Sirs:

The mechanism of CO₂ fixation in pigeon liver extracts has remained rather obscure. We have now obtained from pigeon liver an enzyme preparation that, in the presence of manganese ions, catalyzes the reversible reaction,

\[ l\text{-Malic acid} + \text{TPN}_\text{ox} \rightleftharpoons \text{pyruvic acid} + \text{CO}_2 + \text{TPN}_\text{red} \]  

(1)

As is shown in Fig. 1, Reaction 1 can be followed spectrophotometrically in either direction at the wave-length 340 m\(\mu\).

![Spectrophotometric course of Reaction 1](image)

Fig. 1. Spectrophotometric course of Reaction 1. All samples contained 0.04 M glycylglycine buffer, pH 7.4, liver enzyme with 88 \(\gamma\) of protein, and 1 mm of MnCl₂ in a volume of 3.0 cc.; temperature 22°. Curve 1, 0.135 mm of TPN₉ and 0.448 mm of l-malate at 0 time. Curve 2, either 0.135 mm of TPN₉ and 0.448 mm of fumarate at 0 time and, at arrow A, 0.448 mm of l-malate (○); or 0.21 mm of DPN₉ and 0.448 mm of l-malate at 0 time, and, at arrow A, 0.135 mm of TPN₉ (○). Curve 3, 0.135 mm of TPN₉ and 0.0895 mm of l-malate at 0 time; at arrow B either 12 mm of pyruvate (Curve 3a), or 0.3 cc. of 0.1 M NaHCO₃ saturated with CO₂ and 12 mm of pyruvate (Curve 3b).

The new enzyme has been purified 30- to 40-fold by fractionation with

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alcohol at low temperature and fractional adsorption on alumina gel. It is specific for triphosphopyridine nucleotide (TPN). There is no reaction with diphosphopyridine nucleotide (DPN), fumarate, or phosphopyruvate. Neither inorganic phosphate nor adenosine triphosphate participates in this reaction.

The preparation still contains oxalacetic carboxylase and some of the lactic dehydrogenase present in liver extracts. Since lactic dehydrogenases from different sources can react slowly with TPN, TPN$_{red}$ is oxidized by the preparation on addition of pyruvic acid, but the rate of oxidation is markedly enhanced in the presence of NaHCO$_3$ saturated with CO$_2$ (see Fig. 1). Such acceleration does not occur with pure lactic dehydrogenase and indicates that the liver enzyme catalyzes Reaction 1 in either direction.

$l$-Malic acid is formed through the TPN-linked dismutation, pyruvate + CO$_2$ + glucose-6-phosphate = $l$-malate + 6-phosphogluconate, when the liver preparation is incubated with Mn$^{++}$, pyruvate, CO$_2$, glucose-6-phosphate, and glucose phosphate dehydrogenase.

Reaction 1 does not take place, whether in the presence of DPN or TPN, with a combination of pure malic dehydrogenase, purified oxalacetic carboxylase (from M. lysodeikticus), and manganese ions.

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6 Division of Physiology, National Institute of Health, United States Public Health Service.
REVERSIBLE OXIDATIVE
DECARBOXYLATION OF MALIC ACID
Severo Ochoa, Alan Mehler and Arthur
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