Adaptive regulation of intestinal folate uptake: effect of dietary folate deficiency

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University of California Irvine-Long Beach Veterans Affairs Medical Program, Long Beach 90822; University of California Davis, Davis 95616; Veterans Affairs Greater Los Angeles Medical Program, University of California Los Angeles, Los Angeles, California 90073; Karmanos Cancer Institute, Detroit, Michigan 48201; and Memorial Sloan-Kettering Cancer Center, New York, New York 10021

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Said, Hamid M., Nabendu Chatterjee, Riaz ul Haq, Veedamali S. Subramanian, Alvaro Ortiz, Larry H. Matherly, F. M. Sirotnak, Charles Halsted, and Stanley A. Rubin. Adaptive regulation of intestinal folate uptake: effect of dietary folate deficiency. Am J Physiol Cell Physiol 279: C1889–C1895, 2000.—Folate is an essential micronutrient that, in mammals, must be obtained from exogenous sources via intestinal absorption. Previous studies have characterized different aspects of the mechanism of the intestinal folate uptake process. Much less, however, is known about regulation of this process. In this study, we examined the effect of dietary folate deficiency on intestinal folate uptake using the rat as an animal model. The results showed that dietary folate deficiency leads to a significant (P < 0.01) and specific upregulation in the transepithelial transport of folate acid. The upregulation in transepithelial folate transport 1 was found to be due to an induction in carrier-mediated folate uptake across the brush-border membrane (BBM) and was mediated via a significant (P < 0.01) increase in the maximal velocity but not the apparent Michaelis constant of the uptake process, 2 was associated with a marked increase in the steady-state mRNA level of reduced folate carrier-1 and in the level of the expressed protein at the intestinal BBM, and 3 was associated with a marked (>10-fold) increase in the activity of the intestinal BBM form of folate hydrolase. Results of this study demonstrate, for the first time, that dietary folate deficiency leads to a marked upregulation in intestinal folate uptake and in the activity of folate hydrolase. Furthermore, the upregulation in folate uptake is associated with an increase in mRNA and protein levels of folate carrier, suggesting possible involvement of a transcriptional regulatory mechanism(s) in the upregulation.

intestinal folate uptake; transport regulation; folate deficiency

THE COENZYME DERIVATIVES of folic acid (referred to here as folate) are necessary for the synthesis of purine and pyrimidine precursors of nucleic acids, for the metabolism of several amino acids (including homocysteine), and for the initiation of protein synthesis in mitochondria (2). An adequate supply of folate is therefore necessary for normal human health and well-being. This is manifested by the variety of clinical abnormalities that occur as a result of folate deficiency (that include megaloblastic anemia and growth retardation; see Refs. 2 and 10) and by the health-promoting/disease-preventing effects that result from supplementation with folic acid (e.g., prevention of neural tube defects; see Refs. 1, 14, 18, 21, 24, 37). Folate deficiency is a highly prevalent vitamin deficiency throughout the world (10, 41), and in the Western Hemisphere it often occurs as a result of impairment in the intestinal absorption process of the vitamin (6, 13, 15, 35).

Humans and other mammals cannot synthesize folate and thus must obtain the vitamin from exogenous sources via absorption in the intestine. Therefore, the intestine plays a central role in controlling and regulating folate body homeostasis. The intestine is exposed to folate from two sources: a dietary source, where the vitamin is absorbed in the small intestine, and a large intestine bacterial source, where the vitamin is synthesized by the normal microflora and absorbed by the large intestine. With regard to dietary folate, this folate exists mainly in the polyglutamate form that must be hydrolyzed to the monoglutamate form before absorption. This is accomplished by the specific action of the brush-border enzyme folate hydrolase (4). Absorption of folate monoglutamates then proceeds via a specialized, carrier-mediated system mainly in the proximal part of the small intestine (25, 30–32). The molecular identity of the intestinal folate uptake system has been recently delineated following its cloning from human and mouse small intestine (5, 22, 29). The open reading frame of the cloned intestinal cDNA was found to be similar (or identical in the case of the mouse) to that of the reduced folate carrier (RFC) of nonintestinal epithelial cells of the respective spe-
cies (8, 20, 23, 39, 40). As to the bacterially synthesized folate in the large intestine, recent studies from our laboratory have shown the existence of an efficient, carrier-mediated system for uptake of this source of folate at the apical membrane of colonocytes (9, 17). This system was found to be very similar (or identical) to that of the small intestine (25, 31, 32).

Compared with our current understanding of the mechanism of the intestinal folate uptake process, very little is known about its regulation. Recent studies have shown that transport of a variety of other nutrients in intestinal and other epithelia, including that of other water-soluble vitamins, is regulated by their dietary levels (11, 26, 28). This regulation, however, has been shown to be substrate specific. Dietary deficiency of biotin and riboflavin, for example, has been shown to lead to upregulation in intestinal uptake of these vitamins (11, 28), whereas a decrease in D-glucose and amino acid intake leads to downregulation in their intestinal uptake (11). In addition, the regulation of a nutrient transport process by dietary substrate level was also found to be tissue specific, as in the case of the Na\(^{+}\)-dependent phosphate cotransporter, which is upregulated in dietary phosphate deficiency in intestinal epithelial cells but not in pulmonary epithelial cells (34). The aim of the present study was to examine the effect of dietary folate deficiency on the intestinal folate uptake process using rats as the animal model.

**MATERIALS AND METHODS**

\[^{3}H\]folic acid (specific activity 25–30 Ci/mmol; radiochemical purity >97%) and all other radioactive materials were obtained from NEN and Amersham. All other chemicals, reagents, and kits used in this study were of analytical/molecular biology grade and were obtained from commercial sources. Cellulose nitrate filters (0.45 μm pore size) for use in uptake studies with intestinal brush-border membrane vesicles (BBMV) were purchased from Sartorius Filters (Hayward, CA). Rat folate-deficient and control diets were purchased from Dyets (Bethlehem, PA).

**Induction of folate deficiency in rats.** Folate deficiency was induced in male Sprague-Dawley rats by feeding weaning (28 days old) animals (for a period of 6–8 wk) a folate-deficient diet that contained no folate but with added succinyl sulfathiazole (1% wt/wt). Control rats were pair fed the same diet but with added 2 mg folic acid/kg diet. Rats were maintained individually in wire-bottomed cages (to decrease coprophagy) and had free access to water. Animals were kept in a light- and temperature-controlled room in our animal vivarium. At the end of the study period, rats were weighed, samples of their blood were taken, and their intestines were removed for further processing. The use of rats in this study was approved by the institutional Animal Review Board, and animal experiments were conducted following all regulatory guidelines.

**Preparation of intestinal everted sacs and mucosal-to-serosal transport.** Everted sacs were prepared from rat jejunum as described by us previously (30). Sacs were incubated in 5 ml of continuously oxygenated Krebs-Ringer phosphate buffer (in mM: 133 NaCl, 4.93 KCl, 1.23 MgSO\(_4\), 0.85 CaCl\(_2\), 5 glutamine, 5 HEPES, and 5 MES, pH 5.8). Incubation was performed for 15 min (i.e., initial rate; see Ref. 30) at 37°C in a shaking water bath (75 oscillations/min). At the end of incubation, sacs were removed and washed with ice-cold buffer, and their seminal content was drained into individual scintillation vials and counted for radioactivity.

**Preparation of intestinal BBMV and uptake studies.** BBMV were isolated from the jejunum of folate-deficient and pair-fed control rats by a modification of Kessler's divalent cation (Mg\(^{2+}\)) precipitation technique (16), as described by us previously (25). The final vesicular pellets were suspended in a buffer of 280 mM mannitol and 20 mM HEPES-Tris (pH 7.4) to achieve a final protein concentration of 5–10 mg/ml. Uptake studies were performed on the day of isolation by a rapid filtration technique described previously (25). All incubations were performed at 37°C in the following buffer (final concentrations in mM): 100 NaCl, 80 mannitol, 10 HEPES, and 10 MES, pH 5.5. Protein concentrations were determined using a Bio-Rad DC Protein Assay kit (Bio-Rad, Hercules, CA).

**Measurement of the activity of the brush-border membrane (BBM) marker enzyme alkaline phosphatase in isolated purified BBMV and in initial mucosal homogenates** (prepared to determine the relative purity of the final BBMV preparations in the two rat groups) was performed using a commercially available kit (Sigma Chemical, St. Louis, MO). Activity of the intestinal BBM form of the folate hydrolase was determined in jejunal mucosal samples of folate-deficient and pair-fed control rats as previously described (4).

**Northern blot analysis.** Total RNA was isolated from different areas of the intestinal tract and from the renal cortex of folate-deficient and pair-fed control rats using TRIzol reagent following the protocol described by the manufacturer (Life Technologies, Rockville, MD). Poly(A)\(^{+}\) RNA was then isolated from total RNA using Message Maker Reagent Assembly (Life Technologies). Northern blot analysis was performed using 5 or 10 μg poly(A)\(^{+}\) RNA samples as described by us previously (22, 27, 29). The blots were hybridized separately overnight at 42°C using probes of \(^{32}\)P-labeled full-length cDNAs of RFC transcripts (cloned previously in our laboratory and demonstrated to recognize rat RFC transcripts; see Ref. 29), the folate-binding protein (FBP; kindly provided by Dr. Stephen Lacey of the University of Texas, Dallas, TX; demonstrated to recognize rat transcripts; see Ref. 27), and β-actin (Clontech, Palo Alto, CA). The blots were washed at high stringency (29), and the RNA levels were detected by autoradiography using Kodak X-AR film (Sigma) at −80°C using intensifying screens. Band intensities were quantitated using the Eagle Eye II system (Stratagene, La Jolla, CA). The intensities of the resulting bands of RFC and FBP were then normalized relative to the intensities of the constitutively expressed β-actin.

**Western blot analysis.** BBM was isolated as described earlier in the presence of 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, and 0.5 μg/ml leupeptin. BBM protein (150 μg) was treated with Laemmli buffer and was resolved on 10% SDS-PAGE. After electrophoresis, the proteins were electroblotted on an Immob-Blot polyvinylidene difluoride membrane (Bio-Rad) overnight. The blots were washed two times in PBS-Tween 20 for 10 min, blocked with 5% dried milk in PBS-Tween 20 for 1 h at room temperature, and washed again with PBS-Tween 20. BBM were then probed with anti-rat RFC polyclonal antibodies (1:1,000 dilution in PBS-Tween 20) for 1 h at room temperature, washed two times for 10 min at room temperature in PBS-Tween 20 (Sigma), and reacted with goat anti-rabbit IgG secondary antibodies conjugated to horseradish peroxidase (1:5,000 dilution in PBS-Tween 20; Sigma) for 1 h at room temperature. The blots were finally washed two times in PBS-Tween 20 for 10 min each time, and color was developed using an en-
hanced chemiluminescence kit (Amersham, Arlington Heights, IL). To determine the effect of dietary folate deficiency on the level of expression of the unrelated membrane protein, sodium-dependent glucose transporter (SGLT-1) probing was performed using commercially available anti-SGLT-1 polyclonal antibodies (Chemicon, Temecula, CA). Specific bands were quantitated using the Eagle Eye II System (Stratagene).

The anti-RFC polyclonal antibodies used in this study were initially obtained from Dr. Frank Sirotnak but subsequently were raised by us against a synthetic peptide of rat RFC in rabbits using a commercial vendor (Alpha Diagnostic, San Antonio, TX). Identical results were obtained with both polyclonal antibodies used. In raising the polyclonal antibodies, we designed the antigenic peptide as follows: protein sequence of rat RFC was searched for any similarities with other known proteins using the Blastp algorithm and Swissport database. Because the recently cloned thiamine transporter (12) has been reported to have some degree of homology with RFC, the sequence of this protein was therefore also aligned alongside the corresponding rat RFC using the Clustal W algorithm to detect the regions of lowest homology. The regions of rat RFC protein showing the lowest homology were then analyzed for hydrophilicity (Kyte-Doolittle hydrophathy plot), antigenicity (Hopp/Woods and Protusion Index Antigenicity profiles), and accessibility. A specific region of rat RFC that corresponds to amino acids 494–512 of the rat sequence (PEDGSVEDGRADLVEAKA) was determined to be specific for rat and was therefore used for raising the polyclonal antibodies. The selected peptide was synthesized as keyhole lympet-hydrolysate conjugate and was used for immunization in two rabbits.

Statistical analysis. All transport studies were the results of multiple separate determinations using different everted sacs or BBMV preparations isolated from different rats on different occasions. Data are expressed as means ± S.E. in mole per gram weight or milligram protein per unit time. Data were analyzed using the Student’s t-test. Kinetic parameters of the saturable component of the intestinal folate uptake process [i.e., maximal velocity (Vmax) and apparent Michaelis constant (Km)] were determined using a computerized model of the Michaelis-Menten equation as described by Wilkinson (38). Northern and Western blotting were performed on three separate occasions using three independently isolated samples from different rats; representative blots are presented in this report.

RESULTS

At the time of death, body weights of rats fed the folate-deficient diet were found to be significantly (P < 0.01) lower than body weights of pair-fed controls (249.5 ± 7.1 and 294.3 ± 3.3 g, respectively). Similarly, plasma folate levels of rats on the folate-deficient diet were significantly (P < 0.01) lower than those of pair-fed controls at the time of death (1.2 ± 0.2 and 18.5 ± 0.5 ng/ml, respectively).

Effect of dietary folate deficiency on transepithelial (mucosal-to-serosal) transport of folic acid. In this experiment, we examined mucosal-to-serosal transport of folic acid (0.1 μM) in jejunal everted sacs prepared from folate-deficient and pair-fed control rats. The results showed a significantly (P < 0.01) higher folic acid uptake in everted sacs of folate-deficient compared with pair-fed control rats (9.04 ± 0.82 and 4.93 ± 0.80 pmol·g tissue⁻¹·15 min⁻¹ in folate-deficient and pair-fed control rats, respectively). We also examined mucosal-to-serosal transport of the unrelated water-soluble vitamin biotin (0.1 μM) in jejunal everted sacs prepared from the two rat groups. The results showed no significant difference in mucosal-to-serosal carrier-mediated transport of biotin between folate-deficient and pair-fed control rats (11.98 ± 0.87 and 11.08 ± 0.55 pmol·g tissue⁻¹·15 min⁻¹, respectively).

Effect of dietary folate deficiency on folinic acid uptake by purified intestinal BBMV. The effect of dietary folate deficiency on folinic acid uptake across the intestinal BBMV was examined using isolated purified jejunal BBMV. The results showed folinic acid (0.1 μM) uptake to be significantly (P < 0.01) higher in BBMV prepared from folate-deficient compared with those prepared from pair-fed control rats (0.53 ± 0.08 and 0.21 ± 0.03 fmol·mg protein⁻¹·10 s⁻¹, respectively). The relative purity of the final BBMV preparations was found to be similar in the two rat groups as indicated by the similar (8- to 9-fold) enrichment in the activity of the BBMV marker enzyme alkaline phosphatase in the final BBMV preparations compared with initial mucosal homogenates.

To better define the mechanism involved in the up-regulation in folinic acid uptake in BBMV of folate-deficient rats, we determined whether the effect of folate deficiency is mediated through changes in the Vmax and/or the apparent Km of the folinic acid uptake process. This was performed by examining the initial rate of folinic acid uptake (i.e., 10 s; see Ref. 25) as a function of concentration (0.1–20 μM). Kinetic parameters of the saturable component were then determined as described in MATERIALS AND METHODS. The results (Fig. 1) showed that dietary folate deficiency leads to a significant (P < 0.01) increase in the Vmax of the folinic acid uptake process with no significant changes in the apparent Km (Vmax of 34.38 ± 2.34 and 19.1 ± 1.1 pmol·mg protein⁻¹·10 s⁻¹, respectively; apparent Km of 5.34 ± 0.87 and 6.42 ± 0.7 μM for folate-deficient and pair-fed control rats, respectively).

Effect of dietary folate deficiency on steady-state mRNA level of RFC in intestinal mucosa. In this study, we examined the steady-state mRNA level of RFC in the mucosa of different segments of the intestinal tract (jejunum, ileum, colon) of folate-deficient rats and compared the findings with the levels in corresponding segments of pair-fed control rats. This was performed by Northern blot analysis using poly(A)+ RNA isolated from the particular segment and a randomly radiolabeled full-length cDNA probe of RFC as described in MATERIALS AND METHODS. The results (Fig. 2) showed a marked increase in the steady-state mRNA level of the RFC in folate-deficient rats compared with pair-fed controls in all intestinal segments examined (3.43 ± 0.24-fold increase in the jejunum; 4.1 ± 0.2-fold increase in the ileum; and 3.3 ± 0.10-fold increase in the colon; all data were normalized relative to β-actin).

Effect of dietary folate deficiency on expression of RFC protein. The effect of dietary folate deficiency on the level of expression of the RFC protein at the intestinal BBMV was examined by Western blot analysis.
Polyclonal antibodies raised against a synthetic peptide of the RFC protein and purified jejunal BBM preparations isolated as described in MATERIALS AND METHODS were used. We also determined the level of expression of an unrelated BBM transport protein, that of the sodium-dependent glucose transporter SGLT-1 in folate-deficient and pair-fed control rats to establish the specificity of any observed effect of folate deficiency on the level of RFC protein. The results (Fig. 3) showed a marked (3.13 ± 0.08-fold) induction in the level of expression of the RFC protein at the intestinal BBM of folate-deficient compared with pair-fed control rats. On the other hand, no increase in the level of expression of SGLT-1 protein was found; rather, the expression was similar in the two rat groups (Fig. 3).

Dietary folate deficiency and possible induction of expression of FBP. In this investigation, we tested the possibility that, under the folate-deficient condition, the intestine may express the FBP. This has been reported to occur in the case of other nonintestinal cells (3). This was tested by Northern blot analysis of poly(A)⁺ RNA isolated from the intestine of folate-deficient and pair-fed control rats and a randomly radiolabeled cDNA probe of FBP. Poly(A)⁺ RNA from the kidney cortex of the two rat dietary groups was also run alongside the intestinal RNA to serve as positive controls (renal epithelial cells are known to express significant amounts of the FBP; see Ref. 27). The results (Fig. 4) showed lack of expression of the FBP in the lanes containing intestinal poly(A)⁺ RNA of folate-deficient and pair-fed control rats. On the other hand, expression of the FBP was observed in both lanes containing renal poly(A)⁺ RNA. Interestingly, a markedly (1.91 ± 0.06-fold) higher level of expression of the FBP was found in renal cortex of folate-deficient rats compared with pair-fed controls.

Effect of dietary folate deficiency on the activity of intestinal BBM folate hydrolase. In this study, we tested for the existence of the BBM form of the enzyme folate hydrolase in rat intestine and determined whether its activity is affected by dietary folate deficiency. The results showed that this form of the enzyme indeed exists in rat intestine and that its activity

Fig. 1. Uptake of folic acid by jejunal brush-border membrane vesicles (BBMV) of folate-deficient (○) and pair-fed control (□) rats as a function of concentration. BBMV were preloaded with buffer of 280 mM mannitol and 20 mM HEPES-Tris, pH 7.4. Incubation was performed for 10 s (i.e., initial rate; see Ref. 25) at 37°C in buffer of 100 mM NaCl, 80 mM mannitol, 10 mM MES, and 10 mM HEPES, pH 5.5. Different concentrations of [³H]folic acid were added to the incubation medium at the onset of incubation. Data are means ± SE of 4–8 separate uptake determinations from 3 different BBMV preparations isolated from 6–8 different rats.

Fig. 2. steady-state mRNA levels of the reduced folate carrier (RFC) in different intestinal segments of folate-deficient and pair-fed control rats. Northern blot analysis was performed as described in MATERIALS AND METHODS using 10 µg poly(A)⁺ RNA isolated from mucosal scraping of different intestinal segments and randomly radiolabeled full-length cDNA probe of RFC. C, jejunal brush-border membrane (BBM) from pair-fed control rats; D, jejunal BBM from folate-deficient rats. Data are representative of 3 separate sets of experiments.

Fig. 3. Western blot analysis of RFC and SGLT-1 proteins in jejunal apical membrane of folate-deficient and pair-fed control rats. Analysis was performed as described in MATERIALS AND METHODS. Data shown are representative of 3 separate sets of experiments.

Fig. 4. Northern blot analysis of the level of mRNA of the folate receptor in intestinal mucosa of folate-deficient and pair-fed control rats. Analysis was performed as described in MATERIALS AND METHODS using poly(A)⁺ RNA isolated from intestinal mucosa of folate-deficient and pair-fed control rats and randomly radiolabeled cDNA of the folate receptor [folate-binding protein (FBP)] as probe. Poly(A)⁺ RNA from the kidneys of folate-deficient and pair-fed control rats were also run as positive controls. Data shown are representative of 3 separate sets of experiments.
is significantly \((P < 0.01)\) induced in dietary folate deficiency \((1.5 \pm 0.66\) and \(15.13 \pm 6.27\ \text{pmol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}\) for jejunum of pair-fed control and folate-deficient rats, respectively).

**DISCUSSION**

The aim of the present study was to examine the effect of dietary (nutritional) folate deficiency on the ability of the intestine to absorb folate. The study was performed using the rat as an animal model because previous studies have established the suitability of this species for such investigations (25, 30, 32). Induction of folate deficiency was accomplished by feeding rats a folate-deficient diet that contained the antibiotic succinyl sulfathiazole. The inclusion of the antibiotic into the diet together with keeping the rats in individual wire-bottomed cages was intended to decrease the contribution of the bacterially synthesized folate toward the folate body level. The bacterially synthesized folate in the large intestine can get access into the body through direct absorption in the colon, utilizing the recently identified folate uptake carrier at the apical membrane of colonocytes (9, 17), and through coprophagy where the vitamin becomes available for absorption in the small intestine. This feeding regimen was successful in inducing a folate-deficient state in the rats as indicated by the severe drop in plasma folate level in the group that was fed the folate-deficient diet compared with the group that was pair fed the control diet.

In examining the effect of dietary folate deficiency on the intestinal folate absorption process, we first examined the effect of folate deficiency on the overall transepithelial transport of the vitamin. We did so using a well-established in vitro intact intestinal tissue preparation, i.e., everted sacs (30). The results showed that dietary folate deficiency leads to a significant upregulation in transepithelial transport of a physiological concentration of folic acid. This upregulation was found to be specific for folic acid as uptake of the unrelated water-soluble vitamin biotin was not significantly affected by dietary folate deficiency. The upregulation in transepithelial transport of folate in folate-deficient rats was found to be mediated via an induction in the folate transport event at the BBM of the intestinal absorptive cells. This conclusion is based on the findings with the isolated purified jejunal BBMV that showed a significantly higher folic acid uptake in folate-deficient compared with pair-fed control rats. The upregulation in folate uptake across the intestinal BBM was found to be mediated via a significant increase in the \(V_{\max}\) of the uptake process with no significant changes in its apparent \(K_{m}\). These findings suggest that dietary folate deficiency leads to an induction in the number (and/or activity) of the intestinal folate uptake carriers with no changes in their affinity.

The upregulation in intestinal folate uptake observed in folate-deficient rats was found to be associated with a marked increase in the intestinal steady-state mRNA level of RFC (RFC is the folate uptake carrier that is believed to be responsible for intestinal folate absorption; see Refs. 5, 22, 29). This increase in the steady-state mRNA level of RFC was observed not only in the jejunum (the preferential site of absorption of dietary folate) but also in the ileum, an area of the small intestine that is not usually involved in the normal absorption process of dietary folate (25, 30–32). Assuming that this increase in the mRNA level of RFC in the ileum translates into an increase in functional activity of the folate transport process in that region of the gut in folate-deficient rats, this would then suggest that the small intestine also employs a “recruitment” mechanism along its longitudinal axis to maximize its ability to extract the limited amount of folate presented to the animal under a folate-deficient condition. Of similar interest was the observed significant up-regulation in the steady-state mRNA level of RFC in the colon of folate-deficient rats. Again, if we assume that this induction translates into an increase in functional activity of the folate uptake process in rat colon under the folate-deficient condition, this would then suggest that, when faced with the challenge of folate deficiency, the animal also tries to maximize its uptake of the bacterially synthesized folate in the large intestine. The observation of a similar upregulation in RFC mRNA level in the small and large intestine of folate-deficient rats suggests the possible involvement of a similar regulatory mechanism in the two distinct regions of the intestinal tract.

The increase in intestinal folate uptake and in the steady-state mRNA level of RFC in folate-deficient rats was also found to be associated with a marked increase in the level of the RFC protein expressed at the BBM of enterocytes. This was shown by the results of the Western blot analysis. This induction in the level of RFC protein was specific for the folate transporter as similar levels of expression of the unrelated BBM transport protein SGLT-1 were found in folate-deficient rats and pair-fed controls.

Previous studies with mouse leukemia L-1210 cells have shown that growing these cells under the folate-deficient condition leads to expression of the FBP, i.e., the folate receptor, a folate uptake mechanism that is not usually expressed in these cells (3). For this reason, we tested whether the intestinal absorptive cells could also express the folate receptor under such a physiologically stressful condition, i.e., that of folate deficiency. Our study was performed using Northern blot analysis. In that study, we also included RNA samples from the renal cortex of both folate-deficient and pair-fed rats to serve as a positive control, since renal epithelial cells are known to express significant amounts of the folate receptor (27). The results showed that, unlike the L-1210 cells, the intestinal epithelial cells do not express the folate receptor even when faced with the condition of folate deficiency. Interestingly, however, a significantly higher level of expression of that folate receptor was found in the renal cortex of folate-deficient rats compared with pair-fed controls. This finding shows that, when faced with the condition of folate deficiency, the animal also tries to minimize...
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Folate losses in the urine. The finding also demonstrates differences in the way intestinal and renal epithelial cells handle folate transport. Our finding on the upregulation of expression of the folate receptor in rat kidneys in folate deficiency is in contrast to the recently published findings of da Costa et al. (7), who did not find an upregulation in the steady-state level of mRNA of this receptor. The cause of this discrepancy is not clear but could be due to differences in the length of time rats of the test group were kept on the folate-deficient diet in the two studies.

As stated earlier, dietary folate exists mainly in the form of polyglutamates, a form that must be hydrolyzed to folate monoglutamates before intestinal absorption. This process is accomplished by the specific action of the enzyme folate hydrolase. This enzyme has been shown to exist at the BBM of enterocytes of species like humans and pigs (36) but is barely detectable at the BBM of rat enterocytes (36). Our finding in the present study, however, shows that rat intestine also expresses the BBM form of the enzyme. More interesting, however, was the finding that the activity of this enzyme is also significantly upregulated in folate deficiency. Thus it appears that both of the critical steps that are involved in the normal assimilation of dietary folate, i.e., the hydrolysis of polyglutamates to monoglutamates and the transport of the resulting monoglutamates are upregulated in folate deficiency. Such concerted upregulation ensures maximum absorption of dietary folates by the animal under folate-deficient conditions. Further studies are required to determine the molecular mechanisms involved in the upregulation of these events.

In summary, our study demonstrates that the activities of the intestinal folate uptake process and those of the BBM folate hydrolase are both upregulated in folate deficiency in rats. Furthermore, the upregulation in the folate uptake process is associated with a parallel increase in the steady-state mRNA level of RFC and its protein. The latter findings raise the possibility that transcriptional regulatory mechanisms(s) may be involved in this adaptive regulation in intestinal folate transport in folate deficiency. This issue is currently being investigated in our laboratory.

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