RESTORATION OF BIOTIN-DEFICIENCY-INDUCED DEPRESSION OF PROPIONYL CARBOXYLASE ACTIVITY IN VIVO AND IN VITRO*

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The participation of biotin in the enzymatic carboxylation of propionate, first observed by Lardy and Adler (1956), has been verified with highly-purified propionyl carboxylase preparations (Halenz and Lane, 1960a; Kaziro et al., 1960; and Lane et al., 1960). Biotin deficiency in the rat leads to a reduced ability of mitochondrial acetone powder extracts to carboxylate propionate (Lardy and Adler, 1956) or propionyl CoA (Kosow et al., 1960). Attempts to reverse this enzymatic lesion in vitro were unsuccessful. In this report a rapid restoration of propionyl carboxylase activity lowered by biotin deficiency is demonstrated both in vivo and in vitro.

In curative experiments it is considered desirable to use animals in the early stages of a deficiency, therefore the length of time rats must be maintained on a biotin-deficient diet to markedly reduce propionyl carboxylase activity was investigated. Weanling male rats were fed (ad lib) the biotin-deficient diet containing 30% desiccated egg white described by Rubin et al. (1945) and modified to include a different salt mixture (Phillips and Hart, 1935) and vitamin B_{12}. Positive controls were fed the same diet to which d-biotin had been added (45 μg. per g. diet). After feeding the diets for varying lengths of time (11-39 days), rats from both groups were sacrificed, liver mitochondrial acetone powders prepared, and propionyl carboxylase assays conducted on extracts of the acetone powders using methods described by Halenz and Lane (1960a). The results, summarized in Table I, show that propionyl

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carboxylase activity was greatly reduced compared to the positive controls in the early stages of biotin deficiency, i.e. as early as 11 days on the biotin-deficient regime. Similar results obtained in pair-feeding experiments (Halenz and Lane, 1960b) rule out food intake as a factor effecting propionyl carboxylase activity.

TABLE I

Propionyl Carboxylase Activity at Different Stages of Biotin Deficiency

Duration of Feeding	Propionyl-CoA-dependent $HC^{14}O_3^-$ fixation[1]			
	Biotin-deficient	Positive controls	Ratio	Positive Control / Biotin-deficient
days	μmoles/hour[2]	μmoles/hour[3]		
11	.015	.060		4.0
18	.012	.077		6.4
25	.020	.120		6.0
32	.012	.083		6.9
39	.012	.076		6.3

[1] Reaction mixture for propionyl carboxylase assays included (in μmoles): tris, pH 8.5, 50; glutathione, 2.5; $MgCl_2$, 2; ATP, 2; $KHC^{14}O_3$, 7.5 (specific activity, 50,000 c.p.m. per μmole); propionyl-CoA, 0.5; and extract of 0.5 mg. of mitochondrial acetone powder. Total volume 0.75 ml. and 20-minute incubation at 37°.
[2] Average of duplicate assays conducted on acetone powders from 3 rats.
[3] Average of duplicate assays conducted on acetone powders from 2 rats.

Investigation of the rapidity with which the reduced propionyl carboxylase activity could be restored by biotin administration was conducted to determine the feasibility of attempting restoration *in vitro*. Rats fed the biotin-deficient diet for 26 days each received an intraperitoneal injection of 100 μg. of d-biotin and were sacrificed at various time intervals after injection. As shown in Table II, propionyl carboxylase activity very rapidly increased following biotin injection, the most rapid rate of increase occurring during the first 3 hours.

The rapid restoration of enzyme activity achieved by biotin injection in the preceding experiment suggested that a similar reversal might be accomplished *in vitro* using liver slices. From rats maintained on the biotin-deficient diet for 30 days, liver slices were prepared, pooled, and incubated aerobically either in the presence or absence of 100 μg. of d-biotin for 1, 2

TABLE II

Restoration of Propionyl Carboxylase Activity In Vivo

Time after d-biotin injection	Propionyl-CoA-dependent $HC^{14}O_3^-$ fixation[1]
hours	μmoles/hour[2]
0[3]	.021
3	.077
6	.095
9	.091
12	.133
24	.130

[1]Reaction mixture for carboxylase assays was the same as in Table I. Extract of 0.5 mg. of appropriate mitochondrial acetone powder used for each assay.
[2]Average of duplicate assays conducted on acetone powders from 2 rats.
[3]Negative control, no biotin injection.

TABLE III

Restoration of Propionyl Carboxylase Activity In Vitro

Treatment of liver slices		Propionyl carboxylase activity of mitochondrial acetone powder extracts prepared from liver slices[1]	
d-biotin addition	Length of incubation	Individual values[2]	Average
μg.	hours	μmoles propionyl-CoA-dependent $HC^{14}O_3^-$ fixation per hour	
Biotin-deficient[3]			
0	1, 2 or 3	.066, .067, .070, .074, .069	.072
100	1	.108, .115	.112
100	2	.146, .134, .179, .160	.155
100	3	.209, .212, .224, .237	.220
Biotin-adequate[4]			
0	3	.480, .480	.480
100	3	.490, .410	.450

[1]Reaction mixture for carboxylase assay was the same as in Table 1. Extract of 0.5 mg. of appropriate mitochondrial acetone powder used for each assay.
[2]Each value represents an individual flask of liver slices from which an acetone powder was prepared and assayed in duplicate.
[3]Liver slices from biotin-deficient rats pooled and randomly assigned to the treatments shown. Slices from the equivalent of one-half rat liver per flask incubated in Krebs-Ringer phosphate, pH 7.4, 37°, under 95% O_2 - 5% CO_2 in a Dubnoff metabolic shaker for time indicated after which mitochondria were isolated and acetone powders prepared.
[4]Liver slices from rats on stock diet injected with 200 μg. of d-biotin 15 hours prior to incubation then treated as indicated in footnote 3.

or 3 hours. Mitochondrial acetone powder extracts were prepared from the slices and assayed for propionyl carboxylase activity. It is apparent from the data in Table III that the reduced propionyl carboxylase activity associated with biotin deficiency was increased at least 3-fold by incubating liver slices with d-biotin for 3 hours.

To date all attempts in our laboratory to reverse the enzymatic lesion in cell-free liver systems have been unsuccessful.

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