On the reversibility of succinic dehydrogenase*

In 1937 FISCHER AND EYSENBACH[1] described an enzyme in yeast preparations which catalyzed the reduction of fumarate to succinate in the presence of suitable reduced dyes, such as leucomethylene violet and leucodiethylsafranin[2]. In the intervening years there has been considerable speculation on the possible identity of FISCHER's "fumaric hydrogenase" and succinic dehydrogenase, but, probably because of the use of unsuitable electron acceptor dyes, succinic dehydrogenase activity was not demonstrable in purified preparations of the FISCHER enzyme. More recently, "fumaric hydrogenase" activity was also observed in particulate[3] as well as soluble[4] preparations from heart muscle. The availability of a reliable assay and of essentially homogeneous preparations of succinic dehydrogenase from animal tissues[5] afforded an opportunity to test whether succinic dehydrogenase can reduce fumarate.

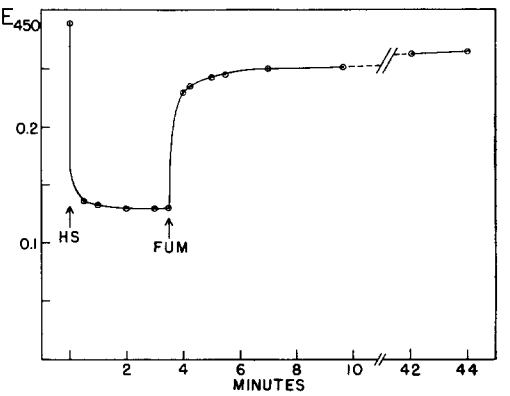

Fig. 1. The reoxidation of reduced succinic dehydrogenase by fumarate. Conditions: the main compartment of a Thunberg tube which fitted the Beckman spectrophotometer contained 9.4 mg succinic dehydrogenase (about 70% pure), 100 μM tris(hydroxymethyl) aminomethane buffer pH 8.3, and 150 μM phosphate, pH 7.6, in a volume of 3 ml. The side arm contained 100 μM neutral dried fumarate. Hydrosulfite (HS) was added in concentrated solution in very slight excess over that needed for reduction of the enzyme, then the tube was twice evacuated and filled with pure N_2. Fumarate (FUM) was added at the point indicated by the arrow. The E_{450} values shown are corrected for the slight dilution caused by the addition of hydrosulfite.

It has been shown[6] that the dehydrogenase isolated from beef heart is in the form of the *ferro*flavoprotein, and that the color due to the flavin component may be bleached by hydrosulfite. This appears to be a true reduction of the flavin inasmuch as aeration causes return of the original amber color. In the absence of excess hydrosulfite and under anaerobic conditions the leucoferroflavoprotein is stable, but if fumarate is tipped in, an immediate reoxidation occurs, with an eventual return of the normal absorption spectrum of the oxidized enzyme. The initial rapid recolorization (Fig. 1), as measured at 450 mμ, does not always reach completion: it is followed by a slow secondary reaction. Since the enzyme used in these experiments had lost some activity on standing, the slower secondary reaction may be due to a reaction between active succinic dehydrogenase and its inactive leuco form, an explanation offered by MORELL for the similar behavior of xanthine oxidase[7].

The reversibility of succinic dehydrogenase has also been demonstrated by the use of this enzyme as a catalyst for the oxidation of leucodiethylsafranin by fumarate. The reaction was followed in special Thunberg tubes which fitted a spectrophotometer. Under suitably anaerobic

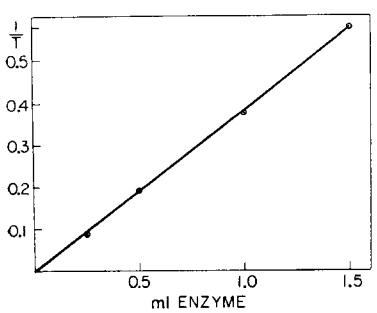

Fig. 2. Relation of enzyme concentration to fumaric hydrogenase activity. Abscissa, enzyme added (0.17 mg/ml solution); ordinate, reciprocal time (min^{-1}) for reoxidation of the leucodiethylsafranin. The latter is the time required for the transition from optical density = 0.05 (92% reduction) to optical density = 0.30 (54% reduction) in the Klett colorimeter, equipped with a green filter. Conditions: as in Fig. 1 except that phosphate was the only buffer present; leucodiethylsafranin concentration = $3.6 \cdot 10^{-5} M$. The tubes were evacuated prior to addition of just sufficient hydrosulfite to decolorize the dye. Temperature, 30°. The blank without enzyme was zero and the initial rate was linear in each case.

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conditions the reoxidation of the dye was negligible in the absence of substrate during the experimental period; with the substrate present the initial rate of recolorization was proportional to the concentration of the dehydrogenase (Fig. 2). At pH 7.6 and 30° in the presence of the dehydrogenase the rate of reduction of fumarate by leucodiethylsafranin was about 1/20th as fast as the oxidation of succinate by phenazine methosulfate. It must be emphasized that these rates have been determined with different dyes and thus may not reflect the true rates of the respective primary enzymic reactions. Like the oxidation of succinate, the reduction of fumarate is inhibited by malonate and *p*-chloromercuribenzoate at the expected concentrations. With the heart enzyme, neither the forward nor the reverse reaction is accelerated by the addition of FAD or of ferrous iron. Thus we are unable to confirm HARRISON's finding[3] that "fumaric hydrogenase" action in dialyzed Keilin-Hartree preparations requires activation by added iron. We have, however, observed a non-enzymic side reaction involving the accelerated reoxidation of the leuco dye by residual O_2 in the presence of Fe^{+2}, which was possibly not noted by HARRISON under his experimental conditions.

It appears clear from this data that the action of succinic dehydrogenase is readily reversible and thus there is no further reason to assume the existence of a separate fumaric hydrogenase, at least in animal tissues.

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BOOK REVIEWS

Protein Metabolism, par R. B. FISHER, Methuen & Co. Ltd., London, 1954, vi + 198 pp., 24 figs., 11s. 6d.

Il s'agit ici d'un grand sujet, traité dans un petit livre. Aussi l'auteur a-t-il choisi certains seulement des aspects du métabolisme des protéines chez les animaux supérieurs, pour y concentrer plus particulièrement son attention. Le thème autour duquel est construit l'ouvrage en question est que le métabolisme des protéines consiste essentiellement en la dépendance les uns des autres des métabolismes des acides aminés qui les constituent, plutôt qu'en les métabolismes individuels de chacun de ces acides aminés considérés indépendamment. Après un premier chapitre concernant la digestion et l'absorption des protéines, l'auteur donne un aperçu général du métabolisme de l'azote. Il étudie ensuite les réactions de désamination et de transamination, ainsi que celles qui conduisent à la formation de l'urée; il passe à la description des aspects généraux du métabolisme des acides aminés; puis consacre un chapitre à l'utilisation des isotopes dans les recherches concernant le métabolisme des protéines; après l'étude des actions hormonales sur ce métabolisme, il termine par une discussion de la valeur nutritive des protéines. Chaque chapitre est suivi d'une liste de références judicieusement choisies. Il est remarquable, combien, en un nombre restreint de pages, l'auteur a pu grouper autant de données expérimentales et d'aperçus personnels. Il est certain que ce petit volume sera lu avec profit par tous ceux qui désirent avoir, présenté d'une façon à la fois originale, suggestive et claire, l'essentiel des connaissances actuelles sur le métabolisme des protéines.

C. FROMAGEOT (Paris)