heterogeneity of proteoglycans from various connective tissues (Abstract). Scand. J. Clin. Lab. Invest. 29 (suppl. 123): 4 (1972).

3. Cooper, R. R., Ponseti, I. V., and Maynard J. A.: Pseudo-achondroplastic dwarfism: A rough-surfaced endoplasmic reticulum storage disorder. J. Bone Joint Surg., 53A: 475 (1973).

4. Deshmukh, K., and Nimni, M. E.: Effects of lysosomal enzymes on the type of collagen synthesized by bovine articular cartilage. Biochem. Biophys. Res. Commun., 424 (1973).

5. McDevitt, C. A., and Muir, H.: Gel electrophoresis of proteoglycans and glycosaminoglycans on large-pore composite polyacrylamide agarose gels. Anal. Biochem., 44: 612 (1971).

6. Miller, E. J.: Structural studies on cartilage collagen employing limited cleavage and solubilization with pepsin. Biochemistry, 11: 4903 (1972).

7. Miller, E. J., Martin, G. R., Piez, K. A., and Powers, M. J.: Characterization of chick bone collagen and compositional changes associated with maturation. J. Biol. Chem., 242: 5481 (1967).

8. Nimni, M. E., and Deshmukh, K.: Differences in collagen metabolism between normal and osteo-arthritic human articular cartilage. Science, 181: 751 (1973).

9. Rosenberg, L., Hellmann, W., and Leinschmidt, A. K.: Macromolecular models of proteinpolysaccharides from bovine nasal cartilage based on electron microscopic studies. J. Biol. Chem. 245: 4123 (1970).

10. Sajdera, S. W., and Hascall, V. C.: Proteinpolysaccharide complex from bovine nasal cartilage: A comparison of low and high shear extraction procedures. J.

Biol. Chem., 244: 77 (1969).

11. Stanescu, V., Bona C., and Ionescu, V.: The tibial growing cartilage biopsy in the study of growth disturbances. Acta Endocrinol., 64: 577 (1970).

12. Stanescu, V., Maroteaux, P., and Sobczak, E.: Gel electrophoresis of the proteoglycans of the growth and of the articular cartilage from various species. Biomedicine, 19: 460 (1973).

13. Stanescu, R., and Stanescu, V.: Unpublished results.

14. Stanescu, V., Stanescu, R., and Szirmai, J. A.: Microchemical analysis of the human tibial growth cartilage in various forms of dwarfism. Acta Endocrinol., 69: 659 (1972).

15. Stark, M., and Kuhn, K.: The properties of molecular fragments obtained on treating calfskin collagen with collagenase from clostridium histolyticum. Eur. J. Biochem., 6: 534 (1968).

16. The technical assistance of Mrs. E. Sobczak and Miss M. P. Richard is gratefully acknowledged.

17. The research contained in the present article was performed with the informed consent of the patients or of the parents of the patients.

18. Portions of this paper were presented at the 22nd Annual Colloquium on Protides of the Biological Fluids, May 1974, Brugge, Belgium.

19. Requests for reprints should be addressed to: V. Stanescu, M.D., Unité de Recherches de Génétique Médicale, Hôpital des Enfants-Malades, 149, rue de Sèvres, Paris (France).

20. Accepted for publication June 13, 1975.

Glycine   ketotic hyperglycinemia
glycine synthase system   vitamin B_{12} deficiency

The Vitamin B_{12}-deficient Rat as a Possible Model of Ketotic Hyperglycinemia

BARBARA O'NEILL ROWLEY, VIRGINIA BROTHERS, AND THEO GERRITSEN[40]

Departments of Pediatrics and Physiological Chemistry, Waisman Center on Mental Retardation and Human Development, University of Wisconsin Center for Health Sciences, Madison, Wisconsin, USA

Extract

The rate of oxidation to respiratory CO_2 of both carbon 1 of propionate and carbon 1 of glycine was decreased significantly in vitamin B_{12}-deficient rats, to 50% and 82% of the control rate, respectively. The activity of the glycine synthase system was reduced during vitamin B_{12} deficiency to 25% of control activity. Serine hydroxymethyltransferase activity was similar for vitamin B_{12}-deficient and control rats. Plasma glycine concentration in vitamin B_{12}-deficient rats (253 ± 16 nmol/ml) did not differ significantly from that of control rats (226 ± 12 nmol/ml). Propionate oxidation was significantly impaired in biotin-deficient rats. However, this impairment, to 66% of the control rate, was not as large as that generated by vitamin B_{12} deficiency. In contrast to the result obtained in vitamin B_{12}-deficient animals, no significant decrease in glycine oxidation could be demonstrated in biotin-deficient animals Plasma glycine concentration of fasted biotin-deficient rats (339 ± 26 nmol/ml) did not differ significantly from that of their controls (371 ± 32 nmol/ml).

Speculation

Activity of the glycine synthase system is reduced in both the ketotic and nonketotic forms of hyperglycinemia. The decrease in glycine synthase system activity in vitamin B_{12}-deficient rats may be generated by a mechanism similar to that in ketotic hyperglycine-

mia, and therefore vitamin B_{12}-deficient rats may be useful to study this mechanism.

In 1961, Childs *et al.* (5) described a patient with a disorder with episodic vomiting, lethargy, ketosis, developmental retardation, and hyperglycinemia and hyperglycinuria. Later studies demonstrated that this disease resulted from an inherited deficiency of propionyl-CoA carboxylase (ATP hydrolyzing) (EC. 6.4.1.3) (13). Similar symptoms may be present in patients with a deficiency in methylmalonyl-CoA mutase (EC. 5.4.99.2) (17, 19) and presumed β-ketothiolase deficiency (acyl-CoA:acetyl-CoA C-acyl transferase, EC. 2.3.1.16) (9). The role of these enzymes in propionate metabolism is shown in Figure 1.

The defect in glycine metabolism in the different forms of ketotic hyperglycinemia appears to be secondary to the impairment in organic acid metabolism. The mechanism of interaction of organic acid metabolism with glycine metabolism is as yet unexplained. The major pathway for glycine degradation in mammals appears to be via conversion to methylenetetrahydrofolate and CO_2, catalyzed by the glycine synthase system (EC. 2.1.2.10) (28). The ketotic hyperglycinemia syndrome differs clinically from the metabolic disease, nonketotic hyperglycinemia, a disorder first described by Gerritsen *et al.* (7). Nonketotic hyperglycinemia is characterized principally by severe mental retardation and caused by an inherited primary defect in the glycine synthase system (26).
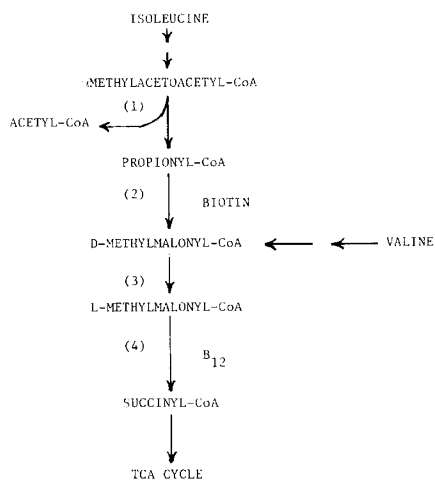
ISOLEUCINE

↓

METHYLACETOACETYL-CoA

(1)

ACETYL-CoA ←

PROPIONYL-CoA

(2)   BIOTIN

D-METHYLMALONYL-CoA ← ← VALINE

(3) ↓

L-METHYLMALONYL-CoA

(4) B_{12}

SUCCINYL-CoA

↓

TCA CYCLE

Fig. 1. The enzymatic pathway of propionate and methylmalonate metabolism in mammals. The numbered reactions refer to the following enzymes. *(1):* β-ketothiolase; *(2):* propionyl-CoA carboxylase; *(3):* methylmalonyl-CoA racemase; *(4):* methylmalonyl-CoA mutase.

In this report a possible animal model for the ketotic hyperglycinemia syndrome is proposed that can be used to study the interaction of organic acid metabolism with glycine metabolism in the intact animal. To produce this animal model, rats were fed a diet deficient in vitamin B_{12}. As shown in Figure 1, propionyl-CoA carboxylase *(enzyme (2))* and methylmalonyl-CoA mutase *(enzyme (4))* are enzymes which depend on cofactors derived from the vitamins biotin and B_{12}, respectively. The activity of propionyl-CoA carboxylase has been shown to be reduced in rats fed a biotin-deficient egg white diet (2, 15). Methylmalonyl-CoA mutase activity is reduced during vitamin B_{12} deficiency in rats (20). Thus, deficiency of biotin or of vitamin B_{12} generates an enzyme defect in propionate metabolism comparable with that found in the respective forms of ketotic hyperglycinemia, and may be a useful animal model for the investigation of glycine metabolism in this syndrome. The present investigation reports studies on the oxidation of propionate and glycine to respiratory CO_2 in rats fed diets deficient in biotin or vitamin B_{12} and on glycine synthase system activity *in vitro* in the livers of vitamin B_{12}-deficient rats. The evidence to be presented here supports the use of vitamin B_{12}-deficient rats as a possible animal model of ketotic hyperglycinemia. The biotin-deficient rats did not demonstrate an impairment in glycine metabolism.

MATERIALS AND METHODS

REAGENTS

Sodium [1-^{14}C]propionate and [1-^{14}C]glycine were purchased from New England Nuclear (29); L-[*ring*-2-^{14}C]histidine and L-[3-^{14}C]serine from Amersham/Searle Corporation (30). The ^{14}C-labeled glycine and serine were purified before use on a cation exchange resin. All radioactive compounds were mixed with amounts of the unlabeled compound to produce the desired specific activity and dosage.

Folic acid and vitamin B_{12} were obtained from Nutritional Biochemicals (31); biotin from Calbiochem (32); NAD, pyridoxal phosphate, dithiothreitol, and digitonin from Sigma Chemical Company (33); and 5,5'-dimethyl-1,3-cyclohexanedione (dimedone) from Eastman Kodak (34). Tetrahydrofolate was synthesized from folate according to the method of Davis (6). The purity and concentration of tetrahydrofolate was measured by quantitative conversion to 5,10-methylidyne tetrahydrofolate using a molar extinction coefficient at 350 nm of 26×10^3 (21).

ANIMALS AND DIETS

The vitamin B_{12}-deficient basal diet (formula of Jaffe and Elvehjem (14)) was purchased from Teklad Mills (35). Two diets

were prepared from the basal diet. The vitamin B_{12}-deficient diet contained 995 g/kg basal diet, 5 g/kg L-methionine, and 10 mg/kg folate; the vitamin B_{12}-supplemented diet contained an additional 50 μg/kg vitamin B_{12}. Beginning at 25 days of age *(day 1)*, male Holtzman rats (36) were fed 10 g diet at the same time daily. The daily amount of diet was gradually increased to 14 g by *day 15* and maintained at that level. The body weight during the series of *in vivo* CO_2 studies ranged from 100 to 150 g.

The biotin-deficient diet was purchased from Nutritional Biochemicals and was prepared according to the formula of Rubin *et al.* (22). The supplemented diet contained 10 mg/kg biotin. Beginning at 25 days of age *(day 1)*, rats were fed 8 g of this diet at the same time daily. Experiments were performed after the rats were fed the diet for 26-36 days and weighed 100-125 g.

$^{14}CO_2$ STUDIES *IN VIVO*

Excretion of respiratory $^{14}CO_2$ after injection of ^{14}C-labeled compounds was used as a measure of the ability of the animals to catabolize the compounds. For analysis of respiration patterns, the rats were fed 5 g diet at the usual time. Three hours later 0.5 μCi in 0.10, 0.25, or 0.50 mmol/100 g body wt of the ^{14}C-labeled compound to be tested was injected intraperitoneally and CO_2 was collected at 10- or 15-min intervals. The percentage of the injected dose excreted as respiratory $^{14}CO_2$ in each 10- or 15-min fraction was determined as described previously (4).

ENZYME ASSAYS

Enzyme assays in the livers were performed 1-3 weeks after completion of the respiration pattern experiments. Rats were fed 5 g diet at the normal feeding time, decapitated 2-3 hr later, and the livers were removed immediately and placed on ice.

For assay of glycine synthase, 1 g liver was homogenized in a Potter-Elvehjem homogenizer in 10 volumes cold 0.25 M sucrose, 10 mM Tris-HCl buffer (pH 8.0). The homogenate was centrifuged at $900 \times g$ for 5 min at 4°. The $900 \times g$ supernatant was centrifuged at $12,000 \times g$ for 20 min to obtain a mitochondrial pellet. The mitochondria were resuspended in 0.25 M sucrose to a concentration of 80 mg protein/ml and then treated with digitonin by the method of Schnaitman and Greenawalt (23) in order to obtain the inner membrane-matrix fraction. A cold 2% (w/v) suspension of digitonin in 0.25 M sucrose was added to the mitochondrial suspension with constant stirring to give a final concentration of 1.5 mg digitonin/10 mg protein. After continuous stirring on ice for 20 min, the suspension was diluted with 5 volumes 0.25 M sucrose, homogenized by hand, and then cen-
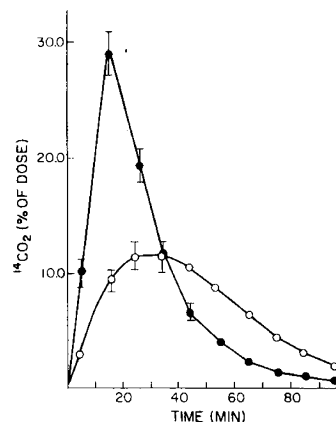
Fig. 2. Oxidation of carbon 1 of propionate to CO_2. [1-^{14}C]Propionate (0.5 μCi in 0.25 mmol/100 g body wt) was administered intraperitoneally. The percentage of the dose oxidized to CO_2/10 min is shown for vitamin B_{12}-deficient (O) and control (●) rats. Each point is the mean for three animals, and the bar represents the SE of the mean. Where no bar is shown, the SE of the mean was less than 1.0%.

Table 1. *Excretion of respiratory $^{14}CO_2$ by vitamin B_{12}-deficient and control rats after intraperitoneal injection of ^{14}C-labeled propionate, glycine, or histidine*

| | | % of dose excreted (mean ± SEM) | | |
	Total dose, mmol/100 g	Vitamin B_{12}-deficient rats	Control rats	Significance (P)
Compound				
Sodium [1-^{14}C]propionate[2]	0.25	36.1 ± 3.6 (3)[3]	72.1 ± 2.3 (3)	<0.001
[1-^{14}C]Glycine[4]	0.50	23.1 ± 0.7 (8)	28.2 ± 1.2 (6)	<0.001
L-[*ring*-2-^{14}C]Histidine[4]	0.10	10.8 ± 0.8 (4)	12.5 ± 2.1 (4)	NS[1]

[1] Not significant.

[2] Data for propionate are for 40 min.

[3] The number of animals is shown in parentheses.

[4] The data for glycine and histidine are for 60 min.

Table 2. *Activities of glycine synthase system and serine hydroxymethyltransferase in liver from vitamin B_{12}-deficient and control rats*

	Vitamin B_{12}-deficient rats	Control rats	Significance (P)
Glycine synthase system[1]	43 ± 6　(4)[2]	169 ± 23 (6)	<0.001
Serine hydroxymethyl-transferase[3]	412 ± 56　(4)	481 ± 54 (6)	NS[4]

[1] Expressed as nanomoles of CO_2 per mg of protein per hr.

[2] Mean ± SEM for the number of animals indicated in parentheses.

[3] Expressed as nanomoles of HCHO per mg of protein per hr.

[4] Not significant.

trifuged at 12,000 × g for 10 min. The supernatant was removed and discarded. The pellet was resuspended in 0.25 M sucrose to give a final protein concentration of approximately 10 mg/ml. This suspension was assayed for glycine synthase activity.

Glycine synthase was assayed in a system similar to that of Motokawa and Kikuchi (18). Assays were performed in triplicate in closed 25 ml conical flasks fitted with a plastic well suspended from the stopper (37). In a final volume of 2.5 ml the incubation mixture contained: 125 μmol Tris-HCl buffer (pH 8.0), 0.625 μmol pyridoxal phosphate, 0.625 μmol NAD, 25 μmol dithiothreitol, 1.25 μmol tetrahydrofolate, 25 μmol glycine containing 0.5 μCi of [1-^{14}C]glycine, and an aliquot of the digitonin-treated enzyme preparation containing 5–10 mg protein. All components except tetrahydrofolate and glycine were preincubated for 5 min at 37°. The reaction was initiated by the simultaneous addition of tetrahydrofolate and glycine and the incubation was continued for 30 min. The center well contained 0.2 ml of 1 M hydroxide of Hyamine in methanol. The reaction was stopped by injection of 0.1 ml of 10 N H_2SO_4 into the incubation mixture. The flasks were shaken for 1 hr after acidification to trap the released CO_2 in the Hyamine. Radioactivity in each Hyamine trap was counted in 10 ml Bray's scintillator (3). For the blanks, acid was added before incubation. The reaction rate was linear with enzyme concentration and time under these conditions.

Serine hydroxymethyltransferase activity was measured by a modification of the method of Taylor and Weissbach (27). The major modifications were the use of saturating levels of L-serine and pH 8.0. One gram of liver was homogenized in a Potter-Elvehjem homogenizer in 20 volumes cold 0.25 M sucrose, 10 mM Tris-HCl buffer (pH 8.0). Aliquots of the homogenate were assayed in triplicate. Blanks consisted of the homogenate heat-inactivated for 5 min at 100°. The assay system contained 50 μmol potassium phosphate buffer (pH 8.0), 0.1 μmol pyridoxal phosphate, 40 μmol 2-mercaptoethanol, 0.8 μmol tetrahydrofolate, and 2 μmol L-serine containing 0.2 μCi of L-[3-^{14}C]serine in an

incubation mixture volume of 0.3 ml. Fifty microliters of water were added to each tube so that the final volume after addition of enzyme was 0.4 ml. The reagents were preincubated for 5 min at 37°. The reaction was initiated by the addition of 50 μl homogenate and the incubation was continued for 15 min. The reaction was terminated and the H^{14}CHO isolated according to the method of Taylor and Weissbach (27). The reaction rate was linear with enzyme concentration and time under these conditions.

Propionyl-CoA carboxylase activity was measured by the method of Giorgio and Plaut (8).

Protein was determined by the method of Lowry *et al.* (16) using crystalline bovine serum albumin as a standard.

Quantitative amino acid analyses were determined using a Beckman/Spinco automatic amino acid analyzer (24).

RESULTS

The rate of oxidation of a loading dose of sodium [1-^{14}C]propionate to respiratory $^{14}CO_2$ was examined as a measure of the extent of derangement of propionate metabolism *in vivo* in vitamin B_{12} deficiency. The respiration patterns for the vitamin B_{12}-deficient and control rats are illustrated in Figure 2. Apparently oxidation of propionate depends on the vitamin B_{12} status of the animals. The total excretion of $^{14}CO_2$ in the first 40 min after injection of the dose of propionate for both groups of animals after 21 days on the diet is tabulated in Table 1.

The cumulative $^{14}CO_2$ excretion from [1-^{14}C]glycine in the first 60 min after injection is shown in Table 1. The rate of oxidation to respiratory CO_2 of both carbon 1 of propionate and carbon 1 of glycine was significantly decreased in vitamin B_{12}-deficient rats, in agreement with earlier studies in this laboratory (4). The rate of oxidation of L-[*ring*-2-^{14}C]histidine to $^{14}CO_2$, which is generally accepted as an index of folate deficiency, was unchanged, suggesting normal folate metabolism in the vitamin B_{12}-deficient group.

The activity of the glycine synthase system (Table 2) was reduced during vitamin B_{12} deficiency. This is consistent with the oxidation of [1-^{14}C]glycine to $^{14}CO_2$ by intact rats. Serine hydroxymethyltransferase activity was similar for vitamin B_{12}-deficient and control rats.

The concentration of glycine was measured in plasma from fasted rats which had been fed the vitamin B_{12}-deficient diet or control diet. Plasma glycine concentration in vitamin B_{12}-deficient rats (253 ± 16 nmol/ml, n = 4) did not differ significantly from that of control rats (226 ± 12 nmol/ml, n = 7).

The ability of biotin-deficient rats to oxidize propionate and glycine *in vivo* was examined. In order to check the extent of biotin deficiency in rats fed the diet used in this study, a group of rats was fed the biotin-deficient or control diets *ad libitum* for 9 weeks, after which liver propionyl-CoA carboxylase activity was determined. It was found that the propionyl-CoA carboxylase activity, expressed per gram of liver, in biotin-deficient animals was about 12% of the activity of control animals. Maximal reduction of propionyl-CoA carboxylase to approximately 15% of control

Table 3. *Excretion of respiratory $^{14}CO_2$ by biotin-deficient and control rats after intraperitoneal injection of ^{14}C-labeled propionate or glycine*

Compound	Total dose, mmol/100 g	% of dose excreted (mean ± SEM)		Significance (P)
		Biotin-deficient rats	Control rats	
Sodium [1-^{14}C]propionate[1]	0.50	24.0 ± 2.1 (3)[2]	36.5 ± 3.4 (3)	<0.05
[1-^{14}C]Glycine[3]	0.50	22.4 ± 0.7 (7)	23.9 ± 0.6 (7)	NS[4]

[1] Data for propionate are for 45 min.

[2] Mean ± SEM for the number of animals shown in parentheses.

[3] Data for glycine are for 60 min.

[4] Not significant.

activity within 3 weeks of feeding biotin-deficient egg white diets has been found by Arinze and Mistry (2) and Kosow and Lane (15). Therefore the time period of 26–36 days was considered adequate for development of biotin deficiency in the following experiments, in which the rats were fed as described under *Methods*. Table 3 shows the rate of formation of respiratory $^{14}CO_2$ from [1-^{14}C]propionate and from [1-^{14}C]glycine. Propionate oxidation was significantly impaired in biotin-deficient rats. However, this impairment was not as large as that generated by vitamin B_{12} deficiency. Apparently, the residual propionyl-CoA carboxylase activity (approximately 15% of control) in biotin-deficient rats is sufficient to permit nearly normal propionate metabolism *in vivo*. In contrast to the results obtained in vitamin B_{12}-deficient animals, no significant decrease in glycine oxidation could be demonstrated in biotin-deficient animals.

Plasma glycine concentration of fasted biotin-deficient rats (339 ± 26 nmol/ml, n = 5) did not differ significantly from that of controls (371 ± 32 nmol/ml, n = 4). It was concluded, therefore, that biotin deficiency in rats could not be used as a model for ketotic hyperglycinemia.

The biotin control rats differed from the vitamin B_{12} control rats in plasma glycine concentration and in rate of oxidation of propionate and glycine. Because these two control diets differ in protein content and source, amount fed, and growth response elicited, metabolism of glycine, propionate, and other intermediates would not be expected to be comparable. The only valid comparisons are those between the deficient animals and their matched controls.

DISCUSSION

The evidence reported here supports the use of vitamin B_{12} deficiency in rats as an animal model for the ketotic hyperglycinemia syndrome. It may be summarized as follows. (*1*) In vitamin B_{12}-deficient rats, propionate metabolism is impaired *in vivo*. This can be demonstrated by a reduced rate of oxidation of propionate to respiratory CO_2. In patients with ketotic hyperglycinemia due to propionic or methylmalonic acidemia, propionate metabolism is impaired, as demonstrated by defective oxidation of tracer doses of [1-^{14}C]propionate to respiratory $^{14}CO_2$ (1). (*2*) In vitamin B_{12}-deficient rats, glycine metabolism is impaired, as can be demonstrated by a reduced rate of oxidation of [1-^{14}C]glycine to respiratory $^{14}CO_2$. In patients with ketotic hyperglycinemia, glycine metabolism is impaired *in vivo* as was demonstrated by reduced oxidation of a tracer dose of [1-^{14}C]glycine to respiratory $^{14}CO_2$ (1). (*3*) In vitamin B_{12}-deficient rats, the activity of the liver glycine synthase system is reduced to approximately 25% of the control level. However, activity of liver serine hydroxymethyltransferase is unchanged. These results are comparable with those obtained from the liver of a patient with methylmalonic acidemia, which contained a normal activity of serine hydroxymethyltransferase but significantly reduced activity of the glycine synthase system (25).

It is not clear at this point whether the changes in the glycine synthase system activity reflect changes in enzyme (protein) concentration or the possible presence of inhibitors of enzyme activity which may have accumulated because of diminished methylmalonyl-CoA mutase activity in vitamin B_{12} deficiency. The identity of the possible inhibitors was not investigated in this work. Hillman *et al.* (11) have demonstrated that glycine oxidation to CO_2 was markedly inhibited when cultured fibroblasts from a patient with β-ketothiolase deficiency were incubated with isoleucine. Further studies by Hillman and Otto (10) have suggested that a metabolite of isoleucine, perhaps tiglyl-CoA, inhibited the glycine-serine interconversion. Activity of serine hydroxymethyltransferase, which catalyzes this interconversion, was the same in vitamin B_{12}-deficient rat livers as in control livers *in vitro*. It is possible that inhibition of this enzyme *in vivo* by an abnormal metabolite would not be detected *in vitro* under these assay conditions, in which saturating concentrations of tetrahydrofolate and serine were used. Under the conditions of the glycine synthase system assay, which employs a lower concentration of tetrahydrofolate, serine hydroxymethyltransferase activity might be inhibited. This could cause a decreased conversion of methylenetetrahydrofolate back to tetrahydrofolate, reducing the amount of this substrate present, and could lead to a lower glycine synthase system activity in the vitamin B_{12}-deficient livers.

Vitamin B_{12} deficiency in rats is not fully consistent with the ketotic hyperglycinemia syndrome in that the plasma glycine concentration in fasted vitamin B_{12}-deficient rats is the same as that in vitamin B_{12}-supplemented controls. Elevated plasma glycine levels, however, are not a consistent feature of propionic acidemia (12) or methylmalonic acidemia (17). Decreased glycine synthase system activity may be reflected in elevated plasma glycine levels only when residual activity is lower than that generated by vitamin B_{12} deficiency. A differential in plasma glycine concentration between vitamin B_{12}-deficient and vitamin B_{12}-supplemented animals might be generated experimentally by glycine loading.

SUMMARY AND CONCLUSION

Vitamin B_{12}-deficient rats demonstrate impaired oxidation of carbon 1 of glycine to CO_2 *in vivo* and a decreased liver glycine synthase system activity *in vitro*. Biotin-deficient rats do not demonstrate a reduced oxidation of carbon 1 of glycine to CO_2 *in vivo*. These results in vitamin B_{12}-deficient rats are comparable with those observed in patients with ketotic hyperglycinemia.

REFERENCES AND NOTES

1. Ando, T., Nyhan, W. L., Connor, J. D., Rasmussen, K., Donnell, G., Barnes, N., Cottom, D., and Hull, D.: The oxidation of glycine and propionic acid in propionic acidemia with ketotic hyperglycinemia. Pediat. Res., *6:* 576 (1972).
2. Arinze, J. C., and Mistry, S. P.: Activities of some biotin enzymes and certain aspects of gluconeogenesis during biotin deficiency. Comp. Biochem. Physiol., *38B:* 285 (1971).

3. Bray, G. A.: A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Anal. Biochem., 1: 279 (1960).
4. Brothers, V., Rowley, B. O., and Gerritsen, T.: Oxidation of compounds metabolized through folate coenzyme pathways in vitamin B_{12}-deficient rats. Arch. Biochem. Biophys., 166: 475 (1975).
5. Childs, B., Nyhan, W. L., Borden, M., Bard, L., and Cooke, R. E.: Idiopathic hyperglycinemia and hyperglycinuria: a new disorder of amino acid metabolism. I. Pediatrics, 27: 522 (1961).
6. Davis, L.: A simple method for the synthesis of tetrahydrofolic acid. Anal. Biochem., 26: 459 (1968).
7. Gerritsen, T., Kaveggia, E., and Waisman, H. A.: A new type of idiopathic hyperglycinemia with hypo-oxaluria. Pediatrics, 36: 882 (1965).
8. Giorgio, A. J., and Plaut, G. W. E.: The effect of univalent cations on activities catalyzed by bovine-liver propionyl-CoA carboxylase. Biochim. Biophys. Acta, 139: 487 (1967).
9. Hillman, R. E., Feigin, R. D., Tenenbaum, S. M., and Keating, J. P.: Defective isoleucine metabolism as a cause of the "ketotic hyperglycinemia" syndrome. Pediat. Res., 6: 394 (1972).
10. Hillman, R. E., and Otto, E. F.: Inhibition of glycine-serine interconversion in cultured human fibroblasts by products of isoleucine catabolism. Pediat. Res., 8: 941 (1974).
11. Hillman, R. E., Sowers, L. H., and Cohen, J. L.: Inhibition of glycine oxidation in cultured fibroblasts by isoleucine. Pediat. Res., 7: 945 (1973).
12. Hommes, F. A., Kuipers, J. R. G., Elema, J. D., Jansen, J. F., and Jonxis, J. H. P.: Propionic acidemia, a new inborn error of metabolism. Pediat. Res., 2: 519 (1968).
13. Hsia, Y. E., Scully, K. J., and Rosenberg, L. E.: Inherited propionyl-CoA carboxylase deficiency in "ketotic hyperglycinemia." J. Clin. Invest., 50: 127 (1971).
14. Jaffe, W. G., and Elvehjem, C. A.: Fractionation of growth-stimulating factor in liver. J. Biol. Chem., 169: 287 (1947).
15. Kosow, D. P., and Lane, M. D.: Restoration of biotin-deficiency-induced depression of propionyl carboxylase activity in vivo and in vitro. Biochem. Biophys. Res. Commun., 4: 92 (1961).
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265 (1951).
17. Morrow, G., III, Barness, L. A., Auerbach, V. H., DiGeorge, A. M., Ando, T., and Nyhan, W. L.: Observations on the coexistence of methylmalonic acidemia and glycinemia. J. Pediat., 74: 680 (1969).
18. Motokawa, Y., and Kikuchi, G.: Glycine metabolism in rat liver mitochondria. V. Intramitochondrial localization of the reversible glycine cleavage system and serine hydroxymethyltransferase. Arch. Biochem. Biophys., 146: 461 (1971).

19. Oberhalzer, V. G., Levin, B., Burgess, E. A., and Young, W. F.: Methylmalonic aciduria—an inborn error of metabolism leading to chronic metabolic acidosis. Arch. Dis. Childhood, 42: 492 (1967).
20. Reed, E. B., and Tarver, H.: Urinary methylmalonic acid and hepatic methyl-malonyl-CoA mutase activity in the vitamin B_{12}-deficient rat. J. Nutr., 100: 935 (1970).
21. Rosenthal, S., Smith, L. C., and Buchanan, J. M.: Enzymatic synthesis of the methyl group of methionine. J. Biol. Chem., 240: 836 (1965).
22. Rubin, S. H., Drekter, L., and Moyer, E. H.: Biological activity of synthetic d,1-desthiobiotin. Proc. Soc. Exp. Biol. Med., 58: 352 (1945).
23. Schnaitman, C., and Greenawalt, J. W.: Enzymatic properties of the inner and outer membranes of rat liver mitochondria. J. Cell Biol., 38: 158 (1968).
24. Spackman, D. H., Stein, W. H., and Moore, S.: Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem., 30: 1190 (1958).
25. Tada, K., Corbeel, L. M., Eeckels, R., and Eggermont, E.: A block in glycine cleavage reaction as a common mechanism in ketotic and non-ketotic hyperglycinemia. Pediat. Res., 8: 721 (1974).
26. Tada, K., Narisawa, K., Yoshida, T., Konno, T., Yokoyama, Y., Nakagawa, H., Tanno, K., Mochizuki, K., Arakawa, T., Yoshida, T., and Kikuchi, G.: Hyperglycinemia: A defect in glycine cleavage reaction. Tohoku J. Exp. Med., 98: 289 (1969).
27. Taylor, R. T., and Weissbach, H.: Radioactive assay for serine transhydroxymethylase. Anal. Biochem., 13: 80 (1965).
28. Yoshida, T., and Kikuchi, G.: Major pathways of glycine and serine catabolism in rat liver. Arch. Biochem. Biophys., 139: 380 (1970).
29. Boston, Mass.
30. Arlington Heights, Va.
31. Cleveland, Ohio.
32. Los Angeles, Calif.
33. St. Louis, Mo.
34. Rochester, N. Y.
35. Division of ARS/Sprague-Dawley, Madison, Wisc.
36. Holzman Co., Madison, Wisc.
37. Kontes Glass Co., Vineland, N. J.
38. The authors thank Dr. James Bloomer for use of the apparatus for the respiration pattern experiments and Alan Hamstra for the amino acid analyses.
39. This study was made possible by Training Grant HD00131 from the National Institutes of Health.
40. Requests for reprints should be addressed to: T. Gerritsen, D.Sc., Professor of Pediatrics and Physiological Chemistry, University of Wisconsin, 613 Waisman Center, 2605 Marsh Lane, Madison, Wisc. 53706 (USA).
41. Accepted for publication June 25, 1975.

Uptake, Activation, and Esterification of Fatty Acids in the Small Intestine of the Suckling Rat

PHILIP G. HOLTZAPPLE,[46] GLEN SMITH, AND OTAKAR KOLDOVSKÝ

Department of Pediatrics, University of Pennsylvania School of Medicine, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA

Extract

The small intestinal mucosal phase of fatty acid absorption was studied in suckling and adult rats. Fatty acid binding protein (FABP) is present in the cytosol of jejunal mucosa of 6-day-old rats in amounts equivalent to that found in mucosal cytosol of adult rats (16.4% and 15.0%, respectively). The percentage of oleic acid binding to FABP is the same in 6-day-old and adult rats (13.9% and 10.2%, respectively). The specific activity of jejunal microsomal oleoyl-CoA synthetase is high in the fetus, falls abruptly after birth by the third day of life to remain constant thereafter into adult life. In contrast the specific activity of acyl-CoA:monoglyceride acyltransferase is low in the fetal jejunum, gradually increases, and is significantly higher in the 6- and 12-day-old rat than in the adult. Uptake of oleic acid by jejunal slices of 6- and 11-day-old animals is three- to fivefold higher than uptake by jejunal slices prepared from adults rats. The rate of esterification of oleic acid is higher in jejunal slices from 6- and 11-day-old rats, reflecting the enhanced uptake of oleic acid.

Speculation

In the suckling rat, the increased intestinal mucosal epithelial cell capacity for fatty acid esterification coincides with a diminished lipolytic activity within the lumen. This paradox suggests that the fatty acid esterification process in the small intestine of the suckling