GLUCONEOGENESIS IN BIOTIN DEFICIENCY: IN VIVO SYNTHESIS OF PYRUVATE HOLOCARBOXYLASE IN BIOTIN DEFICIENT RAT LIVER

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SUMMARY. The activities of key gluconeogenic enzymes in livers of biotin-deficient rats under fed and fasting condition were examined. PEP carboxykinase, FDPase and G6Pase increased significantly on fasting and were not affected by the deficiency. There was a 10- to 20-fold decrease in pyruvate carboxylase activity in the deficiency and fasting had no effect on the activity. Within 1 hour after the administration of biotin to deficient animals there was a 5-fold increase in pyruvate carboxylase activity. Since actinomycin D or puromycin had no effect on the restoration of the activity in vivo it appears that pyruvate apocarboxylase and the holoenzyme synthetase were present in the deficient liver and once biotin was available, in vivo synthesis of pyruvate holocarboxylase occurred.

In an earlier study on gluconeogenis in biotin-deficient rat liver we showed metabolic blocks at the carboxylation of pyruvate and at the reductive synthesis of glyceraldehyde-3-phosphate as evidenced from the incorporation of various labeled precursors into blood glucose and from a cross-over plot of gluconeogenic intermediates (1). Futhermore, within 2 hours after the administration of biotin, gluconeogenesis was completely restored as a result of improved utilization of pyruvate and an increase in the reducing power in the cytosol (2). These results indicated that pyruvate carboxylase activity was restored to the normal level.

In view of these findings we have examined the activity of pyruvate carboxylase and other key gluconeogenic enzymes in the livers of biotin-deficient and control rats. Using protein synthesis inhibitors we have shown in vivo synthesis of pyruvate holocarboxlyase in liver within 1 hour after the administration of biotin to deficient rats.

METHODS. Using male Sprague-Dawley rats (initial weight 35 to 40 g) biotin deficiency was produced in 6 to 7 weeks by feeding an egg-white diet (3).

Pyruvate carboxylase was assayed as described by Ballard and Hanson (4) except that acetyl CoA was generated in situ (5). Liver homogenate (10%) was prepared in a medium containing 0.02 M Tris buffer pH 7.4, 0.25 M sucrose, 1 mM EDTA and 1 mM reduced glutathione, using a Potter-Elvehjem homogenizer for 3 minutes. The homogenate, immersed in crushed ice, was sonicated for 90 seconds with a Branson sonifier model S-125 (125 w/20KC). The sonicate was centrifuged at 20,000 x g in a refrigerated centrifuge (Sorvall RC2-B) for 20 minutes and the supernatant was used for the assay.

ABBREVIATIONS: PEP, phosphoenolpyruvate; FDPase, fructose-1, 6-diphosphatase; G6Pase, glucose-6-phosphatase; EDTA, ethylenediaminetetraacetate.

The reaction mixture contained: 25 μmoles Tris buffer pH 7.4, 2 μC/50 μmoles $NaH^{14}CO_3$, 10 μmoles Na pyruvate, 2.5 μmoles ATP, 5 μmoles $MgSO_4$, 0.43 μmole CoA, 2.5 μmoles acetyl phosphate, 2.4 units phosphotransacetylase, 0.3 unit citrate synthase and 0.1 ml supernatant in a final volume of 1 ml. After incubation at 37° for 10 minutes the reaction was stopped with 0.5 ml trichloroacetic acid (10%) and the excess $NaH^{14}CO_3$ was flushed out by adding pieces of dry ice. An aliquot of the deproteinized supernatant was counted in a Packard liquid scintillation counter. The values were corrected for $NaH^{14}CO_3$ fixation in the absence of the acetyl CoA generating system.

PEP carboxykinase was assayed as described by Seubert and Huth (6), FDPase according to Weber and Cantero (7), G6Pase as described by Arion and Nordlie (8) and glucokinase according to Sharma et al. (9). Other experimental procedures are described under each table.

RESULTS AND DISCUSSION. As will be seen from Table I, in agreement with earlier reports (10-12) the activities of the rate limiting gluconeogenic enzymes increased markedly in control livers after the animals were fasted for 24 hours. Similar increases were observed in biotin-deficient livers except in the case of pyruvate carboxylase. The activity of this enzyme in control liver was 10- to 20-fold higher than in deficient liver. Although Wagle (13) reported a decrease in pyruvate carboxylase activity in biotin

TABLE I

Activities of key gluconeogenic enzymes in livers of biotin deficent and pair-fed control rats

Enzyme	Biotin Status	Fed	Fasted	P
Pyruvate carboxylase μmoles CO_2 fixed/ g liver/minute	Control	4.36 ± 0.59 (4)	8.46 ± 0.34 (4)	< 0.001
	Deficient	0.38 ± 0.04 (4)	0.46 ± 0.04 (4)	
		P < 0.001	P < 0.001	
PEP carboxykinase μmoles PEP formed/ g liver/minute	Control	2.00 ± 0.22 (4)	5.13 ± 0.40 (4)	< 0.001
	Deficient	2.05 ± 0.17 (4)	5.41 ± 0.24 (4)	< 0.001
FDPase μmoles Pi liberated/ g liver/minute	Control	12.84 ± 0.75 (7)	19.43 ± 1.71 (6)	< 0.01
	Deficient	12.39 ± 1.08 (7)	17.58 ± 0.79 (6)	< 0.01
G6Pase μmoles Pi liberated/ g liver/minute	Control	4.54 ± 0.91 (8)	10.50 ± 1.26 (5)	< 0.01
	Deficient	5.77 ± 1.00 (8)	9.16 ± 1.05 (5)	< 0.05

Where indicated the animals were fasted for 24 hours. Pair-fed control animals receiving biotin were used (1). Results are average values ± standard error. The number of animals are given in parentheses. P denotes probability of differences being significant.

TABLE II

Restoration of hepatic pyruvate carboxylase activity in vivo

Group	Hours after biotin administration	No. of rats	Pyruvate carboxylase μmoles CO_2 fixed/ g liver/minute	P
I	0 (no biotin)	8	0.30 ± 0.06	
II	1	4	1.58 ± 0.49	< 0.05
III	2	4	3.31 ± 0.32	< 0.001
IV	4	3	3.47 ± 0.17	< 0.001

Biotin deficient rats were fasted for 24 hours before the experiment. 200 μg of biotin was administered intraperitoneally to each animal and the animals were killed at specific intervals. The results are average values ± standard error. P value denotes probability of differences being significant when compared with group I.

TABLE III

Effect of actinomycin D and puromycin on the de novo synthesis of glucokinase

Group	Treatment after fasting	No. of rats	Glucokinase μmoles glucose phosphorylated/ g liver/ minute
I	None	3	0.57 ± 0.03
II	Actinomycin D	2	0.56
III	Actinomycin D + glucose	3	0.57 ± 0.01
IV	Puromycin	2	0.60
V	Puromycin + glucose	3	0.65 ± 0.03
VI	Glucose	4	1.47 ± 0.03
			P < 0.001

Rats weighing 150 to 200 g fed ad libitum a commercial diet were used. Animals were fasted for 48 hours before the experiment. Where indicated, glucose (5 mmole) was given intragastically and the animals were killed after 3 hours; actinomycin D (250 μg/100 g body wt.) and puromycin (8 mg/100 g body wt.) were given intraperitoneally. Actinomycin D was given 1 hour before glucose (group III) and puromycin was given 45 minutes before glucose (group V). Results are average values ± standard error. P value denotes probability of differences being significant when compared with the result of group I.

deficiency, the values reported even for normal livers were extremely low. This could be due to the method used by him to extract the enzyme since Ballard and Hanson (4) recently pointed out that homogenizing the tissue at 25° without additional treatment was not sufficient to extract mitochondrial pyruvate carboxylase.

The activities of PEP carboxykinase, FDPase and G6Pase were not affected by the deficiency. This is in keeping with our earlier findings (1) that there was no lesion at the site of PEP synthesis from oxalacetate and beyond glyceraldehyde-3-phosphate dehydrogenase step on the pathway of gluconeogenesis in biotin deficiency.

The results of a time study of the restoration of pyruvate carbosylase activity in vivo are given in Table II. Within 1 hour after the administration of biotin to deficient animals there was a 5-fold increase in the activity. It is interesting to note that although restoration of the carboxylase activity at 2 hours after the administration of biotin was still considerably below the level observed in fasted control animals (see Table I), under identical conditions in an earlier study we showed (2) that gluconeogensis

TABLE IV

Effect of actinomycin D and puromycin on the restoration of hepatic pyruvate carboxylase in vivo

Group	Treatment after fasting	No. of Rats	Pyruvate carboxylase μmoles CO_2 fixed/ g liver / minute
I	None	4	0.32 ± 0.10
II	Actinomycin D	3	0.29 ± 0.07
III	Actinomycin D + biotin	3	2.95 ± 0.31
			P < 0.001
IV	Puromycin	2	0.25
V	Puromycin + biotin	3	3.26 ± 0.126
			P < 0.001
VI	Biotin	4	3.31 ± 0.32
			P < 0.001

24-hour fasted biotin-deficient rats were used. Where indicated, biotin (200 μg) was given intraperitoneally and the animals were killed after 2 hours; actinomycin D (250 μg/100 g body wt.) and puromycin (8 mg/100 g body wt.) were administered intraperitoneally. Actinomycin D was given 1 hour before biotin (group III) and puromycin was given 45 minutes before biotin (group V). Results are average values ± standard error. P value denotes probability of differences being significant when compared with the result of group I.

was completely restored to normal within the same period as seen from the incorporation of labeled alanine into blood glucose (6.5% of the dose incorporated). In a study on propionyl CoA carboxylase, another biotin containing enzyme, Kosow and Lane (14) showed a similar restoration of the enzyme activity after the administration of biotin to deficient rats.

In order to determine whether the increase in pyruvate carboxylase activity was the result of de novo enzyme synthesis, deficient rats were given actinomycin D or puromycin before biotin injection. In a preliminary study it was shown (Table III) that the amounts of protein synthesis inhibitors employed in the present study were adequate to block enzyme synthesis. As will be seen from Table IV, actinomycin D or puromycin per se did not have an effect on pyruvate carboxylase. Also, these inhibitors had no effect on the restoration of the activity in vivo.

It has been shown in earlier studies (15-18) that biotin is activated in the presence of the holocarboxylase synthetase and biotin becomes covalently bonded to lysyl ϵ-amino groups of the apoenzyme to form the holoenzyme. It seems, therefore, that there was no de novo synthesis of pyruvate apocarboxylase or the synthetase after the administration of biotin and that these proteins were already present in the deficient liver. Biotin-deficient rat liver also has been shown to contain propionyl CoA apocarboxylase and the synthetase (19-21). Thus it appears that once biotin is available, in vivo synthesis of pyruvate holocarboxylase occurs.

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REFERENCES

1. Deodhar, A. D., and Mistry, S. P., Arch. Biochem. Biophys. 129, 321 (1969).
2. Mistry, S. P., and Deodhar, A. D., Fed. Proc. 27, 283 (1968).
3. Patel, M. S., and Mistry, S. P., J. Nutrition 96, 409 (1968).
4. Ballard, F. J., and Hanson, R. W., J. Lip. Res. 8, 73 (1967).
5. Henning, H. V., and Seubert, W., Biochem. Z. 340, 160 (1964).
6. Seubert, W., and Huth, W., Biochem. Z. 343, 176 (1965).
7. Weber, G., and Cantero, A., Cancer. Res. 19, 763 (1959).
8. Nordlie, R. C., and Arion, W. J., in "Methods in Enzymology" (Colowick, S. P., Kaplan, N. O., and Wood, W. A., eds.) Vol. IX, p. 621. Academic Press, New York (1966).
9. Sharma, C., Manjeshwar, R., and Weinhouse, S., J. Biol. Chem. 238, 3840 (1963).
10. Freedman, A. D., and Kohn, L., Science 145, 58 (1964).
11. Foster, D. O., Ray, P. D., and Lardy, H. A., Biochemistry 5, 555 (1966).
12. Weber, G., and Cantero, A., Proc. Can. Physiol. Soc. 20, 62 (1956).
13. Wagle, S. R., Proc. Soc. Exp. Bio. Med. 121, 15 (1966).
14. Kosow, D. P., and Lane, M. D., Biochem. Biophy. Res. Commun. 4, 92 (1961).
15. Lynen, F., and Rominger, K. L., Fed. Proc. 22, 537 (1963).
16. Lane, M. D., Young, D. L., and Lynen, F., J. Biol. Chem. 239, 2858 (1964).
17. Lane, M. D., Rominger, K. L. Young, D. L., and Lynen, F., J. Biol. Chem. 239, 2865 (1964).
18. Hopner, T., and Knappe, J., Biochem. Z. 342, 190 (1965).
19. Kosow, D. P., and Lane, M. D., Biochem. Biophys. Res. Commun. 5, 191 (1961).
20. Kosow, D. P., Huang, S. C., and Lane, M. D., J. Biol. Chem. 237, 3633 (1962).
21. Siegel, L., Foote, J. L., Christner, J. E., and Coon, M. J., Biochem. Biophys. Res. Commun. 13, 307 (1963).