Glycogen Synthesis in Biotin-Deficient Rat Liver[1,2] (34773)

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A decrease in glycogen synthesis in livers of biotin-deficient rats has been observed in many studies (1–6). However, Mistry and Singh (7) found no change in the activities of the enzymes of the glocogen cycle in the livers of these animals. In view of these observations we have examined the synthesis of hepatic glycogen and its level in rats during various stages of biotin deficiency.

Materials and Methods. Chemicals. Glucose-U-^{14}C (sp act 3.83 mCi/mole) obtained from New England Nuclear Corporation, Boston, Mass. was diluted with nonradioactive glucose of analytical grade.

Animals. Nineteen-day-old weanling male Sprague-Dawley rats weighing 33–38 g were used in all studies. The animals were housed individually in metal cages with raised wire screens in a temperature-regulated room. Biotin deficiency was produced by feeding *ad libitum* a basal diet (8) containing 20% spray-dried egg white. After 4–7 weeks on the basal diet as indicated in legends the deficient animals were divided into two groups. One group continued on the basal diet for another 2 weeks and served as the deficient group. The animals in the second group were cured of the deficiency by injecting each animal with 100 μg of biotin in physiological saline three times a week during the 2-week curative period and the animals were fed *ad libitum*. These cured-normal animals served as the control group. During the curative treatment visible symptoms of biotin deficiency progressively disappeared. Normal animals fed the basal diet *ad libitum* received the same biotin treatment but from the beginning of the experiment. Water was available to animals at all times.

Level of glycogen in liver. In the fed condition, control and deficient animals after a total of 6, 7, and 9 weeks on the basal diet were decapitated during the day and the night as indicated in legends to various tables. A portion of the liver was rapidly removed and glycogen was determined as described below. The weight of total liver was also recorded.

In vivo *synthesis of glycogen.* In these studies, control and deficient animals which were on the basal diet for a total of 6, 7, and 9 weeks were used. The animals were fasted for 24 hr and were given intragastrically 5 mmoles of glucose containing 1.5 or 3 μCi of glucose-U-^{14}C in 3 ml of water. In one experiment 11.1 mmoles (2 g) of nonradioactive glucose instead of 5 mmoles was used. The animals were killed by decapitation after 3 hr or at periods indicated in the figure. Portions of the liver (about 300 mg) were rapidly removed and glycogen was estimated as described below.

In the study of glucose incorporation into glycogen, injection of a trace dose of labeled glucose leads to varying degrees of dilution of the injected precursor depending upon the size and the metabolic state of the animal. In the present study, in order to avoid these sources of error, a sufficiently large glycogenic dose (5 mmoles) of labeled glucose was administered to 24-hr fasted animals. With

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TABLE I. Diurnal Variation in Glycogen Content of Livers of Biotin-Deficient and Control Rats Fed ad Libitum the Basal Diet.

Status of animals[a]	Time when animals were killed	Body weight (g)	Liver glycogen in fed rats			
			mg/g liver		mg/100 g body weight	
Deficient	Day[b]	109 ± 2[d]	39 ± 6.2		159 ± 28	
Cured-normal		176 ± 5	54 ± 2.6	$p < .05$	231 ± 15	$p < .05$
Deficient	Night[c]	115 ± 2	68 ± 4.7		303 ± 23	
Cured-normal		198 ± 4	65 ± 4.1	NS	300 ± 18	NS

[a] All animals were on the experiment for a total of 6 weeks.
[b] Groups of six rats were killed between noon and 4 PM.
[c] Groups of six rats were killed between midnight and 4 AM.
[d] Each result is the mean ± standard error of the mean of 12 animals.

this technique it has been shown (9) that the specific activity of glucose in blood remains nearly the same for the first 4 hr after the administration of the precursor.

Determination of glycogen. Immediately after decapitation a small piece of the tissue was removed, quickly weighed on a Roller-Smith torsion balance, transferred to a tube containing 2 ml of hot 30% KOH, and digested in a boiling water bath for 30 min. Glycogen was coprecipitated with Na_2SO_4 from the KOH digest using 95% ethanol (10), and the precipitate was washed once with 65% ethanol, as suggested by Fong *et al.* (11). In experiments where the incorporation of labeled glucose into glycogen was studied, the precipitate was dissolved in water and reprecipitated with 95% ethanol. After repeating this treatment, the precipitate was dissolved in water and glycogen was estimated by the anthrone method (12). The radioactivity was determined by counting an aliquot with 15 ml of Bray's scintillation solution (13) in a Packard Tri-Carb scintillation counter.

Results and Discussion. The effect of biotin deficiency on the level of glycogen in livers of fed rats is shown in Table I. Livers of deficient animals contained significantly less glycogen than livers of control animals when examined during the day but there was no difference in the level of glycogen during the night. In view of this, the effect of biotin deficiency on the food intake of animals during the day and the night was investigated. The results (Table II) show that deficient and control animals consumed about 80% of their daily food during the night (6 PM to 6 AM). It is possible, therefore, that the marked reduction in food intake (about 2 g between 6 AM and 6 PM) by the deficient animal resulted in a decrease in hepatic glycogen. To test this assumption, 5 mmoles of labeled glucose were given intragastrically to *ad libitum*-fed control and deficient animals during the day. As seen from Table III, although the level of glycogen was significantly lower ($p < .01$) at zero hour in livers of deficient animals than in controls, there was no difference in the level of glycogen between the two groups 3 hr after the administration of a large dose of glucose. As seen from the radi-

TABLE II. Effect of Day and Night on Food Intake of Biotin-Deficient and Control Rats Fed ad Libitum the Basal Diet.

Status of animals	Body weight (g)	Food intake[a]	
		Night (6 PM–6 AM) (g)	Day (6 AM–6 PM) (g)
Deficient	116 ± 4[b]	7.3 ± 0.3	1.9 ± 0.2
Normal	295 ± 9	16.4 ± 0.8	4.8 ± 0.5

[a] Food cups were completely filled daily at 6 PM for 1 week before food intake was recorded. The cups were weighed at 6 AM but not refilled or disturbed in any way. All animals were on the experiment for 7 weeks.
[b] Each result is the mean ± standard error of the mean of 10 animals.

TABLE III. Glycogen Synthesis from Glucose-U-^{14}C in Livers of Biotin-Deficient and Control Rats in the Fed Condition.

Status of animals[a]	Body weight (g)	Glycogen/g liver after hours:[b] 0	3	3
		mg	mg	dpm × 10^{-3}
Deficient	113 ± 4 (4)[c]	40 ± 3.6 (10)	58 ± 11 (4)	132 ± 12 (4)
Cured-normal	196 ± 9 (4)	55 ± 3.6 (11)	57 ± 5 (4)	70 ± 20 (4)

[a] All animals were on the experiment for a total of 6 weeks.

[b] Each animal received by stomach tube 5 mmoles (3 μCi) of labeled glucose in 3 ml of water during the day.

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

oactivity data, actually more glucose was incorporated in hepatic glycogen of deficient animals than in controls. The reduction in the synthesis of hepatic glycogen in biotin-deficient rats observed in earlier studies (1–4) could have been due to trace amounts of glucose injected.

The effect of different stages of biotin deficiency on the *in vivo* synthesis of glycogen from labeled glucose was further investigated using the same experimental conditions except that 24-hr fasted animals were used. As seen from Table IV, there was no difference in the repletion of hepatic glycogen in control and deficient animals that were on the basal diet for 6 weeks. However, after 7 weeks, when they became markedly deficient, these animals were unable to synthesize significant amounts of glycogen when killed 3 hr after the administration of glucose. This was also observed in another study with deficient animals that were on the basal diet for 8 weeks. As will be seen from the radioactivity data in Fig. 1, the rate of glycogen synthesis in the livers of control animals was linear for about 3 hr and soon afterwards reached a plateau. Glycogen synthesis in the livers of deficient animals was negligible during the first 3 hr but at the end of 4.5 hr, considerable amount of glycogen was deposited. Furthermore, the rate of glycogen synthesis in the livers of deficient animals was now comparable to that in the controls. In view of this lag period in the synthesis of hepatic glycogen in severely deficient animals we repeated the experiment and examined glycogen synthesis over a period of 6 hr. In this study we gave intragastrically to 24-hr fasted control and deficient animals which were on the basal diet for 8 weeks, about twice the amount of glucose (11.1 mmoles) in order to ensure an adequate supply of the substrate over this pro-

TABLE IV. Glycogen Synthesis from Glucose-U-^{14}C in Livers of Biotin-Deficient and Control Animals During Various Stages of Biotin Deficiency.

Exp.[a]	Status of animals	Weeks	Body weight (g)	Glycogen/g liver mg	Glycogen/g liver dpm × 10^{-3}	p
1	Deficient	6	100 ± 4[b]	23 ± 4.7	189 ± 25	
	Cured-normal		170 ± 1	23 ± 2.2	178 ± 17	NS
2	Deficient	7	104 ± 4	2 ± 1.1	7 ± 6	
	Cured-normal		176 ± 8	31 ± 3.0	87 ± 7	<.001

[a] Twenty-four-hour fasted rats were given labeled glucose (3 μCi and 1.5 μCi/5 mmoles/3 ml of water in Exp. 1 and 2, respectively) by stomach tube and the animals were killed after 3 hr. In Exp. 2, two deficient animals died during the 3-hr experimental period.

[b] Each result is the mean ± standard error of the mean of four to six rats.

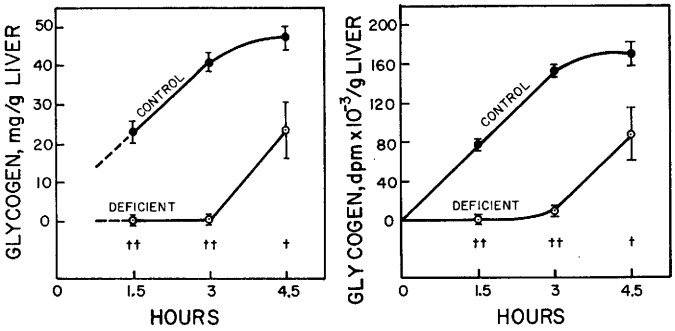

FIG. 1. Rate of synthesis of hepatic glycogen from glucose-U-^{14}C. Control and biotin-deficient rats were on the basal diet for 8 weeks. After a 24-hr fast each animals was given by stomach tube 5 mmoles (3μCi) of labeled glucose in 3 ml of water. The vertical bar gives the standard error of the mean of three or four animals. The dagger indicates a death in the deficient group. Average body weights of control and deficient animals were 192±4 and 103±3 g, respectively.

longed period. Although no glycogen was deposited in the liver of deficient animals for the first 3 hr, as before, substantial repletion occurred in the next 3 hr (results of this repeat experiment are not given). It is important to note that these severely deficient animals which were on the basal diet for 7 or more weeks were not able to withstand the 24-hr fast and the intubation of the dose as judged from the mortality rate of this group during the repletion study (see Fig. 1 and Table IV, Expt. 2). Therefore, it is very likely that the lag period in the repletion of hepatic glycogen observed in these severely deficient animals was the result of experimental stress of the fast and also the stress of the administration of glucose by stomach tube. That glycogen synthesis is not affected in biotin-deficient rat liver was confirmed in another study. The results given in Table V conclusively establish that even severely deficient animals which were on the basal diet for 9 weeks were able to maintain normal levels of hepatic glycogen during the night when they ate most of their daily food. This is in keeping with our earlier findings (7) that the hepatic activity of phosphoglucomutase, UDP-glucose pyrophosphorylase, glycogen synthetase, and glycogen phosphorylase is not altered in the biotin-deficient rat.

TABLE V. Glycogen Level in Livers of Biotin-Deficient and Control Rats Fed ad Libitum the Basal Diet.[a]

Status of animals	Weeks	Body weight (g)	Glycogen/g liver (mg)
Deficient	7	112 ± 3[b]	60 ± 2.0
Deficient	9	111 ± 4	54 ± 2.2
Cured-normal	9	277 ± 6	55 ± 2.1

[a] The animals were killed at midnight.

[b] Each result is the mean ± standard error of the mean of eight animals.

Summary. The effect of biotin deficiency on the *in vivo* synthesis and the level of glycogen in livers of rats during various stages of the deficiency was investigated. A glycogenic dose of labeled glucose was administered by stomach tube to 24-hr fasted control and deficient animals and glycogen synthesis in liver was measured after 3 hr. The deficient animals which were on the basal diet for 6 weeks were able to synthesize the same amount of hepatic glycogen as that by the controls. A marked reduction in the level of glycogen in the livers of deficient animals compared to controls was observed during the day but there was no change in the level between the two groups when examined during the night at all stages of biotin deficiency. The observed decrease during the day was the result of diurnal variation. The results show that in the rat the synthesis of hepatic glycogen is not impaired in biotin deficiency.

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