may be the result of a non-enzymic electron transfer from reduced DPN to TPN via BV. This contention is further supported by the observation that catalytic amounts of BV do not stimulate the rate of DPN reduction.

Further support for the contention that a pyridine nucleotide mediates a secondary reduction of these other H-acceptors is given by the effect of nucleotide pyrophosphatase (NPP)* on the reduction of MB by a crude hydrogenase preparation (Fig. 2). This crude enzyme preparation catalyzes a rapid MB reduction. The addition of NPP markedly inhibited the rate of dye reduction, and the inhibition was reversed by a catalytic amount of DPN.

LESTER PACKER
Department of Microbiology, Yale University, New Haven, Connecticut (U.S.A.) WOLF VISHNIAC

[1] LESTER PACKER AND WOLF VISHNIAC, *Bacteriol. Proc.*, May, (1954).
[2] LESTER PACKER AND WOLF VISHNIAC, *J. Bacteriol.*, in press.
[3] LESTER PACKER AND WOLF VISHNIAC, *Bacteriol. Proc.*, May, (1955), in press.
[4] D. E. GREEN, BRUCE MACKLER, R. REPASKE AND H. R. MAHLER, *Biochim. Biophys. Acta*, 15 (1954) 435.
[5] HARRY D. PECK AND HOWARD GEST, *ibid.*, 15 (1954) 587.

Received March 15th, 1955

* Kindly supplied by Dr. I. KRIMSKY and Dr. E. RACKER.

Isolation and properties of succinic dehydrogenase*

In previous communications the authors have described the solubilization, assay, and partial purification of succinic dehydrogenase[1-3]. The purpose of the present note is to outline the preparation of the enzyme in an essentially homogeneous state and to designate its salient properties. This enzyme is the primary dehydrogenase in the chain of proteins which link the oxidation of succinate to the reduction of cytochrome *c* in mitochondrial or purified particulate preparations[4]. Since this dehydrogenase catalyzes the one-step oxidation of succinate to fumarate in the presence of a suitable dye (phenazine methosulfate) and shares all the important characteristics usually associated with succinic dehydrogenase action, the demonstration that this reaction is catalyzed by a single, discrete protein molecule permits the identification of this protein as succinic dehydrogenase. A corollary of this conclusion is that the other components (protein or otherwise) which functionally link the oxidation of succinate to ferricyanide, cytochrome, methylene blue, *etc.* are members of an electron transport chain in the original particle.

The enzyme has been isolated from beef heart mitochondrial acetone powders[1,2]. Following extraction with 0.06 *M* tris(hydroxymethyl)aminomethane buffer, pH 8.9 and the removal of impurities by precipitation with protamine sulfate, the enzyme was precipitated by 0.5 saturated $(NH_4)_2SO_4$. After a brief dialysis the protamine and $(NH_4)_2SO_4$ precipitation steps were repeated; the enzyme was again dialyzed, and then subjected to two cycles of fractionation with calcium phosphate gel. The precipitate obtained between 0.3 and 0.40 saturated $(NH_4)_2SO_4$ was ultracentrifuged for 4 hours at 144,000 × *g* and the brown pellet was diluted, yielding a clear, amber solution of the dehydrogenase.

Examination in the analytical ultracentrifuge revealed the presence of a single component (Fig. 1). Electrophoretic analysis at pH 7.6 confirmed the above conclusion; only a trace of impurity was detectable. The sedimentation velocity (S_{20} = 6.5 S, determined at several protein concentrations) and a preliminary estimation of the diffusion constant indicate a molecular weight of the order of 110,000 to 140,000. The Q_{O_2} of the best preparations, isolated from fresh acetone powders, is 14,000 under the conditions of assay outlined elsewhere[5], and such preparations contain two atoms of Fe per mole of enzyme (1 atom Fe per 68,000 g protein). On the other hand a number of preparations have been obtained from somewhat older acetone powders with a Q_{O_2} of 7,500 and containing 1 atom

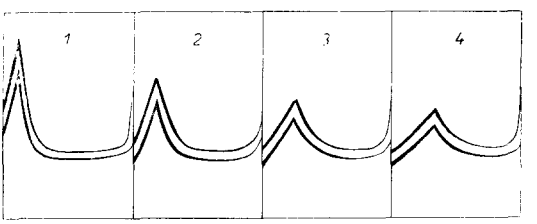

Fig. 1. Sedimentation pattern of succinic dehydrogenase in the ultracentrifuge. Protein concentration, 9.1 mg per ml; buffer, 0.1 *M* NaCl — 0.005 *M* phosphate, pH 7.6; temperature, 4.65°; speed, 59,771 r.p.m.; bar angle, 45°. Centrifugation was continued until the sedimenting peak reached the bottom of the cell. Each of the four exposures was spaced 16 minutes apart.

* Supported by a research grant from the National Heart Institute, U.S. Public Health Service.

Fe per 135,000 g protein. Such preparations appeared none the less homogeneous by physical criteria. The significance of these findings will be discussed in the following note[6].

The Q_{O_2} of 14,000, referred to above, does not necessarily reflect the maximal activity of the enzyme for two reasons. First, the dehydrogenase is quite unstable in the more purified stages and some inactivation may occur in the course of preparation. Second, with phenazine methosulfate as the carrier, the rate of reduction of the dye may well be the limiting factor. It is certainly possible that when the prosthetic group of the dehydrogenase donates electrons to hemin derivatives, as in particulate preparations, its turnover may be greater.

The behavior of the pure dehydrogenase with respect to the effect of pH, inhibitors, and its affinity constants are essentially the same as in mitochondrial preparations of heart. The K_M for succinate is $1.3 \cdot 10^{-3}$ M; the pH optimum is about 7.6, and the enzyme is highly sensitive to inhibition by all types of –SH reagents (mercaptide-forming, oxidizing, and alkylating agents). *p*-Chloromercuribenzoate appears to react almost stoichiometrically with the dehydrogenase and the resulting inhibition is completely reversible by glutathione. The substrate protects the enzyme from the effect of –SH combining substances. The inactive *p*-chloromercuribenzoate derivative of the enzyme may be fractionated by the procedure outlined above in the same way as the intact enzyme and the activity may be completely regenerated at any stage of purification by treatment with HCN which dissociates the mercurial compound. As would be expected from past knowledge of the particulate enzyme, malonate is a competitive inhibitor, whereas cyanide, BAL, and antimycin A are without effect on the enzyme. The effect of iron-complexing agents is described in the succeeding note[6].

One important distinction between the isolated, soluble dehydrogenase and particulate preparations of the "succinic dehydrogenase complex" is the unique requirement of the soluble enzyme for inorganic phosphate[5]. The possible reasons for this interesting behavior have been discussed elsewhere[5].

The isolated dehydrogenase is less stable than its particulate counterpart and appears to undergo several types of inactivation, which are at present not well understood. At —20° as much as 20% of the activity may be lost in 24 hours and prolonged dialysis at 0° results in variable loss of activity. Ethylenediaminetetraacetate, glutathione, and cysteine neither protect to a significant extent from inactivation on storage nor do they reverse the loss of activity. The *p*-chloromercuribenzoate derivative undergoes inactivation on storage to the same extent as the intact enzyme. Thus, this type of inactivation does not appear to be due to a loss of essential –SH groups.

THOMAS P. SINGER*
EDNA B. KEARNEY
NORMA ZASTROW

Edsel B. Ford Institute for Medical Research, Henry Ford Hospital, Detroit, Michigan (U.S.A.)

1 T. P. SINGER AND E. B. KEARNEY, *Biochim. Biophys. Acta*, 15 (1954) 151.
2 T. P. SINGER AND E. B. KEARNEY in W. D. MCELROY AND B. GLASS, *A Symposium on Amino Acid Metabolism*, The Johns Hopkins Press, Baltimore, 1955, p. 558.
3 T. P. SINGER AND E. B. KEARNEY, *Federation Proc.*, 14 (1955) in press.
4 D. E. GREEN, P. M. KOHOUT AND S. MII, *Biochim. Biophys. Acta*, 14 (1954) 295.
5 E. B. KEARNEY, T. P. SINGER AND N. ZASTROW, *Arch. Biochem. Biophys.*, in press.
6 E. B. KEARNEY AND T. P. SINGER, *Biochim. Biophys. Acta* (to be submitted).

Received March 19th, 1955

* Established Investigator of the American Heart Association.

On the density and the optics of silk fibroin

In their important investigation of the structure of silk fibroin MARSH, COREY AND PAULING[1] assume a density of 1.45 g/cm³ for a crystalline polypeptide consisting only of glycine, alanine and serine in the proportions 3:2:1. That figure is derived from the densities of crystals of glycine (1.607), D,L-alanine (1.40), D,L-serine (1.537) and some simple peptides of glycine and alanine (1.280–1.57).

Silk fibroin is a porous system[2] with such small submicroscopic interfibrillar spaces that imbibition with penetrating liquids is accompanied by considerable swelling. Therefore, it is difficult to verify experimentally the predicted density. But it can be derived from the molecular refraction, since the refractive indices of silk fibroin have been measured accurately in this laboratory[3,4]. The result of such a calculation includes the influence of the tyrosine component in silk fibroin.

The refractive indices of the highly anisotropic silk fibroin, considered as an optically uniaxial body, are at 25° C $(n_D)_\varepsilon = 1.5960$ and $(n_D)_\omega = 1.5454$. They yield a medium refractive index[5]