Folic Acid Conjugase: Inhibition by Unconjugated Dihydroxy Bile Acids[1] (34388)

LESLIE H. BERNSTEIN, SIDNEY GUTSTEIN, AND SIMON WEINER
(Introduced by T. H. Spaet)
Department of Medicine, Lincoln Hospital Division, Albert Einstein College of Medicine, Bronx, New York 10454

Naturally occurring folic acid in both plant (1) and animal tissue (2) has been shown to occur in the form of polyglutamates containing two to seven L-glutamic acid residues in gamma carboxy alpha amino linkage. The terminal glutamic residue possesses two free carboxyl groups. These polyglutamates are apparently storage forms of folic acid.

In the mammal hydrolysis of these compounds occurs within the small bowel either intraluminally (3) or within the mucosal brush border (4). The enzyme presumably responsible for the hydrolysis and absorption of dietary folate is gamma glutamyl carboxypeptidase (conjugase) (5). Since certain patients with malabsorption may respond to microgram quantities of free folic acid (6), but not to larger quantities of the vitamin in the diet, it appeared reasonable to consider the possibility that intestinal conjugase activity in such patients was either absent or inhibited. We examined tri- and dihydroxy bile acids for their effect on conjugase because of the finding of altered bile salts in some malabsorptive states in which malabsorption is due to bacterial proliferation (7).

Method. Folic acid polyglutamate was prepared by a modification of the method of Schertel (8). Yeast extract was chromatographed on a column of anionic acrylamide resin (BIO-GEL DM-2) using a zero to 0.5 *M* phosphate buffer pH 6.8 as a gradient eluant. Mercaptoethanol, 0.5%, was added to prevent oxidation of folate compounds.

Tubes containing the folic acid heptaglutamate were pooled and concentrated by pressure ultrafilatration through a synthetic polymeric membrane (DIAFLO U M-2) which allows passage of compounds with molecular weights of 1000 or less. After the filtrate was discarded, the remaining material was virtually free of folic acid and mercaptoethanol but yielded (after exposure to chicken pancreas) 1×10^{-5} g/ml of folic acid (*L. casei*). This material was used as substrate.

The enzyme was prepared as follows: Adult guinea pigs were anesthetized with chloroform and then killed by exsanguination. The small bowel was rapidly removed and washed clean with 0.9 *M* NaCl solution at 4°. The mucosa was removed by compression of the bowel between a porcelain spatula and a glass plate. This material was suspended by mixing with glass beads in 3 vol of 0.001 *M* bicarbonate buffer, pH 7.4; the material was decanted, filtered through nylon gauze, and subjected to a spin of 122*g* for 10 min. This was sufficient to bring down particles of brush border and nuclear material and increased specific activity about 20 times. Sonication in 0.1 *M* PO_4 buffer, pH 6.1, produced a supernatant with about a 6-fold increase in specific activity. This supernatant solution was used as a source of conjugase.

Assays were run for 15 min at 37°. Tubes contained 1.0 ml of 0.1 *M* phosphate buffer at varying pH values or at pH 6.1 when inhibition studies were done. Folic polyglutamate 0.1 ml and 50 μl of 2-mercaptoethanol were added. The assays were stopped by exposure of the tubes to 100° for 10 min. Absence of mercaptoethanol led to large losses of folate at this step. The folic acid freed was assayed by the method of Herbert (9).

[1] Supported by U. S. Army Grant DADA17-68-C-8112.

Cholic, deoxycholic, and chenodeoxycholic acids were dissolved in absolute alcohol to yield a concentration of 20 mg/ml and from 1 to 50 μl were added to the assay tubes. Equivalent amounts of alcohol were added to control tubes, and a second set of control tubes containing a standard quantity of free folic acid were also run to guard against any effect of bile acid on the subsequent *L. casei* assay.

Results. Studies of pH optima of guinea pig conjugase showed a sharp peak at pH 6.1 in phosphate buffer. Using phosphate citrate buffer the pH shifted slightly to pH 5.3 (Fig. 1).

When bile acid studies were run at pH 6.1 in phosphate buffer the following was obtained: Cholic acid showed no suppression of conjugase below 2.0 μmoles/ml. At 2.0 μmoles/ml, suppression was minimal (Fig. 2). Deoxycholic acid showed marked inhibition between 0.5 and 1.0 μmoles/ml. At 2.0 μmoles/ml enzyme inhibition was complete (Fig. 2). Chenodeoxycholic acid showed early but incomplete inhibition between 0.05 and 0.2 μmoles/ml. With increasing concentration of this bile acid, inhibition of the enzyme increased. At 2.0 μmoles/ml of chenodeoxycholic acid there was no detectible activity of conjugase.

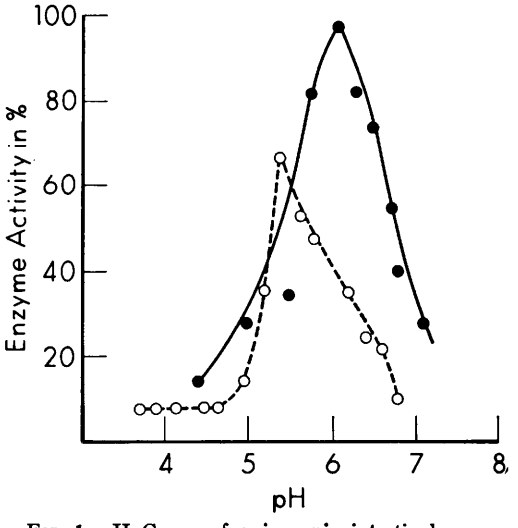

FIG. 1. pH Curve of guinea pig intestinal mucosa conjugase in phosphate (●); and citrate phosphate (○) buffer. Maximum value in phosphate is defined as 100% activity.

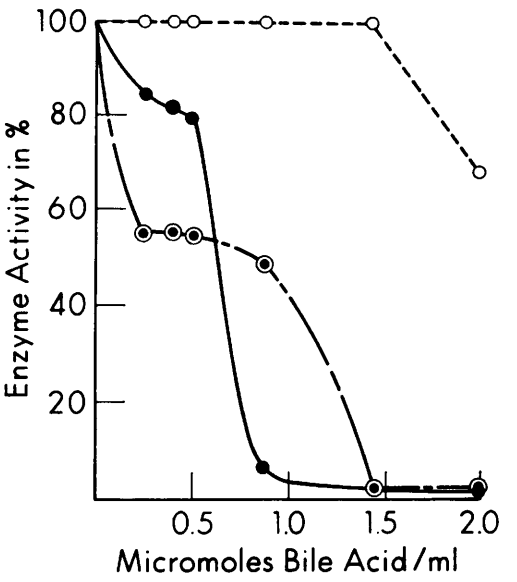

FIG. 2. Inhibition of guinea pig intestinal mucosa conjugase by dihydroxy bile acids: cholic (○); deoxycholic (●); and chenodeoxycholic (⊙).

Discussion. The results of this study show that the dihydroxy bile acids inhibit guinea pig intestinal conjugase while the trihydroxy bile acid, cholic acid, does not. Although inhibition of conjugase in humans has been reported to occur with diphenylhydantoin (10, 11) and oral contraceptives (12), this is the first report of inhibition of this enzyme by naturally occurring compounds.

There are several malabsorptive states in which bacterial proliferation occurs—blind loop syndrome (7), partial gastrectomy (7), small intestinal diverticuli (7), scleroderma (13), and tropical sprue (14). Malabsorption of fats in these disorders is felt to result from bacterial deconjugation and dehydroxylation of bile salts (15). If, indeed, the inhibition of conjugase by dihydroxy bile acids, as demonstrated *in vitro,* occurs *in vivo* as well, then this inhibition might provide an explanation for folic acid deficiency seen in some malabsorption states dominated by small intestinal bacterial proliferation.

Summary. Evidence is presented to show inhibition of guinea pig intestinal mucosa

gamma glutamyl carboxypeptidase (conjugase) by dihydroxy bile acids. Cholic acid, a trihydroxy acid, shows minimal inhibition at physiologic levels, while chenodeoxycholic and deoxycholic acids show striking inhibition.

1. Butterworth, C. E., Brit. J. Haematol. **14**, 339 (1968).

2. Usdin, E., J. Biol. Chem. **234**, 2373 (1959).

3. Santini, J. R., Berger, F. M., Berdasco, G., Sheehy, T. W., Aviles, J., and Davila, I., J. Am. Dietetic Assoc. **41**, 563 (1962).

4. Strief, R. N. and Rosenberg, I. H., J. Clin. Invest. **46**, 1121 (1967).

5. Rosenberg, I. H., Streiff, R. R., Godwin, H. A., and Castle, W. B., New Engl. J. Med. **280**, 985 (1969).

6. Sheehy, T. W., Perez-Santiago, E., and Rubini, M., New Engl. J. Med. **265**, 1232 (1961).

7. Tabaqchali, S. and Booth, C. C., Lancet **2**, 12 (1966).

8. Schertel, M. E., Bochne, J. W., and Libby, D., J. Biol. Chem. **240**, 3154 (1965).

9. Herbert, V., J. Clin. Pathol. **19**, 12 (1966).

10. Hoffbrand, A. V. and Necheles, T. F., Lancet **2**, 528 (1968).

11. Rosenberg, I. H., Streiff, R. R., Godwin, H. A., and Castle, W. B., Lancet **2**, 530 (1968).

12. Streiff, R. R., Clin. Res. **17**, 71 (1969).

13. Kahn, I. J., Jeffries, G. H., and Sleisenger, M. H., New Engl. J. Med. **274**, 1339 (1966).

14. Gorbach, S. L., Mitra, R., Jacobs, B., Banwell, J. G., Chatterjee, B. D., and Guha Mazumder, D. N., Lancet **1**, 74 (1969).

15. Tabaqchali, S., Hatzioannou, J., and Booth, C. C., Lancet **2**, 12 (1968).

Received July 28, 1969. P.S.E.B.M., 1969, Vol. 132.