The dehydrogenase solubilized from *Claviceps* shows a kinetic behavior similar to that from heart particles. Fumarate, malonate and pyrophosphate competitively inhibits the oxidation of succinate with inhibition constants of $9.3 \cdot 10^{-4}$ *M*, $3.0 \cdot 10^{-5}$ *M* and $4.2 \cdot 10^{-4}$ *M*, respectively (Fig. 1). *o*-Phenathrolin partially inhibits the oxidation in an apparently non-competitive manner whereas α,α'-dipyridyl even at $3 \cdot 10^{-3}$ *M* does not show inhibition. The Michaelis constant for succinate is $3 \cdot 10^{-3}$ *M*, which is somewhat higher than that for the heart enzyme but lower than that for the *Micrococcus* succinic dehydrogenase ($5.3 \cdot 10^{-3}$ *M*)[5].

Cultivation of *C. purpurea* was described previously[6]. The adductor muscle was dissected from mussel which was collected from the bays on the Oregon Coast.

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Nature of the two forms of malic dehydrogenase from rat liver

Malic dehydrogenase activity may be found in both the mitochondrial and supernatant fractions obtained by differential centrifugation of rat-liver homogenates[1,2]. Investigation of the nature of the two forms was pertinent to the purification studies of MDH reported earlier from this laboratory[3,4]. The chromatographic and electrophoretic behavior of the two forms (obtained by procedure C, below) is in general agreement with other recent studies[2,5]. The two preparative procedures A and B, outlined below, also yield fractions of MDH which have chromatographic and electrophoretic characteristics similar to either the "mitochondrial" or the "supernatant" forms. However, the experiments to be described in this communication demonstrate that the "supernatant" form can be converted to the "mitochondrial" one on treatment with *n*-butanol.

The three procedures are as follows. *Procedure A*.[3] 1, Homogenization of rat liver with aq. EDTA-NaCl; 2, heat treatment; 3, alcohol fractionation; 4, chromatography on Amberlite XE-64, which yields two fractions. About 30–40 % of the enzyme is not retained and passes through the column along with a large amount of colored proteins

Abbreviations: MDH, malic dehydrogenase; DEAE-, diethylaminoethyl-; EDTA, ethylenediaminetetraacetic acid.

("red peak" MDH); the second fraction is retained and eluted by an ionic-strength gradient. 5, The fraction eluted from the resin in step 4 is purified by starch-paste electrophoresis. *Procedure B.*[4] 1, Homogenization of rat liver with *n*-butanol–aq. EDTA-NaCl; 2, heat treatment 3, $(NH_4)_2SO_4$ fractionation; 4, chromatography on Amberlite XE-64; 5, chromatography on DEAE-cellulose. *Procedure C.* Rat liver was homogenized in 0.25 *M* sucrose and centrifuged essentially according to SCHNEIDER[6]. The 700–5,000 × *g* (10 min) precipitate and the 20,000 × *g* (60 min) supernatant were designated as the "mitochondrial" and "supernatant" fractions, respectively.

TABLE I

CHROMATOGRAPHIC AND ELECTROPHORETIC BEHAVIOR OF MDH FRACTIONS

Chromatography on Amberlite XE-64 was carried out by gradient, 0.02 *M* → 0.1 *M* sodium citrate, pH 6.2–6.3. Starch-paste electrophoresis was performed in 0.05 *M* sodium citrate, pH 5.4, for about 20 h at 300 V, 4°.

*Fraction of MDH**	*Amberlite XE-64*		*Electrophoresis*	
	% bound	*% not bound*	*% at cathode*	*% at anode*
I. Procedure A				
1. MDH from step 3	70	30	70	30
2. MDH from step 4 (bound to resin)	—	—	100	0
3. MDH from step 4 ("red peak")	0	100	0	100
4. "red peak" MDH, butanol-treated	99	1	—	—
II. Procedure B				
5. MDH from step 3	98	2	70	30
6. MDH from step 4 (bound to resin)			98	2
7. Pure MDH from step 5**			100***	
III. Procedure C				
8. "Mitochondrial" fraction	99	1	98	2
9. "Supernatant" fraction	0	100	10	90
10. "Supernatant", butanol-treated	98	2	40	60
11. "Supernatant", butanol-treated and eluted from Amberlite XE-64			90	10

* See text for description of fractions.
** This fraction is unretarded by DEAE-cellulose.
*** Moving-boundary electrophoresis.

As shown in Table I, the chromatographic and electrophoretic properties of the "red peak" MDH (procedure A) are identical to the "supernatant" form (procedure C). Rechromatographing of the red peak MDH on Amberlite XE-64 several times is without effect. However, if the same fraction is stirred with *n*-butanol for 15 min, dialyzed and then applied to the resin, it is bound and purified over 6-fold. The supernatant MDH (procedure C) also binds to the resin after treatment with *n*-butanol.

In contrast to procedures A and C, every step of procedure B yields over 98 % of the enzyme as a single fraction. That one of the forms is not lost through inactivation during purification by procedure B is supported by the following: 1, the initial extraction step yields at least as much total enzyme as does procedure C, and 20–30 % more than does procedure A; 2, over 95 % of MDH is recovered in every step of procedure B; 3, when the enzyme obtained from step 3 (procedure B) as a single fraction is subjected to starch electrophoresis, it separates into two fractions, one of

which moves toward the anode (Table I). Thus, a fraction resembling electrophoretically the supernatant form is still present up to this step. However, if the enzyme from step 3 (procedure B) is chromatographed on Amberlite XE-64, 98 % of the MDH appears as a single peak. The total recovery of MDH is again over 90 %. When eluted from the resin, the enzyme is shown to be electrophoretically homogeneous. Thus, both treatment with butanol and chromatography on Amberlite XE-64 are necessary for the complete conversion of the enzyme to a single form.

The conversion of the "supernatant" form (procedure C) follows the same pattern. Electrophoresis of the butanol-treated "supernatant" shows that 30–40 % has been converted to the "mitochondrial" form. However, the butanol-treated "supernatant" binds to Amberlite XE-64, and the eluted enzyme moves toward the cathode (Table I).

The well known ability of butanol to break up lipid complexes suggests a possible explanation of the results. The "supernatant" form may be a lipoprotein complex of MDH. Treatment with butanol dissociates part of the complex. The remaining complex may be sensitized enough so that the resin can now resolve it completely.

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The isolation and structure of cerebrosides from wheat flour

Our interest in the chemistry and biochemistry of plant lipids led us to a study of the benzene-extractable lipids of wheat flour[1], from which we have now been able to isolate and characterize a mixture of cerebrosides.

Unbleached wheat flour was extracted with benzene. Addition of acetone caused precipitation of an insoluble fraction. The benzene-acetone-soluble material was distributed between *n*-heptane and methanol and the methanol-soluble lipids were purified using a silicic acid column. The fraction eluted with chloroform–methanol (94:6) was rich in cerebrosides, as established by color tests, elementary analyses and infrared spectrum.

Acid hydrolysis of this material and extraction of the basic components by the method of CARTER *et al.*[2] gave a mixture of long-chain bases, which were purified by