Absorption of Liver Folates from Small Intestinal Segments of the Rat[1] (37028)

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Marked differences were found in the intestinal absorption of folate in the rat between milk-bound and free pteroylglutamic acid (PGA) (1). Since in most investigations on folate absorption the "test substance" used was either PGA or other highly purified polyglutamates (2–7), the conclusions arrived at concerning the mechanisms of folate absorption may not be valid for food folate. To obtain more information on food folate absorption, rat liver containing ^{3}H-labeled folate was fed to animals and their absorption from selected intestinal segments was studied. The results of these experiments are described below.

Materials and Methods. Female rats of the Hebrew University strain, weighing 100 g (± 15 g) were used. The animals were kept in individual metabolic cages during the experiment. The control rats were fed Purina chow, while the folate-deficient rats were given a folate-poor diet for a period of 20 days (8). The third group of animals received 1 mg PGA subcutaneously 3 hr before the experiment (PGA loaded rats).

Two hundred microcuries of ^{3}H-PGA[2] were injected subcutaneously into each of 2 fasting rats 3 times at 48 hr intervals. Three days following the last injection, the animals were sacrificed by bleeding, the liver removed, rinsed thoroughly with saline, blotted with filter paper, weighed and homogenized in a pestle-type homogenizer. The folate was extracted from a measured aliquot and its quantity was estimated using *L. casei* as the assay organism according to the method described in detail before (10). The radioactivity of another measured aliquot was estimated and the specific activity of the liver folate was calculated accordingly. In order to determine the nature of the labeled folates in the liver, aliquots were subjected to column chromatography (11) which showed that over 80% of the radioactivity was derived from various forms of folates about half of it having been methyltetrahydrofolate, while about 15% of it could not be accounted for by either *L. casei, S. faecalis* or *P. cerevisiae*. An amount of the liver homogenate corresponding to 300 ng of folate, was fed by stomach tube to fasting rats.

The experimental procedures employed were described in detail (12, 13), and they were briefly as follows. The abdomen of the anesthetized animal was opened and a polyethylene tube, one end of which was attached to an impermeable plastic bag (3–4 ml vol) was inserted into the intestinal lumen at the selected site. Ileal absorption was studied in either jejunoprive or shunted animals. The intestine distal to the insertion was closed with a double ligature. The rats were sacrificed 24 hr after the operation. The radioactivity in the plastic bag, of the carefully emptied intestinal content proximal to it, and of the wall of the corresponding segment was measured. The amount of PGA absorbed was expressed as a percentage of the total administered dose.

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[2] ^{3}H-PGA labeled in the 3- and 5-positions of the *p*-aminobenzoate moiety was obtained from The Radiochemical Centre, Amersham, England. In order to remove degradation products of folate from the preparation, it was purified by chromatography according to the method of Jacquez (9). The purified material was assayed with *L. casei* and its specific activity was calculated accordingly.

TABLE I. Liver Folate Absorption from the Small Bowel.

Form of folate fed		Percentage of folate absorbed from			
		Normal animals		Folate depleted animals	
		Jejunum	Ileum	Jejunum	Ileum
^{3}H-PGA	Mean[a]	60.6	11.3	59.4	24.2
300 ng	Range	56–72	8–19	53–76	21–33
^{3}H-liver folate, raw	Mean	73.5	40.8	72.8	37.6
300 ng	Range	67–82	35–46	65–84	34–42
^{3}H-liver folate,boiled[b]	Mean	71.7	22.9	69.2	25.7
300 ng	Range	51–81	14–29	59–87	20–32
^{3}H-liver folate, fried[c]	Mean	70.4	41.7	67.6	31.0
300 ng	Range	62–78	35–54	65–70	24–46

[a] Each mean is based on at least 6 experiments.

[b] The liver was placed into boiling water for 15 min before homogenization. Further procedures in Methods.

[c] The liver was fried in vegetable oil for 10 min before homogenization. Further procedures in Methods.

Results. The absorption of liver folate was in general more efficient than that of crystalline PGA. This difference was particularly evident when folate was fed directly to the ileum; three times more liver folate was absorbed than free PGA when identical amounts of folate were fed. Frying the liver before feeding did not affect absorption. The ileal absorption of folate from boiled liver was found diminished (Table I). Folate-depleted animals absorbed liver-bound folate similarly to normal rats (Table I). PGA loading had no effect on the absorption of subsequently fed liver folate. By sacrificing the animals at varying time intervals after feeding the degree of absorption could be established. A substantial absorption of liver folate occurred already 2 hr after feeding and the peak was reached after 12 hr (Table II).

Discussion. Methods to assess the absorption of folate from the intestinal tract are based either on changes in the serum folate concentration or in the urinary excretion, or on the measurement of radioactive faecal or urinary folate. These methods have been widely employed in man and in experimental animals (12–24), when the oral test dose consisted mostly of either pteroylglutamic acid or one of its highly purified congeners. In the present study absorption was estimated by feeding "natural" food folates to rats using liver as a source, as compared to PGA and by measuring directly the folate content after passage through various segments (jejunum, ileum) of the intestine to determine the amount absorbed.

Substantial differences were observed in the handling of folates by the intestine depending upon whether they were supplied either as crystalline PGA or were fed as liver folate after it has been incorporated into this tissue. The absorption of the liver folate was more efficient and it was not affected by the folate status (folate depletion or overload) of the animal. These findings were in conformity with our observations in humans reported recently (25) and with those of Markkanen (26), that a much more rapid, pronounced and sustained serum folate elevation was obtained after ingestion of liver folates, than after oral administration of a comparable

TABLE II. Absorption of Liver-Bound Folate from Small Bowel at Varying Intervals After Feeding.

After feeding (hr)	No. of rats	^{3}H-PGA mean	Absorbed range
2	5	29.36	22.5–34.3
5	6	57.01	53.1–61.3
12	5	73.48	67.5–81.7
24	5	76.14	73.7–78.8

amount of crystalline PGA. The bulk of folates are present in the liver as methyltetrahydrofolate (27). The more efficient absorption of liver folates across the intestinal wall may be due to a more rapid transfer of reduced and methylated folates in comparison with PGA.

The marked ileal absorption of liver folates in comparison with PGA may have been the result of the slow passage of the raw undigested liver placed into this portion of the small bowel. The diminished folate absorption noted with PGA bound to a milk protein (1), as against the enhancement observed with liver folate may be due to different forms of folate and may also indicate that the part of the folate in the liver is bound and that the binding is different from that in milk.

The availability of liver folate for absorption was not altered by frying the liver prior to feeding but there was a marked drop in the ileal absorption of boiled liver folate. The diminished ileal absorption of folates from boiled liver may have resulted from the destruction of the liver conjugase(s) or of other liver factors by boiling making the polyglutamates still present in the liver (28) less available for absorption.

Summary. The absorption of ^{3}H-labeled folate incorporated in rat liver from small intestine segments of rats has been studied.

The absorption of liver folate from the jejunum and the ileum was highly efficient. Boiling the liver before feeding did not affect the jejunal absorption, but reduced markedly the availability of folate for ileal absorption, while frying the liver had no such effect.

Contrary to the observation with crystalline PGA, folate depletion or folate overload prior to feeding did not alter the absorption of liver folate.

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