

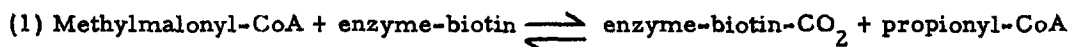
NET SYNTHESIS OF METHYLMALONYL-CoA BY ADP-, ATP-, AND  
Mg-INDEPENDENT TRANSCARBOXYLATION CATALYZED BY  
PROPIONYL CARBOXYLASE \*

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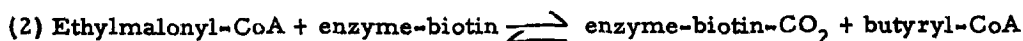
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Received April 13, 1961

Evidence indicating that propionyl carboxylase catalyzes an exchange between  $C^{14}$ -propionyl-CoA and methylmalonyl-CoA, which is independent of  $Mg^{++}$ , ADP, ATP, or  $P_i$ , has been provided by Halenz and Lane, 1961, and by Friedman and Stern, 1961. The following reaction is in accord with these findings:



The present report provides evidence that highly-purified mitochondrial propionyl carboxylase can catalyze the formation of methylmalonyl-CoA from ethylmalonyl-CoA and propionyl-CoA in the absence of added  $Mg^{++}$ , ADP, ATP, or  $P_i$ . It seems likely that the transcarboxylation reaction proceeds via an "enzyme-biotin-CO<sub>2</sub>" intermediate as shown in Reactions 2 and 3:



Bovine liver mitochondrial propionyl carboxylase (specific activity, 554  $\mu$ moles  $HCO_3^-$  fixed per hour per mg. of protein) was prepared as described by Lane *et al.*, 1960. Chemically-synthesized ethylmalonyl-CoA (prepared according to the method of Wieland and Rueff, 1953) and propionyl-CoA-1- $C^{14}$  (prepared according to the method of Simon and Shemin, 1953) were employed in these studies

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\*This study was supported in part by National Science Foundation Research Grant No. G14984.

to avoid adenine nucleotide contaminants. As shown in Table I, the formation of  $C^{14}$ -methylmalonyl-CoA from propionyl-CoA-1- $C^{14}$  was dependent upon the presence of both carboxylase and ethylmalonyl-CoA, but not upon  $Mg^{++}$  or ADP and  $P_i$ .

TABLE I  
Net Synthesis of  $C^{14}$ -Methylmalonyl-CoA From Propionyl-CoA-1- $C^{14}$   
and Ethylmalonyl CoA

Additions to or deletions from the basic reaction mixture*	Propionyl-CoA-1- $C^{14}$ incorporation into methylmalonyl-CoA**
	c. p. m. $\times 10^{-3}$ ***
None	24.0
- Enzyme	2.0
- Ethylmalonyl-CoA	2.0
+ Avidin	2.4
+ $MgCl_2$	26.1
+ $MgCl_2$ + ADP + $P_i$	12.6

\* Basic reaction mixture included (in  $\mu$ moles): Tris, pH 8.5, 100; glutathione, 5; propionyl-CoA-1- $C^{14}$  ( $6.2 \times 10^5$  c.p.m. per  $\mu$ mole), 0.3; ethylmalonyl-CoA, 0.31; and propionyl carboxylase (554 units per mg.), 59 units. Other additions: avidin (2.5 units per mg.), 10 mg.;  $MgCl_2$ , 4  $\mu$ moles; ADP, 4  $\mu$ moles; and  $K_2HPO_4$ , 4  $\mu$ moles. Total volume, 1.1 ml.

\*\* Reaction mixtures were incubated for 30 minutes at  $37^\circ$ . After alkaline hydrolysis (1N NaOH) of thioesters and acidification, free acids were quantitatively extracted, paper chromatographed with unlabeled methylmalonic acid as reference (n-amylformate-water-formic acid solvent system), and the methylmalonic acid spot quantitatively eluted and counted as described by Halenz and Lane, 1960. Radioactivity on chromatograms located with a strip counter corresponded exactly to the methylmalonic acid reference spot.

\*\*\* Average of duplicates.

Pretreatment of propionyl carboxylase with avidin for 10 minutes at  $0^\circ$  completely inhibited the transcarboxylation reaction. The addition of  $Mg^{++}$ , ADP, and  $P_i$  partially inhibited transcarboxylation. This is in agreement with our previous report (Halenz and Lane, 1961) that ADP and  $P_i$  inhibits  $C^{14}$ -propionyl-CoA-methylmalonyl-CoA exchange in the presence, but not in the absence, of  $Mg^{++}$ . In the cases in which transcarboxylation proceeded maximally (see Table I), approximately 0.04  $\mu$ moles of methylmalonyl-CoA were synthesized. This corresponds to 26 per cent "carboxyl transfer" from added ethylmalonyl-CoA. Presumably, only 0.15  $\mu$ mole of the enzymatically active isomer of ethylmalonyl-

CoA was added, since, according to Beck and Ochoa, 1958, only 50 per cent of chemically-synthesized methylmalonyl-CoA is enzymatically active.

It has also been demonstrated in our laboratory that the propionyl carboxylase-catalyzed exchange between C<sup>14</sup>-propionyl-CoA and methylmalonyl-CoA (Halenz and Lane, 1961) is inhibited both by avidin and p-hydroxymercuribenzoate.

#### References

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