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Activities of Pyruvate Carboxylase and Propionyl CoA Carboxylase in Rat Tissues During Biotin Deficiency and Restoration of the Activities After Biotin Administration[1–3] (38035)

GRACE SHEN CHIANG AND S. P. MISTRY
(Introduced by F. A. Kummerow)
Department of Animal Science, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

It is known that the activities of biotin dependent carboxylases are reduced in biotin deficient animals (1–5). Changes in the carboxylase activities during the progress of biotin deficiency has also been studied (2, 5), but they were mainly with respect to liver and adipose tissue. In the present study, we have examined the reduction of pyruvate carboxylase (EC 6.4.1.1.) and propionyl CoA carboxylase (EC 6.4.1.3) activities in liver, kidney, brain and heart of the rat at various stages of biotin deficiency and also the rapidity with which the enzyme activities are restored in these tissues when biotin is supplied to the deficient animals.

Materials and Methods. Materials. Citrate synthase and phosphotransacetylase were purchased from Boehringer Mannheim Corporation. Coenzyme A was obtained from P. L. Biochemicals. ATP, GSH, *d*-biotin and pyruvate were from Sigma Chemical Company. $NaH^{14}CO_3$ and Aquasol were obtained from New England Nuclear. All other chemicals used were of the highest purity commercially available.

Animals. Weanling male Sprague-Dawley rats weighing 35–40 g were fed *ad libitum* a 20% spray-dried egg white diet (6). Control animals received the same diet but were given 200 μg *d*-biotin in saline intramuscularly once a week beginning on the day the animals were first placed on the experimental diet.

Enzyme Preparations. Rats were killed by decapitation. The liver, kidney, brain and heart were quickly removed and dropped into ice-cold homogenizing medium containing 0.02 *M* Tris–HCl buffer pH 7.4, 0.25 *M* sucrose, 1 m*M* GSH and 1 m*M* EDTA. Further operations were done at 0°–4°. Samples for enzyme assays were prepared by homogenizing the tissues in the above medium (10% w/v) with Potter–Elvehjem glass homogenizer for 3 min. Brain was homogenized for only 1 min. One and five-tenths milliliters of the brain homogenates were lyophilized. Liver, kidney and heart homogenates were first diluted 5-fold and 1.5 ml aliquots were lyophilized. The samples were stored in a desiccator at −20°. For the determination of enzyme activities, lyophilized samples were resuspended in 1.5 ml distilled water and assayed.

Enzyme Assays. Pyruvate carboxylase was assayed as described previously (4). Propionyl CoA carboxylase was measured essentially according to the $^{14}CO_2$ fixation assay of Lane and Halenz (7). In both assays, the unreacted $NaH^{14}CO_3$ was removed by repeated addition of small pieces of dry ice to the trichloroacetic acid supernates. Aliquots were then counted in Packard liquid scintillation counter after adding 15 ml of Aquasol.

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[2] A preliminary report of this work has appeared: Fed. Proc., Fed. Amer. Soc. Exp. Biol. 32, 950 Abstr. (1973).

[3] We would like to thank Dr. M. S. Patel for giving us details for brain pyruvate carboxylase assay.

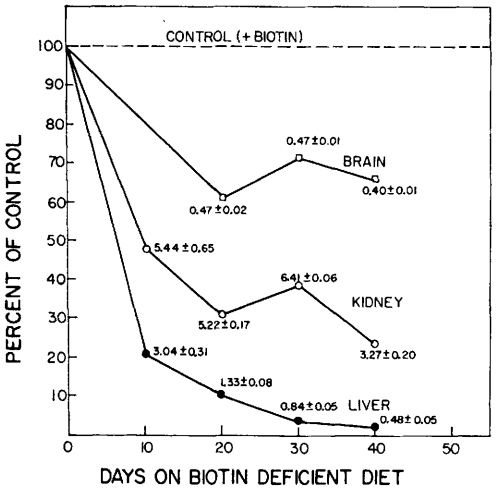

FIG. 1. Pyruvate carboxylase activities of rat liver, kidney and brain at various stages of biotin deficiency. All animals were fed *ad libitum* a 20% egg white diet. Control animals received the same diet but were given 200 μg *d*-biotin in saline intramuscularly once a week beginning on the day they were fed the experimental diet. Each point represents the mean of duplicate determinations for two to four animals. Values indicated are the activities of the deficient rats in units per g tissue ± standard error of the mean.

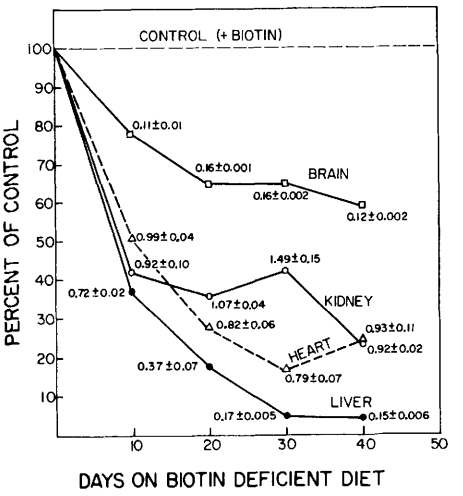

FIG. 2. Propionyl CoA carboxylase activities of rat liver, kidney, brain and heart at various stages of biotin deficiency. Animals were maintained as described in Fig. 1. Each point represents the mean of duplicate determinations for two to four animals. Values indicated are the activities of the deficient rats in units per g tissue ± standard error of the mean.

Enzyme activities are expressed as μmoles of CO_2 fixed per min at 37°.

Results and Discussion. The changes in the activities of pyruvate carboxylase and propionyl CoA carboxylase are shown in Figs. 1 and 2, respectively. The values are expressed as percent of the control activities. Both pyruvate and propionyl CoA carboxylase activities in these tissues decreased with the progress of biotin deficiency. There was a rapid fall in the activity of hepatic pyruvate carboxylase; within 3 wk it was only 10% of the control and decreased further to 2% by the end of the experiment (Fig. 1). Kidney and brain showed less marked effects; even after 40 days, pyruvate carboxylase activity was 25% and 65% of the control, respectively. Negligible activity of the enzyme was observed in the heart of the deficient animal. Control liver and kidney had considerable pyruvate carboxylase activity, 15–20 units per g liver and 10–15 units per g kidney and these agree with those reported by Ballard *et al.* (8). Since these are gluconeogenic tissues, this is to be expected. The activities in brain and heart were low; about 0.7 and 0.35 unit, respectively. The main purpose of pyruvate carboxylase in brain and heart is to provide oxaloacetate for the Krebs cycle. Also, pyruvate carboxylase may play an important role in supplying oxaloacetate to translocate the acetyl moiety as citrate for lipogenesis in the developing rat brain (9).

Propionyl CoA carboxylase activity in liver, kidney and heart was 2–4 units per g tissue. As in the case of pyruvate carboxylase, the activity of propionyl CoA carboxylase was low in the brain. As has been shown in earlier studies (2, 5), propionyl CoA carboxylase activity in liver decreased markedly in biotin deficient rats and reached a value of only 5% of the control at the end of the experiment (Fig. 2). The activity in the heart also decreased markedly. Like pyruvate carboxylase, the decrease in propionyl CoA carboxylase activity was less marked in kidney and brain; 25% and 60% of the control, respectively, at the end of the experiment.

TABLE I. Restoration *in Vivo* of Pyruvate Carboxylase Activities of Tissues From Biotin Deficient Rats.[a]

Time after *d*-biotin injection (hr)	Tissue		
	Liver	Kidney	Brain
	(μM CO_2 fixed/min/g tissue)		
0 (no biotin)	1.40 ± 0.07 (3) [b]	6.07 ± 0.43 (2)	0.46 ± 0.00 (3)
1	3.91 ± 0.35 (2)	8.72 ± 0.04 (3)	0.54 ± 0.04 (3)
2	6.00 ± 0.21 (3)	14.25 ± 0.58 (3)	0.56 ± 0.02 (3)
4	16.35 ± 1.03 (3)	22.15 ± 1.56 (2)	0.64 ± 0.04 (3)
8	18.58 ± 0.25 (3)	23.73 ± 1.17 (3)	0.67 ± 0.02 (3)
12	21.74 ± 0.62 (2)	21.71 ± 2.11 (3)	0.66 ± 0.04 (3)
Control	21.82 ± 1.56 (3)	15.78 ± 0.59 (3)	0.74 ± 0.03 (3)

[a] Animals were fed *ad libitum* a 20% egg white diet for 35 days. Control animals in addition received 200 μg *d*-biotin intramuscularly once a week. A single injection of 200 μg *d*-biotin was given intraperitoneally to each biotin deficient animal and the animals were killed at the intervals indicated.

[b] Each result is the mean ± standard error of the mean. The number of animals are given in parentheses.

With the progress of the deficiency the two enzymes decreased at about the same rate in each tissue (Figs. 1 and 2), although the rates were quite different for different tissues. Liver displayed maximum rate of depletion whereas brain was least affected. It is possible that the two enzymes are turning over at different rates in different tissues.

The rapidity with which the decreased enzyme activities in various tissues are restored to control levels after biotin injection are shown in Tables I and II. The most rapid rates of increase occurred during the first 4 hr. This is similar to the rates reported for hepatic propionyl CoA carboxylase (2, 5) and pyruvate carboxylase (5). Kidney enzyme activities were restored to normal within 2 hr; this is not surprising, since it had higher residual activities. Heart was the only tissue where propionyl CoA carboxylase activity was restored to only about half of the control value 24 hr after biotin administration. The different rates of

TABLE II. Restoration *in Vivo* of Propionyl CoA Carboxylase Activities of Tissues From Biotin Deficient Rats.[a]

Time after *d*-biotin injection (hr)	(μM CO_2 fixed/min/g tissue)			
	Liver	Kidney	Brain	Heart
0	0.21 ± 0.01 (3)	1.36 ± 0.10 (3)	0.16 ± 0.00 (3)	0.68 ± 0.06 (3)
1	1.09 ± 0.19 (2)	3.00 ± 0.21 (3)	0.19 ± 0.02 (3)	0.90 ± 0.03 (3)
2	1.34 ± 0.05 (3)	4.22 ± 0.38 (3)	0.20 ± 0.01 (3)	1.41 ± 0.12 (3)
4	2.39 ± 0.18 (3)	4.90 ± 0.13 (3)	0.23 ± 0.01 (3)	1.19 ± 0.13 (3)
8	2.98 ± 0.20 (3)	4.02 ± 0.34 (3)	0.27 ± 0.01 (3)	1.79 ± 0.12 (3)
12	3.83 ± 0.20 (2)	3.87 ± 0.37 (3)	0.24 ± 0.00 (3)	2.00 ± 0.17 (3)
24	4.43 ± 0.29 (3)	4.68 ± 0.09 (2)	0.22 ± 0.02 (3)	2.40 ± 0.20 (2)
Control	4.08 ± 0.16 (3)	3.57 ± 0.26 (3)	0.27 ± 0.01 (3)	4.59 ± 0.22 (3)

[a] All animals were maintained as described in footnote a in Table I. Each result is the mean ± standard error of the mean. The number of animals are given in parentheses.

restoration may be due to the differences in the availability of biotin to these tissues or that the rates at which the holoenzymes are synthesized are not the same for all tissues (Tables I and II).

Summary. Both pyruvate and propionyl CoA carboxylase activities decreased in liver, kidney, brain and heart of the rats with the progress of biotin deficiency. Enzyme activities in liver were severely affected; kidney and brain showed a less marked effect. Pyruvate carboxylase activity in heart was very low but propionyl CoA carboxylase activity was comparable to that of the liver. The activities of the two enzymes in liver, kidney and brain of the biotin deficient rat were restored to normal within 24 hr after biotin injection, but heart propionyl CoA carboxylase activity was restored to only half of the control activity.

1. Wakil, S. J., J. Lipid Res. **2**, 1 (1961).

2. Kosow, D. P., and Lane, M. D., Biochem. Biophys. Res. Commun. **4**, 92 (1961).

3. Dakshinamurthy, K., and Desjardins, P. R., Can. J. Biochem. **46**, 1261 (1968).

4. Deodhar, A., and Mistry, S. P., Biochem. Biophys. Res. Commun. **34**, 755 (1969).

5. Arinze, J. C., and Mistry, S. P., Comp. Biochem. Physiol. **38B**, 285 (1971).

6. Patel, M. S., and Mistry, S. P., J. Nutr. **96**, 409 (1968).

7. Lane, M. D., and Halenz, D. R., *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. V, p. 576. Academic Press, New York (1962).

8. Ballard, F. J., Hanson, R. W., and Reshef, L., Biochem. J. **119**, 735 (1970).

9. Patel, M. S., and Tilghman, S. M., Biochem. J. **132**, 185 (1973).

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