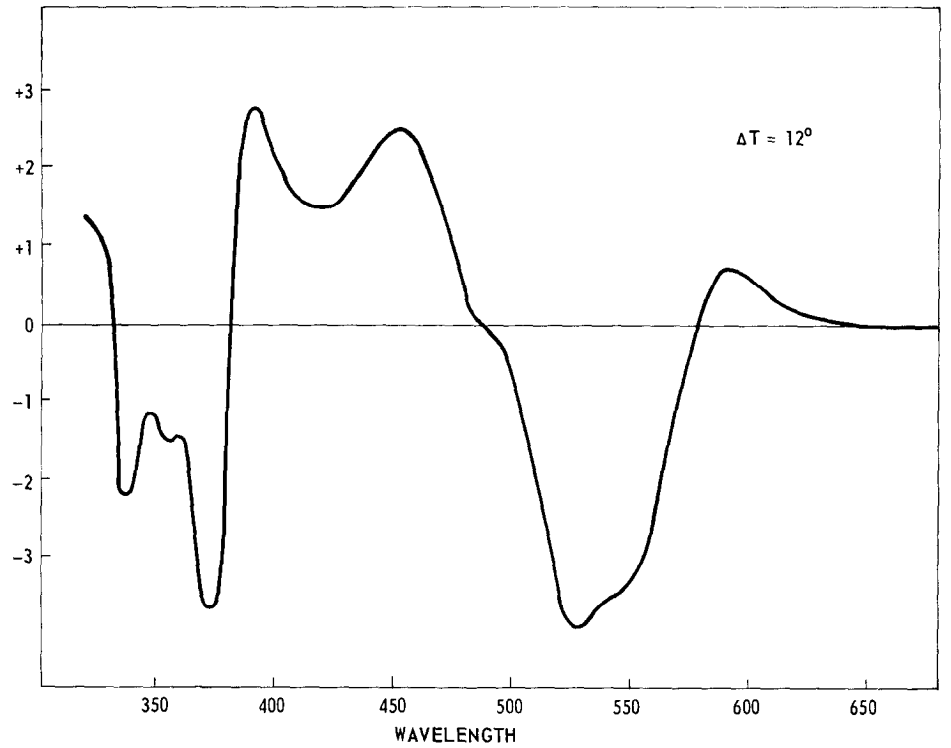

FIG. 5. The thermal difference spectrum of 8.12×10^{-5} M solutions of methyl cobalamin in dimethyl sulfoxide at 25° and 37°. The ordinates are the extinction coefficients for a 1 mM solution.

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Stimulation of Lipoyl Dehydrogenase Diaphorase Activity by Quinine and Optochin Hydrochlorides

Quinine has been reported to inhibit the oxidation of pyruvate by *Plasmodium gallinaceum* obtained from infected, quinine-treated chickens (1), and of α-ketoglutarate by mouse liver and kidney homogenates (2). Since lipoyl dehydrogenase (NADH : lipoamide oxidoreductase, EC 1.6.4.3) is known to be a component of the enzyme complexes functioning in the oxidative decarboxylation of these keto acids (3–6), it was of interest to determine the effect of quinine on this enzyme. This communication reports that quinine and its antipneumococcal analog, optochin, cause a stimulation of the diaphorase activities of pig heart muscle and *Escherichia coli* lipoyl dehydrogenases. With optochin, this effect is associated with the destabilization of the flavin semiquinone form of

the enzyme, resulting in its conversion to the fully reduced state.

Purified lipoyl dehydrogenase from *E. coli* (7) and from pig heart muscle (8) was kindly supplied by Drs. Charles Williams, Jr. and Vincent Massey, respectively, of the Department of Biochemistry, University of Michigan. Both enzymes were assayed for diaphorase activity, with reduced nicotinamide adenine dinucleotide (NADH) as the electron donor and 2,6-dichloroindophenol (DCI) and ferricyanide as the acceptors, as described by Massey (8).

The greatest concentrations of optochin and quinine (8.7×10^{-3} M and 9.6×10^{-3} M, respectively) produced an approximate sixfold and threefold increase, respectively, of the DCI reductase activity of the heart enzyme, but had no effect on its ferricyanide reductase activity (Table I). The same concentrations of drugs produced an approximate fourfold increase in the ferricyanide reductase activity of the *E. coli* enzyme. In addition, concentrations of optochin and quinine (4.3×10^{-3} M and 4.8×10^{-3} M, respectively) which caused only a threefold increase in the heart enzyme DCI reductase activity produced a fivefold increase in the DCI reductase activity of the *E. coli* enzyme. The reason for the difference in sensitivity of the two enzymes to optochin and quinine is not known at this time. Williams has found (personal communication) that *E. coli* lipoyl dehydrogenase is more sensitive to stimulation of its DCI and ferricyanide reductase activities by arsenite than is the pig heart enzyme.

Anaerobic spectral experiments were done (Figs. 1-3) with the apparatus and technique described by Massey *et al.* (9), and absorption spectra were recorded with a Cary model 14 spectrophotometer. They showed that a known stimulatory concentration (5.2×10^{-3} M) of optochin HCl had no effect on the anaerobic absorption spectrum of oxidized heart lipoyl dehydrogenase. However, reduction of the enzyme with either NADH or reduced nicotinamide adenine dinucleotide phosphate (NADPH), in the presence of optochin, resulted in the formation of an unstable flavin semiquinone and, subsequently, fully reduced enzyme. With NADH this is characterized by the reduction in the 530 mμ absorption band and the appearance of a broad absorption band at 720 mμ (10) and, with NADPH, by the loss of the flavin semiquinone absorption band at 530 mμ (11).

Since mercurials and Cu^{2+} are known (12-14) to stimulate the diaphorase activity of lipoyl dehydrogenase, while at the same time inhibiting its dihydrolipoate activity (the physiological function), the observation that quinine stimulates the diaphorase activity of lipoyl dehydrogenase does

TABLE I

STIMULATORY EFFECT OF OPTOCHIN AND QUININE ON THE DIAPHORASE ACTIVITY OF PURIFIED LIPOYL DEHYDROGENASE FROM PIG HEART MUSCLE AND *Escherichia coli*[a]

Drug (conc. × 10^{-3}M)	Δ Absorbance/minute			
	Pig heart enzyme		*E. coli* enzyme	
	DCI system	Ferricyanide system	DCI system	Ferricyanide system
Optochin[b]				
0	0.09	0.13	0.04	0.09
1.1	0.17	n.d.[c]	0.09	0.12
2.2	0.23	n.d.[c]	0.16	0.16
4.3	0.30	0.12	0.21	0.25
8.7	0.56	0.11	n.d.[c]	0.32
Quinine[b]				
0	0.09	0.10	0.04	0.09
1.2	0.12	n.d.[c]	0.07	0.11
2.4	0.17	n.d.[c]	0.13	0.23
4.8	0.23	0.10	0.18	0.33
9.6	0.31	0.10	n.d.[c]	0.37

[a] All assays were done in a Bausch and Lomb Spectronic 20 adjusted to 0.40 absorbance, at the desired wavelength (600 mμ for DCI system and 420 mμ for ferricyanide system), with an electron acceptor blank. The desired amount of drug, contained in 1 ml of glass-distilled water, was added to the reaction mixture, the reaction was started approximately 15–30 seconds later by the addition of NADH (similar results were obtained after 5 minutes exposure to the drugs), and absorbance readings were taken every 15 seconds.

[b] As the hydrochloride.

[c] Not done.

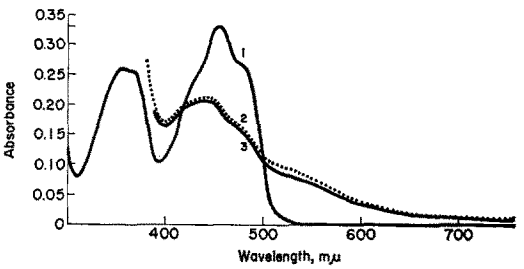

FIG. 1. NADH reduction of pig heart muscle lipoyl dehydrogenase. The experiment was carried out under anaerobic conditions, at 25°, in a volume of 2.55 ml containing 180 μmoles phosphate (pH 7.6), 3 μmoles EDTA, 0.074 μmole lipoyl dehydrogenase, and 0.5 μmole NADH. Curve 1, oxidized enzyme; Curve 2, 3 minutes after addition of NADH; Curve 3, 210 minutes after NADH.

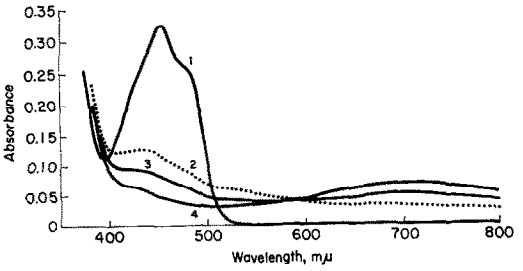

FIG. 2. NADH reduction of pig heart muscle lipoyl dehydrogenase in the presence of optochin HCl. The experiment was carried out under anaerobic conditions, at 25°, in a volume of 2.55 ml containing 180 μmoles phosphate (pH 7.6), 3 μmoles EDTA, 0.074 μmole lipoyl dehydrogenase, 13 μmoles optochin HCl, and 0.5 μmole NADH. Curve 1, oxidized enzyme + optochin HCl; Curve 2, 3 minutes after addition of NADH; Curve 3, 3, 28 minutes after NADH; Curve 4, 171 minutes after NADH.

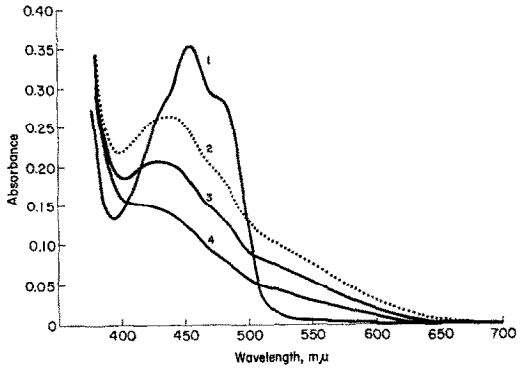

FIG. 3. NADPH reduction of pig heart muscle lipoyl dehydrogenase in the presence of optochin HCl. The experiment was carried out under anaerobic conditions, at 25°, in a volume of 2.55 ml containing 180 μmoles phosphate (pH 7.6), 3 μmoles EDTA, 0.079 μmole lipoyl dehydrogenase, 13 μmoles optochin HCl, and 0.5 μmole NADPH. Curve 1, oxidized enzyme + optochin HCl; Curve 2, 5 minutes after addition of NADPH; Curve 3, 47 minutes after NADPH; Curve 4, 468 minutes after NADPH.

not contradict the idea that it inhibits the oxidative decarboxylation of pyruvate and α-ketoglutarate by inhibiting lipoyl dehydrogenase. Unfortunately, I was unable to determine the effect of quinine and optochin on the enzyme's dihydrolipoate activity because of the strong interfering absorbance by the drugs at the assay wave length.

The catalytic oxidation by Cu^{2+} (15) and the reaction with mercurials (12) of catalytically important sulfhydryl groups is known to result in the formation of an unstable flavin semiquinone and, subsequently, fully reduced form of the enzyme, and the stimulation of diaphorase activity. Whether optochin and quinine exert their effects on this enzyme by a similar mechanism (i.e., reaction with sulfhydryl groups) remains to be determined. Datta and Basu (2) have reported, however, that quinine inhibited the oxidation of α-ketoglutarate in competition with arsenite and that the quinine inhibition was partially reversed by hydrogen sulfide water and 2,3-dimercaptopropanol (BAL).

Whatever the mechanism of the effect, the results are consistent with all previous results that conditions which destabilize the semiquinoid enzyme (arsenite, Cu^{2+} incubation, mercaptide formation) result in enhanced DCI reductase activity of lipoyl dehydrogenase.

Acknowledgments. I thank Dr. Vincent Massey for many helpful discussions, for his gift of purified pig heart muscle lipoyl dehydrogenase, and for determining the anaerobic absorption spectra. I am also grateful to Dr. Charles Williams, Jr. for his generous gift of purified *E. coli* lipoyl dehydrogenase.

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A Method for the Investigation of the Fine Structure of Amylopectin

It is now generally accepted that amylopectin is a multiple-branched molecule (1, 2). The evidence for this has come from enzymic studies (3, 4), and it is proposed here to describe an extension of the enzymic method that permits the further examination of the fine structure of amylopectin in far greater detail than previously.

The method is based on an examination of the distribution of the lengths of the unit chains of 1 → 4-bonded α-glucose units which, joined through 1→6 bonds, comprise the total structure. The most ordered form of multiple branching is regular rebranching, as shown in Fig. 1. In such a molecule it is possible to derive the form that the chain-length distribution should take as follows. The interval at which branching occurs is taken as 1. The number of chains of length 1 (A chains) is assigned the value x, and the basis of the structure is then that the number of chains of any length y is $x/2^{(y-1)}$. The total number of chains is therefore $\sum_{y=\infty}^{y=1} x/2^{(y-1)}$ and the total number of glucose units is $\sum_{y=\infty}^{y=1} xy/2^{(y-1)}$. These are convergent series which sum to $2x$ and $4x$, respectively. The average chain length is 2 (i.e., $4x/2x$). The only unbranched chains are those of average length 1, and the average chain length of the B chains (equal in number to the A chains) is 3. A convenient distinction can be made between the A chains, which form a homogeneous group of average length 1, and the B chains, which, although averaging 3 in length, would follow a distribution in number of the type $x/2^{(y-1)}$.

An experimental investigation of the distribution of the lengths of the unit chains requires methods for (*i*) the fragmentation of the polysaccharide into its constituent unit chains, (*ii*) the fractionation of the chains, and (*iii*) the determination of their average chain lengths. Preliminary studies along these lines have been carried out with waxy-maize, rice, potato, and maize starches. The scission of the branch linkages was

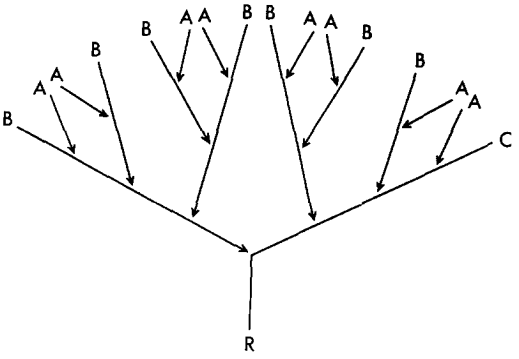

FIG. 1. Amylopectin depicted as a regularly rebranched structure. (—) Chain of 1→4-linked α-glucose units; (→), branch point; (R), reducing chain end. For the definition of A, B, and C chains, see text and Ref. 3.

effected with purified pullulanase (5), an extracellular bacterial enzyme specific for the hydrolysis of α-1→6-glucosidic linkages in amylaceous polysaccharides, to the extent that with amylopectin there is total or near-total debranching (6). Fractionation of the debranched polysaccharides was performed on Sephadex G-50 columns. Total polysaccharide in the column fractions was determined by hydrolysis with fungal amyloglucosidase as by Lee and Whelan (7). A convenient method for the determination of chain length was the measurement of the wavelength of peak absorption of the iodine-stained fractions, as by Bailey and Whelan (8). These authors demonstrated a linear relation between the wavelength of peak absorption and chain length for amylose-type chains of DP 20–60.

The elution pattern for waxy-maize starch is shown in Fig. 2A. The first peak from the left represents material excluded by the gel. The second, central, peak falls within the fractionation range of the gel, and the third peak represents material of low molecular weight (DP < 20). Similar patterns were obtained for the debranched rice, maize, and potato starches. The presence of 20–25% of linear amylose component in the last three starches was reflected in the greater amount of material in the first peak (e.g., Fig. 2B, maize starch).

The third peak can be accounted for in terms of a regularly rebranched structure, for which there would be at least 25% by weight of low-molecular weight material (A chains) of fairly narrow chain-length distribution (average length 12 units for an amylopectin of overall average length 24). However, the feature of most interest in the elution curves for the starches is the central peak. This is seen more clearly in Fig. 3 (curves A–D),