Some Aspects of Carbohydrates Metabolism in Biotin-Deficient Rats* (32699)

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We reported (1) that amino acid incorporation into liver proteins was reduced markedly in biotin-deficient rats and chicks. In these experiments biotin deficiency was produced on a regime where glucose was the sole source of the carbohydrate. Substitution of 10% of the glucose in the diet by sodium succinate restored amino acid incorporation to the normal level. We suggested that biotin deficiency might be accompanied by an impairment of glucose metabolism resulting in a decreased supply of energy for synthetic processes such as protein synthesis. An exogenous supply of succinate by making itself available for energy production ameliorated this defect.

There are conflicting reports (2, 3) about the alleviation of biotin deficiency by sorbitol when it partially replaced glucose in the diet. Using amino acid incorporation (1) into liver proteins as an index we observed that sorbitol as well as fructose showed a definite beneficial effect. These data and the results of a study of oxidative phosphorylation in biotin deficiency are given in this paper.

Animals and basal diet. Using 35–40 gm weanling male Sprague-Dawley rats, biotin deficiency was produced in 6–7 weeks on a 71% glucose, 20% raw egg-white basal diet which was complete in all nutrients except biotin. Since in biotin deficiency rats are unable to synthesize ascorbic acid (4) it was included in the basal diet. Normal animals were given parenterally 100 μg of biotin twice a week. Dietary conditions are described under each table.

Materials. Fructose, sorbitol, ATP, NAD, DL-β-hydroxybutyrate, acetyl CoA, hexokinase, peroxidase, and glucose oxidase were secured from Sigma Chemical Co. and insulin

TABLE I. Effect of Fructose or Sorbitol on the Incorporation of DL- or L-Leucine-2-^{14}C in Rat Liver Microsomal Protein in Biotin Deficiency and Comparison of the Propionyl CoA Carboxylase Activity of the Liver.

		Expt. 1		Expt. 2
Group	Biotin status	Incorporation of DL-leucine-2-^{14}C cpm/mg protein	Propionyl CoA carboxylase cpm CO_2 fixed/mg protein	Incorporation of L-leucine-2-^{14}C cpm/mg protein
1	Normal	395	624	607
2	Deficient	117	98	227
3	Deficient, fructose fed	481	91	590
4	Deficient, sorbitol fed	492	94	

Note—Each result is the average value obtained with two animals. Fructose- or sorbitol-fed rats received the basal diet with 10% fructose or sorbitol at the expense of an equal amount of glucose. Each animal was kept in a tubular cage to prevent coprophagy (6). After an overnight fast, 1 μc of DL- or L-leucine-2-^{14}C in 50 μmoles/100 gm body weight was administered parenterally and the animals were killed after 30 min. Leucine incorporation into microsomal liver protein was measured as described earlier (1) and propionyl CoA carboxylase activity in liver was estimated according to Neujahr and Mistry (7). Animals in groups 1, 3, and 4 received the same amount of food which was consumed by animals in group 2.

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from Eli Lily Co. The DL- and L-leucine-2-^{14}C, L-leucine-U-^{14}C, $NaHCO_3$-^{14}C, and D-glucose-U-^{14}C were purchased from Volk Radiochemical Company and New England Nuclear Corporation. Propionyl CoA was prepared (5) using propionic anhydride and acetyl CoA.

Methods. Experimental procedures are described under each table.

Results. In our earlier studies *in vivo* and *in vitro* with rats and chicks it was established (1) that the rate of amino acid incorporation into various fractions of liver and other tissues was significantly reduced in biotin deficiency. Under similar standardized conditions using pair-fed animals of approximately the same weight, the results given in Table I indicate that the incorporation of leucine into microsomal proteins of liver of sorbitol or fructose-fed biotin-deficient rats was comparable to that in pair-fed normal animals and was three to four times more than in biotin-deficient rats which received glucose as the sole source of the carbohydrate in the diet. This effect of feeding fructose was confirmed in a second experiment. The propionyl CoA carboxylase data definitely established that these animals were biotin deficient.

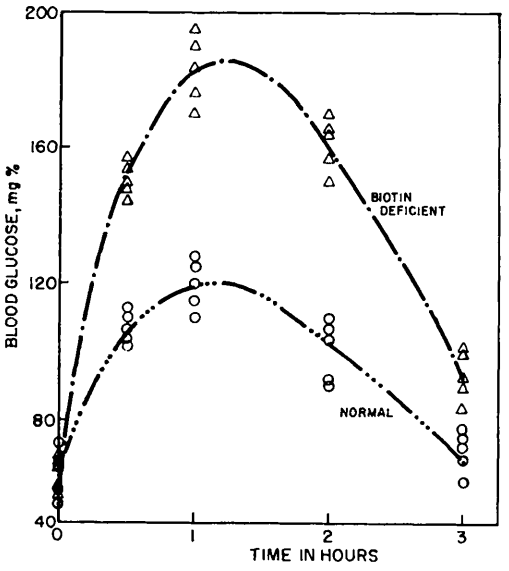

FIG. 1. Oral glucose tolerance of normal and biotin-deficient rats. A dose of 100 mg of glucose/100 gm body wt was given by stomach tube to animals fasted for 18 hours. Blood samples were taken from the tail vein at 0, 0.5, 1, 2, and 3 hours. Five normal and five biotin-deficient rats were used in this study. Blood glucose was estimated according to Huggett and Nixon (8). The animals were pair-fed from the start of the experiment.

The results of an oral glucose tolerance test are shown in Fig. 1. One hour after the administration of glucose, blood sugar rose to a peak of 185 mg/100 ml in the biotin-deficient group compared to a peak of 120 mg/100 ml in the pair-fed normal group. At the end of 4 hours, blood sugar returned to the initial level in both groups.

As will be seen from Table II, the urinary excretion of ketone bodies in a 24-hour collection by biotin-deficient rats was three times greater than the excretion by pair-fed normal animals.

TABLE II. Urinary Excretion of Ketone Bodies by Normal and Biotin-Deficient Rats.

Biotin status	Number of rats	Ketone bodies excreted mg/day/100 gm body wt	p^a
Normal	6	0.70 ± 0.05 (0.60–0.86)	<0.001
Deficient	6	2.20 ± 0.14 (1.70–2.60)	

Note.—Results are average values ± standard error. Minimum and maximum values are shown in parentheses. After fasting for 18 hours, 100 mg of glucose in physiological saline/100 gm body weight was injected intraperitoneally and urine was collected for 24 hours. Ketone bodies were determined according to Michaels *et al.* (9). The animals were pair-fed from the beginning of the experiment.

[a] Probability of differences being significant.

The effect of insulin administration on the incorporation of glucose into liver glycogen in biotin deficiency is shown in Table III. In the deficiency, glycogen synthesis in liver was reduced compared to pair-fed normal rats as seen from the amount present as well as from the radioactivity incorporated from glucose, expressed in terms of the specific activity of glycogen or as incorporation in the entire liver. Administration of insulin for the last 3 days of the experiment restored liver glycogen to the level found in normal pair-fed animals.

The results of a study of the effect of insulin on the incorporation of leucine in rat

TABLE III. Effect of Insulin on the Incorporation of D-Glucose-U-^{14}C into Liver Glycogen in Biotin Deficiency.

Group	Biotin status	Glycogen		
		mg/100 gm Liver	cpm/mg	cpm/Total liver
1	Normal	120	12,400	64,480
2	Deficient	31	9,600	12,480
3	Deficient, insulin treated	96	12,480	59,884

Note.—Each result is the average value obtained with two animals. Insulin was given intramuscularly 0.4 unit/100 gm body wt/day for the last 3 days of the experiment. After an overnight fast, 2 μc of D-glucose-U-^{14}C in 0.67 μmole/100 gm body wt was injected and the animals were killed after 1 hour. Proteins were removed with trichloracetic acid, glycogen was precipitated with 2 volumes of ethanol, dried, weighed, and the radioactivity was determined by solid counting at infinite thinness. Animals in groups 1 and 3 received the same amount of food which was consumed by animals in group 2.

TABLE IV. Effect of Insulin on the Incorporation of L-Leucine-U-^{14}C in Rat Liver Microsomal Proteins during Various Stages of Biotin Deficiency.

Group	Biotin status	Weeks on diet (epm/mg protein)			
		1	2	3	4
1	Normal	361	482	483	403
2	Deficient	292	268	232	203
3	Deficient, insulin treated	385	456	508	410
4	Normal, insulin treated			460	

Note.—Each result is the average value obtained with two animals. Insulin was given intramuscularly 0.4 unit/100 gm body wt/day for the last 3 days of the experiment. After an overnight fast, 0.5 μc of L-leucine-U-^{14}C in 50 μmoles/100 gm body wt was administered parenterally and the animals were killed after 30 min. Leucine incorporation into microsomal liver protein was measured as described before (1). Animals in groups 1, 3, and 4 received the same amount of food which was consumed by animals in group 2.

liver microsomal proteins during various stages of biotin deficiency are given in Table IV. These data confirm and extend the results shown in Table I and secured in our earlier studies (1) that the amino acid incorporation is decreased in biotin deficiency. The difference, between the pair-fed normal and the biotin-deficient animal to incorporate leucine, gradually increased with the progress of the deficiency and in each case administration of insulin to the deficient animal restored the incorporation to the normal level. There was no change in the incorporation of leucine when insulin was administered to normal animals under identical conditions.

In an attempt to locate the lesion we made a preliminary study of the overall glucose phosphorylating activity of liver at high glucose concentration as a criterion for hexokinase and glucokinase. The results given in Table V

TABLE V. Glucose Phosphorylation by Normal and Biotin-Deficient Rat Livers.

Biotin status	Number of rats	Units/gm liver	p[a]
Normal	6	4.60 ± 0.22 (4.05–5.24)	<0.05
Deficient	6	2.88 ± 0.23 (2.16–3.26)	

Note.—Results are average values ± standard error. Minimum and maximum values are shown in parentheses. Overall glucose phosphorylating activity of liver, as an index of hexokinase and glucokinase activities was tested (10) on 105,000g liver supernatant fluid at 26.7 mM glucose concentration. The animals were pair-fed from the beginning of the experiment.

[a] Probability of differences being significant.

point to a difference in overall glucose phosphorylation by preparations from pair-fed normal and biotin-deficient livers.

The results of the effect of various stages of biotin deficiency on oxidative phosphorylation and on the lipid content of rat liver mitochondria are shown in Table VI. Usual P/O

TABLE VI. Effect of Various Stages of Biotin Deficiency on Oxidative Phosphorylation and on the Lipid Content of Rat Liver Mitochondria.

Weeks on biotin-deficient diet	Lipid % dry wt. of mitochondria	ΔO_2 μatoms	Δ Pi μmoles	P:O
0 (normal animals)	30.5	5.25	14.84	2.82 (4)
4	29.0	6.45	14.00	2.17 (4)
6	25.0	6.70	9.75	1.45 (4)
8	21.1	2.40	2.00	0.83 (4)

Note.—The results are mean values and the number of animals are given in parentheses. Twice-washed mitochondria were prepared from liver homogenate in 0.25 *M* sucrose and 1 m*M* EDTA at pH 7.4. The mitochondrial pellet was reisolated in 0.25 *M* sucrose. One ml of the mitochondrial preparation contained the particles from 125 mg of fresh liver. All operations were performed at 0–4°C. β-Hydroxybutyrate was used as the substrate and oxygen consumption was measured by the Warburg technique for 30 min at 30°C after temperature equilibration. Phosphate esterified was the difference in inorganic phosphate between zero time and the end of the incubation period. Inorganic phosphate was measured according to Fiske and Subbarrow (11). Each Warburg vessel contained: 50 μmoles DL-β-hydroxybutyrate, 50 μmoles phosphate buffer pH 7.4, 3 μmoles ATP, 2 μmoles NAD, 150 μmoles glucose, 25 KM units hexokinase, 0.03 μmole cytochrome C, 15 μmoles $MgCl_2$, 3 μmoles EDTA, and 1 ml mitochondrial preparation in a final volume of 2.5 ml.

ratios were obtained for the oxidation of β-hydroxybutyrate with liver mitochondria from animals at the beginning of the experiment, i.e., zero week on the biotin-deficient basal diet. As the deficiency progressed, a gradual decrease in P/O ratio was observed and it correlated with the decrease in the lipid content of mitochondria. Phosphate esterified was markedly reduced at 6 weeks on the biotin-deficient diet.

Discussion. Fructose and sorbitol seem to exert a beneficial effect on biotin-deficient animals as judged from amino acid incorporation studies (Table I). It was shown (12) that the vitamin-sparing effect of sorbitol was due to increased synthesis of vitamins by intestinal microflora stimulated by the slowly absorbing sugar and ingestion by the animal of its own vitamin-rich feces. Since corophagy was prevented in the present experiment, the beneficial effect of sorbitol on amino acid incorporation could not be due to the above reason. Furthermore, liver propionyl CoA carboxylase was decreased in the sorbitol-fed animals to the same extent as in deficient animals fed the basal diet where glucose was the only source of the carbohydrate. This clearly shows that the sorbitol fed animals were biotin-deficient.

Fructose is absorbed better than sorbitol from the gastrointestinal tract. Furthermore, fructose has been shown to have no vitamin-sparing effect (12). Beneficial effect of fructose feeding could then be due to its better utilization by the animal. In the intact animal, sorbitol gives rise to fructose rather than to glucose (13). Therefore, the beneficial effect of sorbitol feeding could be the result of its transformation into fructose.

The glucose tolerance curve (Fig. 1) resembles that of mild diabetes. Increased urinary excretion of ketone bodies (Table II) also gives indication of certain similarities between biotin deficiency and mild diabetes.

Overall glucose phosphorylation appears to be affected in biotin deficiency (Table V) and this could be one of the reasons for the low incorporation of glucose into liver glycogen (Table III). Since fructose enters the glycolytic pathway through a different phosphorylation step, it is possible that it is better utilized than glucose in biotin deficiency. We have studied the effect of various stages of biotin deficiency on the initial steps of glucose and fructose metabolism, the profile of glycolytic and glycogen-cycle enzymes in liver and the patterns of carbohydrate utilization

by organs other than liver and these results will be reported in a separate paper.

Injection of insulin to biotin-deficient animals brought about an improvement in the synthesis of liver glycogen from glucose (Table III) and in the incorporation of leucine into microsomal protein (Table IV). This would suggest lack of insulin in the deficient animal but we found no change in histology of the β-cells of islets of Langerhans. In studies with anti-insulin serum, Wagle (14) reported that the level of insulin was not altered in biotin deficiency.

It is important to note that at about 6 weeks on the biotin-deficient basal diet, which is the usual period employed in our studies, the biotin-deficient animal just reaches maximum body weight (see reference 4). The animal becomes severely deficient, possibly developing secondary effects, only after 8 weeks on the deficient diet. In the study on oxidative phosphorylation (Table VI) at 6 weeks on the deficient diet the mitochondrial lipid in liver was significantly decreased. Correspondingly, the P/O ratio was also lowered. We showed that the reduction in the lipid of mitochondria was due to a reduction in palmitate and stearate (15). It is possible, therefore, that these changes in the fatty acid composition of the lipid might alter the structural integrity of mitochondria with the consequent effect on oxidative phosphorylation. This defect, as reflected by amino acid incorporation studies, was compensated during early stages of the deficiency when the animals received an oxidizable substrate such as succinate (see reference 1) or fructose or sorbitol in the basal diet. It is, therefore, possible that a moderate decrease in oxidative phosphorylation does not limit the efficiency of the biotin-deficient animal as long as adequate levels of oxidizable substrates are maintained. However, in advanced deficiency as observed after 8 weeks on the deficient diet, succinate, sorbitol, or fructose feeding did not help the animal. Only administration of biotin restored fatty acid synthesis and the integrity of mitochondria for normal oxidative phosphorylation. Thus, it would appear that in biotin deficiency energy production is impaired in two ways: by decreased utilization of glucose as evidenced also from a reduction in ascorbic acid synthesis (4) and by decreased oxidative phophorylation.

Summary. In biotin deficiency, energy production was impaired in two ways: by decreased utilization of glucose and by decreased oxidative phosphorylation. The decrease in the utilization of glucose was reflected in low glucose tolerance, a decrease in glycogen synthesis, and amino acid incorporation in liver proteins and an increase in the excretion of ketone bodies. Fructose or sorbitol feeding or insulin administration to the deficient animal brought about an improvement in amino acid incorporation. In biotin deficiency the lipid content of mitochondria was significantly decreased.

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