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.

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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]

$n_{iso} = (n_\varepsilon + 2n_\omega)$: 3 = 1.5623 for isotropic silk fibroin. This figure is used for the determination of the density ϱ according to the formula

$$\varrho = \frac{n_{iso}^2 - 1}{n_{iso}^2 + 2} \cdot \frac{M}{N}$$

where M is the molecular weight and N the molecular refraction of silk fibroin. The increments for the calculation of M are taken from the tables of LANDOLT-BÖRNSTEIN[6].

If the density is estimated in this way for glycine, alanine and serine residues in the proportions 3:2:1, the result of the calculation is $\varrho = 1.51_5$, which figure is appreciably higher than 1.45. But the other amino acids of silk fibroin, especially tyrosine and phenylalanine, must also be taken into consideration in such a determination, since they influence the value of n_{iso}. For their inclusion the residue percents of the amino acids indicated by TRISTRAM[7] have been used: glycine 44.7, alanine 25.7, serine 11.9, tyrosine 5.4, valine 1.6, aspartic acid 1.6, phenylalanine 1.1. The calculation yields the density $\varrho = 1.46_4$. This value differs by only 1% from the assumed density of 1.45, so that it may be near to the real density of the chain lattice of silk fibroin.

It is interesting to compare the optics of this polypeptide chain lattice with those of the native cellulose lattice[8]. The corresponding refractive indices and the light dispersion are listed in Table I.

TABLE I

COMPARISON OF NATIVE CELLULOSE AND CRYSTALLIZED SILK FIBROIN

	Birefringence			*Dispersion*		*Density*	
	$(n_D)_\varepsilon$	$(n_D)_\omega$	Δn_D	$(n_F - n_C)_\varepsilon$	$(n_F - n_C)_\omega$	$(n_D)_{iso}$	ϱ
Native cellulose (20° C)	1.600	1.531	0.069	0.0114	0.0088	1.554	1.55
Silk fibroin (25° C)	1.596	1.545	0.051	0.0130	0.0089	1.562	1.46_4

In both cases the birefringence Δn is many times that of quartz (0.009). The refractive index n_ε parallel to the chain axis is astonishingly high for aliphatic compounds and unexpectedly similar for the carbohydrate cellulose and the polypeptide silk fibroin. Perpendicular to that direction the refractive indices n_ω are much lower, but still considerably higher than in polar aliphatic liquids (butanol 1.40, glycerol 1.47). The aromatic amino acid residue tyrosine in silk fibroin has no influence on n_ε, but it is probably due to the fact that n_ω is significantly higher in silk than in cellulose. The light dispersion is again very similar for both compounds. In either case it is stronger parallel to the chain axis $(n_F - n_C)_\varepsilon$ than perpendicular to it $(n_F - n_C)_\omega$. All this anisotropy is caused by the different type of chemical bonds parallel and perpendicular to the axis of the chain lattice: the chains are formed by main valency bonds, whilst the lateral lattice bonds are partly hydrogen or even mere cohesion bonds. As a result the temperature coefficient of n_ε is much smaller than that of n_ω as shown for cellulose[8].

The density of crystalline silk fibroin is considerably lower than that of crystalline cellulose. In spite of this its mean refractivity n_{iso} is distinctly higher. These relations are probably caused by the presence of tyrosine and phenylalanine in silk fibroin which decrease the density of the chain lattice by their voluminous lateral groups and increase the refractivity by their aromatic ring.

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