# The Absorption of Milk-Bound Pteroylglutamic Acid from Small Intestine Segments[1] (36435)

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Previous studies have shown that crystalline pteroylglutamic acid (PGA) is rapidly absorbed from the rat jejunum and that this absorption is evenly distributed throughout a 25–40-cm length of the proximal small bowel. Folate deficient animals absorbed less folic acid than normals and this defect could be corrected by parenteral injection of PGA (1, 2). PGA absorption was diminished by "saturating" the rats with folic acid prior to the absorption test (1, 2). Since these data were obtained with crystalline PGA, it was decided to examine the ability of rat small bowel to absorb food-bound folate. As milk was found to contain a protein with powerful binding capacity for PGA (3, 4), the absorption of cow and goat milk-bound PGA was investigated and is reported in this paper.

*Materials and Methods.* Female rats of the Hebrew-University strain, weighing 100 (±15) g were used. The animals were kept in individual metabolic cages during the experiment. The normal controls were fed purina, while the folate-deficient animals were given a folate-poor diet for 20 days prior to the experiment, as described previously (5). A third group of rats received 1 mg PGA sc 3 hr before the experiment (PGA loaded animals).

The experimental procedures employed have been described in detail (2). Briefly they were as follows: the abdomen of the anethesized animals was opened and a polyethene tube, one end of which was attached to an impregnable plastic bag (3–4 ml volume) was inserted into the intestinal lumen at the selected site. The intestine, distal to the insertion was closed with a double ligature. The abdomen was closed and the animals were tube-fed 100 ng of $^{3}$H-PGA[2]; they were killed 24 hr after the operation. The radioactivity in the plastic bag, of the carefully emptied intestinal content together with the isotonic saline employed from its repeated washing, and of the wall of the corresponding segment was measured (Packard $\beta$-Liquid scintillation counter, Model 314-Ex). The amount of PGA absorbed was expressed as a percentage of the total administered dose.

The $^{3}$H-PGA bound to milk proteins was prepared as described by Ghitis *et al.* (3, 4). The folate binding capacity of goat milk was found to be about four-fold greater than that of cow milk (Grossowicz, unpublished observations). Milk was lyophilized and redissolved in appropriate volumes of water to permit the complete binding of about 100 ng PGA to a 3-ml sample, which was then fed to the rats.

*Results.* Absorption of milk-bound PGA was about 20% less than that of unbound PGA (Table I). There was no difference between the absorption of PGA bound to cow milk and that bound to goat milk. Contrary to the finding with free PGA, parenteral preloading with unlabeled folic acid did not diminish the subsequent absorption of milk-bound folic acid. The absorption of milk-bound folate from the isolated ileum was al-

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[2] $^{3}$H-PGA labeled in the 3- and 5-positions of the *p*-amino-benzoate moiety was obtained from The Radiochemical Center, Amersham, England. In order to remove degradation products of folate from the preparation, it was purified by chromatography according to the method of Jacques (6). The purified material was assayed with *Lactobacillus casei* and its specific activity was calculated accordingly.

TABLE I. The Absorption of Free and Milk-Bound $^{3}$H-PGA.[a]

| Experimental procedure | Number of animals | Percent of $^{3}$H-PGA absorbed in 24 hr mean | ± SD |
|---|---|---|---|
| A PGA, bag applied to caecum | 6 | 82.1 | 4.8 |
| B PGA, bag applied to end of jejunum | 6 | 71.8 | 5.3 |
| C Milk-bound PGA, bag applied to caecum | 9 | 65.7 | 3.7 |
| D Milk-bound PGA, bag applied to end of jejunum | 6 | 55.9 | 6.4 |
| E Milk-bound PGA, 3 hr after 1 $\mu$g parenteral folic acid, bag applied to caecum | 9 | 62.8 | 4.1 |
| F As E, bag applied to end of jejunum | 6 | 55.0 | 3.6 |

[a] 100 ng administered orally.

most three times higher than that of free PGA (Table II). Folate depleted animals absorbed normal amounts of milk-bound PGA, but the absorption of unbound PGA in such animals was diminished (Table III). Preloading with folic acid did not affect the absorption of milk-bound PGA in folate-depleted rats (Table III).

*Discussion.* The intestinal absorption of folate has been extensively studied in man and in experimental animals (1, 2, 7–10). In most of these studies the test material employed was either crystalline PGA or other mono-, or polyglutamates. In only a few instances has folate absorption been investigated with the vitamin administered in the form in which it occurs in natural foodstuffs (11–13). In the experiments described here PGA was bound to a naturally occurring folate-binding protein.

The absorption from the gut of PGA bound to goat or to cow milk was about 20% less than with unbound folic acid. The similar results obtained with PGA bound to cow milk or to goat milk is remarkable in view of the fact that the folate binding capacity of the latter is about four times greater than that of the former. It seems, therefore, that these differences in the folate binding capacity do not have any effect on the absorption of

TABLE II. Absorption of Free and Milk-Bound $^{3}$H-PGA From the Ileum.[a]

| Experimental procedure | Number of animals | Percent of $^{3}$H-PGA absorbed in 24 hr mean | ± SD |
|---|---|---|---|
| PGA, duodeno-ileal anastomosis, bag applied to caecum | 6 | 13.4 | 2.3 |
| Milk-bound PGA, otherwise as above | 6 | 38.4 | 3.7 |

[a] 100 ng PGA administered orally.

TABLE III. Absorption of Free, and of Milk-Bound $^3$H-PGA in Folate Deficient, and in Folate-loaded Rats.

| Experimental procedure | Number of animals | Percent of $^3$H-PGA absorbed in 24 hr mean | range |
|---|---|---|---|
| A $^3$H-PGA fed to folate deficient animals. Bag applied to end of jejunum | 6 | 58.0 | 51.0–64.4 |
| B Milk-bound $^3$H-PGA fed, otherwise as A | 6 | 59.4 | 53.6–66.8 |
| C As A, but 1 mg PGA given parenterally 3 hours before absorption test | 6 | 49.2 | 43.5–56.3 |
| D As B, but 1 mg PGA given parenterally 3 hr before absorption test | 6 | 61.7 | 54.9–67.3 |

the vitamin.

Since the total absorption of milk-bound folic acid was less than that of free folic acid, while ileal absorption was three times greater, it follows that the jejunal absorption of milk-bound folate was substantially less than that of free folic acid. Whether the decreased jejunal absorption was due to ineffective liberation of the folate from the binding proteins or to some other cause, cannot be established on the basis of the available data. The augmented ileal absorption of milk-bound PGA may have resulted from the slowing of the passage of the intestinal content through this segment with a concomitant prolongation of the exposure of the epithelial surface to the folic acid. Such a possibility is supported by our previous finding that substantial amounts of PGA can be absorbed from the ileum in jejunoprive rats (2).

*Summary.* Binding tritiated pteroylglutamic acid to cow or to goat milk protein was associated with a substantial reduction in its absorption from rat jejunum, while the ileal absorption increased markedly. There was no difference in the amount of folate absorbed whether it was bound to cow milk or to goat milk. Despite the fourfold higher folate binding capacity of goat milk than of cow milk, there was no difference in the amount of folate absorbed after their administration. Folate depletion or folate overload had no effect on the amount of the milk-bound folate absorbed, while such treatments decreased the absorption of free folate.

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