lactate dehydrogenases from rat tissues in 7·5% polyacrylamide gel in tris–borate buffer, pH 8·6.

For both types of enzyme recoveries of nearly 100% of the added activity were obtained. The relative proportions of the lactate dehydrogenase isoenzymes of rat heart extract determined by preparative electrophoresis were in close agreement with those derived by Wieland & Pfleiderer (1961) from ion-exchange chromatograms. By contrast, staining and scanning the zones after separation on analytical agar gel electrophoresis underestimated isoenzyme 1. Human small-intestinal and liver alkaline phosphatases were separated by preparative gel electrophoresis, and the heterogeneity within the zones of each enzyme that had previously been demonstrated on analytical gels was preserved in the fractions collected from the preparative column. The closely migrating bone and liver phosphatase isoenzymes could not be resolved completely, but the heterogeneity of a mixture of the two isoenzymes was demonstrated.

The advantages of the apparatus in studies of isoenzymes are thus in providing quantitative recoveries of enzyme fractions with a degree of resolution comparable with that of analytical gel electrophoresis.

The apparatus (patent applied for) is obtainable from Wright Scientific Ltd., 3 Lower Road, Kenley, Surrey.

Gordon, A. H. & Louis, L. N. (1967). *Analyt. Biochem.* **21**, 190.

Jovin, T., Chrambach, A. & Naughton, M. A. (1964). *Analyt. Biochem.* **9**, 351.

Wieland, T. & Pfleiderer, G. (1961). *Ann. N.Y. Acad. Sci.*, **94**, 691.

The Stoicheiometry of Electron Transfer by Bacterial and Plant Ferredoxins

By M. C. W. Evans, D. O. Hall, H. Bothe and F. R. Whatley. (*Department of Botany, King's College, University of London, 68, Half Moon Lane, London, S.E. 24*)

The number of electrons accepted by spinach ferredoxin on reduction by illuminated chloroplasts was determined by Whatley, Tagawa & Arnon (1963) and by Horio & San Pietro (1964) by measuring the stoicheiometry of the reoxidation of the ferredoxin by $NADP^+$. Both groups found that spinach ferredoxin accepted one electron/molecule on reduction. Sobel & Lovenberg (1966) measured the number of electrons accepted by bacterial ferredoxin from *Clostridium pasteurianum* by using the method of Whatley *et al.* (1963) and several other methods. They found that clostridial ferredoxin accepted two electrons/molecule on reduction. Tagawa & Arnon (1968) redetermined the number of electrons accepted by clostridial ferredoxin by using the stoicheiometry of ferredoxin reoxidation by $NADP^+$ or benzyl viologen, and obtained a value for one electron/molecule of ferredoxin.

In the course of our work on the physical properties of ferredoxins we have determined the number of electrons accepted by two bacterial ferredoxins and two plant ferredoxins. The bacterial ferredoxins were from the photosynthetic bacterium *Chromatium* and from the non-photosynthetic anaerobe *Clostridium welchii*. The plant ferredoxins were from spinach and from the blue-green alga *Anacystis nidulans*. By both the methods described by Tagawa & Arnon (1968), we find that both bacterial ferredoxins accept two electrons/molecule on reduction, and that spinach and *Anacystis* ferredoxins each accept one electron/molecule.

Horio, T. & San Pietro, A. (1964). *Proc. nat. Acad. Sci., Wash.*, **51**, 1226.

Sobel, B. E. & Lovenberg, W. (1966). *Biochemistry*, **5**, 6.

Tagawa, K. & Arnon, D. I. (1968). *Biochim. biophys. Acta*, **153**, 602.

Whatley, F. R., Tagawa, K. & Arnon, D. I. (1963). *Proc. nat. Acad. Sci., Wash.*, **49**, 266.

Flavoprotein Properties of D-2-Hydroxyacid Dehydrogenase Purified from Rabbit Kidney

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

D-2-Hydroxyacid dehydrogenase (EC 1.1.99.6) is a widely distributed enzyme which catalyses the oxidation of the D-stereoisomer of a number of 2-hydroxyacids to the corresponding keto-acids, using a variety of artificial acceptors (Tubbs &

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.

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

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.

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

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