The Oxidation of Glycine and Formate to CO₂ by Rat Liver Homogenates*

MARTIN P. SCHULMAN AND DAN A. RICHERT

From the Department of Biochemistry, State University of New York, Medical College at Syracuse

(Received for publication, February 9, 1959)

Literature on the subject of glycine metabolism has been reviewed by Weinhouse (1) and by Arnstein (2). The three catabolic pathways for which there has been most support are: (a) the deamination of glycine to glyoxylic acid, and dehydroxylation of the latter to CO₂ and formate; in a liver homogenate the formate is oxidized to CO₂ and water by catalase and the hydrogen peroxide produced by the action of xanthine oxidase on xanthine or hypoxanthine; (b) the conversion of glycine to serine and subsequent oxidation of the serine; and (c) the succinate-glycine cycle proposed by Shemin (3).

In the present studies, measurements were made of the rates of CO₂ formation from glycine-1-C₁⁴, glycine-2-C₁⁴, and formate-C₁⁴ by liver homogenates from rats which were fed diets deficient in either vitamin B₆, molybdenum, or folate acid. Studies with vitamin B₆-deficient livers were of interest, because it was found previously that blood from vitamin B₆-deficient ducklings in blood from vitamin B₆-deficient ducklings in evolution from both glycine carbons, but the oxidation of formate to CO₂ was diminished. Omission of folic acid from the diet not from formate was stimulated by the addition of pyridoxal-5'-phosphate. The impaired formate oxidation could be related to the observed decrease of xanthine oxidase in vitamin B₆-deficient livers. Liver homogenates depleted of xanthine oxidase in molybdenum deficiency gave essentially normal rates of CO₂ evolution from both glycine carbons, but the oxidation of formate to CO₂ was diminished. Omission of folate acid from the diet decreased the production of CO₂ from the α-carbon of glycine by about 75 per cent, and from the carboxyl carbon of glycine by about 25 per cent.

EXPERIMENTAL

Weanling male albino rats were fed a purified 24 per cent Labco casein diet (6) containing riboflavin, thiamine, pyridoxine, niacin, calcium pantothenate, and choline, but not added folate acid, vitamin B₁₂ or biotin. This diet produced very low levels of xanthine oxidase in the intestine and moderately decreased levels of xanthine oxidase in the liver. Weanling rats grew well on this diet and did not show symptoms of folic acid or vitamin B₁₂ deficiencies. The basal diet was modified by (a) the addition of 81 mg. of Na₂WO₄ 2 H₂O per kg. to produce very low levels of liver xanthine oxidase (9); (b) the addition of 0.2 mg. of folate acid, 0.025 mg. of vitamin B₁₂, and 0.08 mg. of biotin per 100 gm. of diet together or individually as indicated; and (c) the omission of pyridoxine from the diet containing added folate acid, vitamin B₁₂, and biotin.

The diets were fed for 2 to 3 weeks. The rats were decapitated; livers were immediately chilled in ice and a 10 per cent homogenate was prepared with cold 0.15 M KCl. The homogenate (0.4 ml.) was added to each vessel containing 1.2 ml. of incubation medium plus other additions in water as indicated to a total volume of 3.0 ml. The incubation medium was a modification of the one described by Pardee et al. (10) and was prepared by mixing the following; 1.3 ml. of 0.770 M KCl; 0.5 ml. of 0.0004 M cytochrome c; 1.5 ml. of 0.1 M potassium phosphate buffer, pH 7.4; 0.5 ml. of 0.1 M MgCl₂; and 2.2 ml. of H₂O. Ten μmoles of labeled substrate with the following specific activities were included in each vessel: glycine-1-C₁⁴ (25,600 c.p.m. per μmole), glycine-2-C₁⁴ (100,000 c.p.m. per μmole), or sodium formate-C₁⁴ (40,000 c.p.m. per μmole). The incubation was carried out in a Dubnoff metabolic shaker at 37° in air in a special 20-ml. beaker without lip and covered with a tight rubber cap; a center well contained 0.2 ml. of 5 N NaOH. After 1 hour of incubation with shaking, 0.2 ml. of 6 N HCl was injected into the main compartment to stop the reaction. The vessels were shaken for an additional 10 minutes in order to absorb all the released C₂O₄²⁻ in the center well. The contents of the center well were collected quantitatively by washing with CO₂-free H₂O; 25 μmoles of Na₂C₂O₄ were added, and an aliquot (.1/2 of total) was added to 5 per cent BaCl₂. The resulting BaCO₃ was collected on a filter paper, washed with H₂O, dried with acetone, and assayed for C₁⁴ directly on the filter paper. The samples were assayed in duplicate in a flow counter and corrected to infinite thinness (11), and the corrected counts per minute were calculated to give the number of μmoles of each substrate converted to CO₂.

Liver xanthine oxidase was determined by the procedure of Axelrod and Elvehjem (12) and the activity was expressed as c.mm. of O₂ per 20 min. per 283 mg. of fresh liver.

RESULTS

Molybdenum Deficiency—The oxidation of formate was depressed when the liver xanthine oxidase was depleted (Table 1).
Only 50 mpmoles of formate were converted to CO₂ when the liver xanthine oxidase was "zero," as compared with 208 to 282 mpmoles of formate oxidized to CO₂ by livers from rats fed the purified diet plus folic acid, whereas only 16 to 20 mpmoles were oxidized by livers from rats fed the purified diet with or without biotin and vitamin B₁₂. Apparently the mechanism by which the α-carbon of glycine is converted to CO₂ is very sensitive to the amount of folic acid in the diet. Moreover, the conversion of the carboxyl carbon of glycine to CO₂ was affected much less on a percentage basis by dietary folic acid; the values for the 24 per cent casein diet with and without biotin were 254 and 308, respectively, whereas in the presence of folic acid or vitamin B₁₂ or both they were 357 to 409 mpmoles. With normal liver, the carboxyl carbon of glycine was converted to CO₂ at least 5 times as rapidly as the α-carbon. This is in agreement with the results obtained by Nakada and Weinhouse (16).

A relationship of folic acid to the metabolism of the α-carbon of glycine has been demonstrated previously in several ways. There is a decreased rate of formate and CO₂ production in vivo by folic acid deficient rats (7). Vohra et al. (17) found a decreased incorporation of glycine-2-¹⁴C in the 3 position of serine by liver homogenates of folic acid deficient turkey poult. Krutzer and Lantz (18) could counteract glycine toxicity in pouls by increasing the intake of folic acid. They believed that glycine manifests its toxicity by creating a folic acid deficiency. The results in this paper show that there is much less of a folic acid requirement for decarboxylation of glycine than for the metabolism of the α-carbon.

The folic acid, as tetrahydrofolic acid (19), must transfer much of the α-carbon of glycine to some acceptor in the liver homogenate, because only one-fifth as much α-carbon as carboxyl carbon goes to CO₂. Since the incorporation of glycine-2-¹⁴C into the 3 position of serine is decreased in folic acid deficiency, glycine must be one such acceptor.

Vitamin B₆ Deficiency—A vitamin B₆ deficiency decreased the rate of CO₂ formation from all three of the radioactive substrates.
The addition of pyridoxal-5'-phosphate to the liver homogenate stimulated the formation of CO₂ from both glycine carbons in both the deficient and control homogenates. With the deficient livers the formation of CO₂ from the carboxyl and α-carbons of glycine was increased 75 and 61 per cent, respectively, by the addition of pyridoxal-5'-P in vitro. The oxidation of the two substrates was stimulated 16 and 37 per cent, respectively, in the control homogenates.

These results indicate that pyridoxal-5'-phosphate is required early in glycine metabolism but do not clarify the pathway by which CO₂ is formed. Pyridoxal-5'-P could be involved in a glycine transamination reaction to yield glyoxylate (20) or in the succinate-glycine cycle to form δ-aminolevulinic acid (21, 22). If the metabolism were through serine, pyridoxal-5'-P could be involved in a reversal of the reactions by which serine is converted to glycine, as described by Huennekens et al. (23).

The decreased formate oxidation by vitamin B₆-deficient livers was apparently due to a decreased liver xanthine oxidase. In a comparable experiment, the average liver xanthine oxidase activity of ten rats, weighing 93 gm. after 16 days on the deficient diet, was 7 c.mm. of O₂ per 20 minutes. Control rats receiving the same diet plus vitamin B₆ weighed 137 gm. and had an average liver xanthine oxidase activity of 25. Pyridoxal-5'-P, added in vitro, decreased formate oxidation in both the control and deficient homogenates and acted as an inhibitor of xanthine oxidase.

**SUMMARY**

1. The oxidation of formate to CO₂ by rat liver homogenates depleted of xanthine oxidase was decreased.
2. An impaired conversion of glycine α-carbon and, to a much lesser extent, of carboxyl carbon to CO₂ was found in homogenates of livers from rats fed a purified diet unsupplemented with folic acid.
3. CO₂ formation from both glycine carbons and from formate was decreased in homogenates of livers from vitamin B₆-deficient rats. Pyridoxal-5'-phosphate, added in vitro, enhanced the CO₂ production from both glycine carbons in the control as well as the deficient livers.

**REFERENCES**

The Oxidation of Glycine and Formate to \( \text{CO}_2 \) by Rat Liver Homogenates
Martin P. Schulman and Dan A. Richert