Colonic mucosal concentrations of folate correlate well with blood measurements of folate status in persons with colorectal polyps

Young-In Kim, Karim Fawaz, Tamsin Knox, Young Mee Lee, Richard Norton, Sanjeev Arora, Lori Paiva, and Joel B Mason

ABSTRACT
Background: Estimates of habitual dietary folate intake are known to be imprecisely correlated with systemic measures of folate status. Furthermore, measurements of blood folate concentrations may not accurately reflect the concentration of folate in tissues of interest. This issue is important for assessing folate status in the colorectal mucosa because low dietary intake or blood concentrations of folate are associated with an increased risk of colorectal neoplasia.

Objective: We examined whether conventional measures of folate in blood and a more sensitive, inverse indicator of systemic folate status, serum homocysteine, accurately reflected folate concentrations in human colonic mucosa obtained by endoscopic biopsy.

Design: In 30 persons with colorectal polyps, blood samples were taken and biopsies of normal rectosigmoid mucosa performed at the time of colonoscopic polypectomy. Serum, red blood cell, and colonic mucosal folate and serum homocysteine concentrations were measured.

Results: Serum and red blood cell folate and serum homocysteine concentrations accurately reflected colonic mucosal folate concentrations; among these, serum homocysteine correlated best with mucosal concentrations. Folate concentrations in the normal rectosigmoid mucosa were significantly lower in persons with adenomatous polyps than in those with hyperplastic polyps (P = 0.04). Conventional measures of systemic folate status were not significantly lower in those with adenomas, although serum homocysteine was mildly elevated (P = 0.04).

Conclusions: Our data underscore the ability of systemic measures of folate status, particularly serum homocysteine, to reflect folate concentrations in the colonic mucosa. Nevertheless, future studies that examine the ability of folate to modulate colorectal carcinogenesis may benefit from direct measurement of folate in the colon.


KEY WORDS Folate, homocysteine, colon cancer, adenoma, polyps, rectosigmoid mucosa, colorectal neoplasia

INTRODUCTION
Epidemiologic studies conducted in persons with ulcerative colitis (1–3), a disease associated with an enhanced risk of both folate deficiency and colorectal neoplasia (4), as well as in the general population (5–17) have nearly unanimously established an inverse association between folate status and colorectal neoplasia. The results of animal studies generally support the epidemiologic observations and, in addition, provide strong evidence for a causal relation between folate status and colorectal carcinogenesis (18, 19).

Human studies pertaining to this issue have typically assessed folate status by either estimating the habitual dietary intake of folate (6–17) or by measuring blood folate concentrations directly (1, 2, 5–7). However, each of these methods of assessing folate status has limitations. Habitual dietary folate intake is usually assessed by means of a food-frequency questionnaire, a method known to be imprecisely correlated with systemic measures of folate status (16). Furthermore, even the direct determination of blood folate concentrations may not be sufficiently...
accurate because the target tissue of interest in this field of research is the colonic mucosa and it is well known that each tissue expresses differential susceptibility to folate depletion (20). Note that the gastrointestinal mucosa is among the tissues most prominently affected by folate deficiency (21). Because most carcinogenic events occur at a cellular level, knowledge of the actual folate concentration within the tissue of interest, rather than in the blood, may be of considerable importance. The concept of tissue-specific susceptibility to folate depletion in humans was introduced by Whitehead et al (22), who observed megaloblastic cells of the uterine cervix in women taking a drug associated, despite the absence of signs of systemic deficiencies of folate or vitamin B-12. Localized folate depletion was also shown in the oral mucosa: cigarette smoking was observed to acutely deplete folate concentrations in buccal mucosa cells but to not affect measures of systemic folate status (23).

It is unclear whether blood concentrations of folate accurately reflect concentrations in the colonic mucosa. Studies in which rats were fed different dietary amounts of folate showed that colonic mucosal folate concentrations are significantly correlated with dietary intake and plasma folate concentrations (19, 24). However, recent human studies showed little correlation between folate concentrations in human colonic epithelial cells isolated by endoscopic biopsy and either serum or red blood cell (RBC) folate concentrations, raising a concern that systemic folate indexes might not accurately reflect folate concentrations in the colorectal mucosa (25, 26). Accurate measures of folate status are important for ascertaining the risk of colorectal cancer in epidemiologic studies and for determining the effects of folate supplementation in clinical studies.

We studied whether conventional blood (serum and RBC) measurements of folate accurately reflected folate concentrations in the colonic mucosa as obtained from endoscopic biopsy in humans. In addition, we investigated whether serum homocysteine concentrations were inversely correlated with colonic mucosal concentrations of folate. Serum concentrations of homocysteine increase with folate deficiency because the metabolic pathway of homocysteine depends on a remethylation reaction in which N-5-methyltetrahydrofolate serves as a co-substrate with homocysteine (27); in practice, an elevation of this amino acid has been found to be a more sensitive indicator of cellular folate depletion than blood folate concentrations (28). Because homocysteine is at the intersection of 2 metabolic pathways, remethylation and transsulfuration, deficiencies of other micronutrients, such as vitamin B-12, pyridoxal phosphate, and possibly even flavin adenine dinucleotide (or riboflavin), can affect these metabolic pathways and have been associated with elevated concentrations of serum homocysteine (28).

SUBJECTS AND METHODS

Study design, subjects, and sample collection

The study was reviewed and approved by the institutional investigational review boards of the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University and the New England Medical Center. Consecutively referred patients >18 y of age with known or suspected colorectal polyps according to flexible sigmoidoscopy or a barium enema who were referred to the New England Medical Center for colonoscopy and polypectomy were asked to serve as volunteers. Subjects were excluded from the study if they were taking sulfasalazine or methotrexate, both of which are folate antagonists (29, 30), or had any clinical or laboratory evidence of intestinal malabsorption or maldigestion. Subjects who had been taking folate supplements containing >400 mcg folate/d were excluded from the study; however, subjects were allowed to remain in the study if they were consuming multivitamin preparations containing ≤400 mcg folate/d. The latter individuals are referred as multivitamin users throughout this paper. Written, informed consent was obtained from all subjects.

Information on alcohol intake and tobacco use was obtained from each subject at the time of recruitment. Alcohol and tobacco affect folate absorption and metabolism (31, 32) and are considered to be important environmental factors in colorectal carcinogenesis (33, 34). Current smokers were classified as those smoking <0.5, 0.5–1, or >1 pack/d. Exsmokers were defined as those who had not smoked for >1 y before recruitment. Alcohol intake was classified as minimal (<1 drink/d), moderate (1–2 drinks/d), or heavy (>3 drinks/d). Exdrinkers were defined as those who had not consumed alcohol for >1 y before recruitment. Family history of colorectal cancer or adenoma, which is an important genetic factor in colorectal carcinogenesis (35), was also ascertained.

All patients were prepared for colonoscopy with an orally administered colonic lavage solution (GoLYTELY; Braintree Laboratories, Inc, Braintree, MA). Colonoscopy was performed in the standard fashion and the colonoscope reached the cecum in all study patients. All identifiable polyps were removed, immersed in formalin, and processed for routine histology, after which the polyp was classified as either adenomatous or hyperplastic by a staff pathologist at New England Medical Center. Adenomas are the well-established precursor of adenocarcinoma of the colorectum (35), whereas hyperplastic polyps are not considered to be malignant (36, 37). The size of each polyp was measured by the pathologist. At the time of polypectomy, 3 biopsies of normal-appearing mucosa at the rectosigmoid junction (≈15 cm from the anal verge), ≥10 cm from any polyp or other mucosal abnormality, were also performed with standard biopsy forceps. These biopsy samples were immediately placed in foil packets and frozen in liquid nitrogen and then stored at −70°C for subsequent analysis of colonic mucosal folate concentrations.

After colonoscopy was completed, venous blood was drawn from each subject (while he or she was in a fasting state) for a complete blood count and measurement of serum folate, vitamin B-12, and homocysteine concentrations and RBC folate concentrations. Blood samples for complete blood counts were collected in tubes containing sodium EDTA and analyzed immediately in the medical center’s clinical hematology laboratory (System 9000; Serono Baker Diagnostic Inc, Allentown, PA). Blood for measuring serum folate, vitamin B-12, and homocysteine concentrations was collected into evacuated tubes and centrifuged at 800 × g for 10 min at 4°C; serum was stored at −70°C until assayed. The portion of serum for the folate analysis was stored in 0.5% sodium ascorbate, which is known to retard folate degradation in storage (38). A whole-blood sample, used to measure RBC folate, was stored in 1% ascorbic acid at −70°C for the same reason.

Vitamin and homocysteine assays

Serum vitamin B-12 and RBC folate concentrations were determined by radioimmunoassay (MAGIC kit; Ciba-Corning...
Magnetic Immunochemistries, Medfield, MA). Serum folate was measured by a standard microbiological microtiter plate assay using *Lactobacillus casei* (39). Colonic mucosal folate concentrations were also measured by the microbiological assay, according to a method described previously for the measurement of tissue folates (20), as follows. A rapid determination of biopsy weight was followed immediately by extraction of tissue folates in 20 volumes of freshly prepared folate extraction buffer [5 mmol β-mercaptoethanol/L and 0.1 mol sodium ascorbate/L in 0.1 mol bis(2-hydroxyethyl)iminom-tris(hydroxymethyl)methane/L, pH 7.85] at 95°C for 20 min. Extracts were then treated with chicken pancreas conjugase to convert all folylpolyglutamates to their corresponding diglutamate derivatives (40), thereby enabling us to study the extracts with the microbiological assay. Total serum homocysteine was measured by HPLC according to the fluorometric method of Vester and Rasmussen (41). This method incorporates a treatment of the samples with a reducing agent before analysis and therefore measures the sum of the free, protein-bound, and disulfide forms of homocysteine. All laboratory assays were performed with the investigators blinded to the pathologic diagnosis of the polyp.

**Statistical analysis**

Linear regression was used to assess the correlation between variables. Comparisons of means between subjects with adenomatous polyps and those with hyperplastic polyps were assessed by Student’s *t* test. Analysis of covariance was performed to compare variables between groups after adjustment for covariates (age and multivitamin use). For categorical response variables, differences between groups were assessed by Fisher’s exact test. Statistical analyses were performed by using SYSTAT 5 for Macintosh (Systat, Evanston, IL). All significance tests were two-sided and were considered to be statistically significant if *P* was < 0.05. Results are expressed as means ± SEMs.

**RESULTS**

Thirty subjects were recruited: 10 had hyperplastic polyps (mean number of polyps: 3.2 ± 0.8; range: 1–8) and 20 had adenomas (mean number of polyps: 2.0 ± 0.4; range: 1–7). Six subjects had both adenomatous and hyperplastic polyps. The average size was 0.5 ± 0.03 cm (range: 0.4–0.7 cm) for hyperplastic polyps and 1.2 ± 0.2 cm (range: 0.2–4.0 cm) for adenomatous polyps (*P* = 0.02). Most polyps were located distal to the splenic flexure (62% and 94% of the adenomatous and hyperplastic polyps, respectively; NS).

As shown in **Figure 1**, colonic mucosal concentrations correlated directly with folate concentrations in both serum (*r* = 0.62, *P* < 0.001) and RBCs (*r* = 0.46, *P* = 0.013). Serum and RBC folate concentrations also correlated directly with one another (*r* = 0.41, *P* = 0.027). Serum homocysteine concentrations correlated inversely with folate concentrations in serum (*r* = −0.59, *P* = 0.001), RBCs (*r* = −0.44, *P* = 0.016), and colonic mucosa (*r* = −0.72, *P* < 0.001; Figure 1). The number or size of the polyps was not significantly correlated with the 3 folate determinations or with homocysteine concentrations. Furthermore, the distance from the biopsy site to the nearest polyp was not significantly correlated with colonic mucosal folate concentrations.

Differences between blood and colonic mucosal folate measurements and serum homocysteine concentrations were compared between subjects with hyperplastic polyps and those with

**FIGURE 1.** Correlations between colonic mucosal folate and serum folate, red blood cell (RBC) folate, and serum homocysteine concentrations in subjects with adenomatous (○; *n* = 20) or hyperplastic (●; *n* = 10) polyps. Colonic mucosal folate concentrations correlated directly with serum (*r* = 0.62, *P* < 0.001) and RBC (*r* = 0.46, *P* = 0.013) folate concentrations. Serum homocysteine concentrations, which rise during folate deficiency, correlated inversely with colonic mucosal folate concentrations (*r* = −0.72, *P* < 0.001).
Table 1
Characteristics of the study subjects

<table>
<thead>
<tr>
<th></th>
<th>Subjects with hyperplastic polyps</th>
<th>Subjects with adenomatous polyps</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 6M, 4F)</td>
<td>(n = 11M, 9F)</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>54.1 ± 4.0ⁿ</td>
<td>62.4 ± 1.9</td>
</tr>
<tr>
<td>Multivitamin users [n (%)]</td>
<td>4 (40)</td>
<td>7 (35)</td>
</tr>
<tr>
<td>Alcohol use [n (%)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>2 (20)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Exdrinker</td>
<td>1 (10)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Minimal</td>
<td>4 (40)</td>
<td>9 (45)</td>
</tr>
<tr>
<td>Moderate</td>
<td>3 (30)</td>
<td>5 (25)</td>
</tr>
<tr>
<td>Heavy</td>
<td>0 (0)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Smoking status [n (%)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>3 (30)</td>
<td>8 (40)</td>
</tr>
<tr>
<td>Exsmoker</td>
<td>4 (40)</td>
<td>7 (35)</td>
</tr>
<tr>
<td>&lt;0.5 pack/d</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>0.5–1 pack/d</td>
<td>1 (10)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>&gt;1 pack/d</td>
<td>2 (20)</td>
<td>4 (20)</td>
</tr>
<tr>
<td>No. of polyps</td>
<td>3.2 ± 0.8</td>
<td>2.0 ± 0.4</td>
</tr>
<tr>
<td>Size of polyps (cm)</td>
<td>0.5 ± 0.03</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>History of past adenomas or adenoma [n (%)]</td>
<td>0 (0)</td>
<td>7 (35)</td>
</tr>
<tr>
<td>Family history of colorectal cancer or adenoma [n (%)]</td>
<td>1 (10)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>140.0 ± 6.3</td>
<td>141.0 ± 3.4</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.408 ± 0.013</td>
<td>0.412 ± 0.009</td>
</tr>
<tr>
<td>Mean corpuscular volume (fL)</td>
<td>86.1 ± 1.8</td>
<td>90.3 ± 2.6</td>
</tr>
<tr>
<td>Vitamin B-12 (pmol/L)</td>
<td>424.6 ± 50.0</td>
<td>347.2 ± 28.4</td>
</tr>
</tbody>
</table>

ⁿ± SEM. RBC, red cell.

Table 2
Folate and homocysteine concentrations according to the type of colorectal polyps³

<table>
<thead>
<tr>
<th></th>
<th>Hyperplastic (n = 10)</th>
<th>Adenomatous (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum folate (nmol/L)</td>
<td>66.8 ± 13.7</td>
<td>51.9 ± 12.6</td>
</tr>
<tr>
<td>RBC folate (nmol/L)</td>
<td>800.4 ± 124.4</td>
<td>695.9 ± 50.7</td>
</tr>
<tr>
<td>Colonic folate (nmol/g tissue)</td>
<td>1.18 ± 0.18</td>
<td>0.78 ± 0.10²</td>
</tr>
<tr>
<td>Serum homocysteine (µmol/L)</td>
<td>7.8 ± 0.7</td>
<td>9.5 ± 0.5³</td>
</tr>
</tbody>
</table>

³± SEM. RBC, red cell.

²Significantly different from hyperplastic, P = 0.04.

³Significantly different from hyperplastic, P = 0.006.

Discussion

Our observations indicated that colonic mucosal folate concentrations measured in endoscopic biopsy samples correlated directly with serum and RBC folate concentrations. Furthermore, these data showed that the colonic mucosal folate concentration correlated inversely with the concentration of serum homocysteine, a sensitive, inverse, systemic measure of cellular homocysteine, a sensitive indicator of cellular folate depletion in the hyperplastic polyp group (Table 2, P = 0.04). After multivitamin users were excluded from the analysis, mean serum homocysteine concentrations remained 20% higher in subjects with adenomatous polyps than in those with hyperplastic polyps (P = 0.012).

The serum concentration of total homocysteine is known to rise with folate deficiency (27) and is considered to be a more sensitive indicator of cellular folate depletion than blood folate concentrations (28). The mean concentration of serum homocysteine was 22% higher in those with adenomatous polyps than in those with hyperplastic polyps (Table 2, P = 0.04). After multivitamin users were excluded from the analysis, mean serum homocysteine concentrations remained 20% higher in subjects with adenomatous polyps than in the hyperplastic polyp group (Table 3, P = 0.06). Prior observations suggest that there is a small rise in total homocysteine with age (43). However, after we adjusted the analysis for age, the mean serum homocysteine concentration was still significantly higher in subjects with adenomatous polyps than the control value (P = 0.037).

Table 3
Folate and homocysteine concentrations according to the type of colorectal polyps (multivitamin users excluded)

<table>
<thead>
<tr>
<th></th>
<th>Hyperplastic (n = 6)</th>
<th>Adenomatous (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum folate (nmol/L)</td>
<td>54.8 ± 22.6</td>
<td>28.3 ± 6.3</td>
</tr>
<tr>
<td>RBC folate (nmol/L)</td>
<td>577.2 ± 95.8</td>
<td>632.4 ± 54.6</td>
</tr>
<tr>
<td>Colonic folate (nmol/g tissue)</td>
<td>0.88 ± 0.09</td>
<td>0.58 ± 0.05²</td>
</tr>
<tr>
<td>Serum homocysteine (µmol/L)</td>
<td>8.5 ± 0.8</td>
<td>10.2 ± 0.5³</td>
</tr>
</tbody>
</table>

²± SEM. RBC, red cell.

³Significantly different from hyperplastic, P = 0.006.

³P = 0.06.
These data support previous animal studies in which folate concentrations in colonic mucosal scrapings were shown to be directly correlated with serum and RBC folate concentrations and inversely correlated with serum homocysteine concentrations (19, 24). However, these data do contrast with the results of 2 recent human studies in which no correlations between folate concentrations in colonic epithelial cells and serum and RBC folate measurements were observed (25, 26). In these studies, colonocytes were isolated before folate concentrations were measured. In contrast, our method was designed to determine the folate concentration of all of the contents of a colonscopic biopsy, including epithelial mucus and cells from the lamina propria, both of which are important constituents of the environment in which colonic epithelial cells exist. The fact that colonic folate concentrations in the present study correlated with serum and RBC folate concentrations, as well as with serum homocysteine concentrations, suggests that this method may be more representative of the in vivo environment than the method in which colonocytes are first isolated and then allowed to equilibrate for several hours. Nevertheless, because colonic neoplasms arise only from the colonocyte itself, we cannot exclude the possibility that the method of measuring folate concentrations in isolated colonocytes may be more predictive of susceptibility to neoplasia. Regardless of the interpretation, these data collectively imply that the method of processing colonoscopic biopsy samples may alter the apparent concentration of folate in the colon. Future studies with larger numbers of subjects are warranted to confirm the findings obtained in this study and also to generalize these findings to a larger population of patients with colorectal polyps. Additionally, only subjects with either hyperplastic or adenomatous polyps were included in the present study; hence, future studies are warranted to better define the concentration of folate in colonic mucosa in subjects without polyps.

One interesting secondary finding of this study was that subjects with adenomatous polyps in their colons had significantly lower colonic mucosal folate concentrations in the normal mucosa of the rectosigmoid junction than did those who had hyperplastic polyps. Such differences were not apparent to a significant degree when conventional indicators of systemic folate status were examined. However, a sensitive indicator of systemic folate status, serum homocysteine, did detect a modest reduction in folate status in the group with adenomas. Because hyperplastic polyps are generally regarded as not having malignant potential (36, 37), our data suggest that persons at risk of developing colorectal cancer have significantly lower folate concentrations in the normal colonic mucosa than do those not at risk. The sample size in the present study was small; hence, the finding of no difference in serum and RBC folate concentrations between the groups with adenomatous or hyperplastic polyps may have resulted from a type II error. Despite the small sample size, however, we found significant differences in colonic mucosal folate and serum homocysteine concentrations between the 2 groups, indicating adequate statistical power to reject the null hypothesis of no difference in these 2 indexes between subjects grouped according to the pathology of their polyps. Our findings need to be confirmed in future studies with larger numbers of subjects. Also, future studies comparing colonic mucosal folate concentrations between those with adenomatous polyps and those with no polyps are necessary to unequivocally establish that persons at risk of developing colorectal cancer have localized folate depletion compared with those not at risk. Additionally, the association between the folate concentration of the colonic mucosa and the type of polyp needs to be further explored in a larger population to help identify persons with adenomatous polyps at risk of developing colorectal cancer.

The fact that the folate concentration was lower in the normal rectosigmoid mucosa distant from the polyps in those with adenomatous polyps than in those with hyperplastic polyps suggests that there are widespread alterations in the folate physiology of the colonic epithelium in persons with adenomas. Such a finding is not without precedent: widespread alterations in other biochemical and physiologic phenomena, such as ornithine decarboxylase activity (44–46) and crypt cell proliferation (47–51), were described previously in normal colorectal mucosa of persons prone to colorectal neoplasia. The anatomic extent of this aberration in colonic folate cannot be determined from this study because we sampled only the rectosigmoid junction; however, the fact that reduced folate concentrations were found distant from the adenomas implies that this is a widespread phenomenon that is not confined solely to the immediate vicinity of the adenoma. Colonic mucosal biopsies were not taken from the immediate vicinity of the polyp (they were taken ≥10 cm away) because we were more interested in widespread alterations in folate concentrations than in effects induced by increased local utilization of the vitamin. Our data do contrast with one recent human study in which it was observed that folate concentrations were not significantly different between normal colonic epithelial cells adjacent to neoplasms and colonic epithelial cells from healthy control subjects (26). This study showed that folate concentrations in neoplastic colonic epithelial cells were significantly lower than those in adjacent normal cells. However, this study used the method isolating colonocytes before measuring folate, which, as discussed previously, yields different results from the method we used.

It is unclear why folate concentrations were lower in the colonic mucosa of subjects with adenomatous polyps than in those with hyperplastic polyps, even among the multivitamin consumers, in the absence of overt, systemic folate deficiency. Folylpolyglutamates are derivatives of folylmonglutamates and are generated by the addition of variable numbers of glutamate residues in γ-peptide linkages; the polyglutamates are preferentially retained by tissues (52). Endogenous folate in mammalian tissues consists almost entirely of folylpolyglutamate derivatives, whereas folylmonglutamates are the only circulating forms of folate in plasma and the only form of folate that is transported across the cell membrane (52). The synthesis of folylpolyglutamates plays a major role in the regulation of folate homeostasis and in the regulation of 1-carbon metabolism. Mammalian cells possess 2 enzymes that can potentially directly regulate the state of polyglutamation: folylpolyglutamate synthase (tetrahydrofolylpolyglutamate synthase), which catalyzes the synthesis of folylpoly-γ-glutamates, and γ-glutamyl hydrolase (conjugase or γ-Glu-X carboxypeptidase), a peptidase that can hydrolyze the folate polypeptide chain (52). Thus, either diminished folylpolyglutamate synthase or enhanced γ-glutamyl hydrolase activity will result in decreased retention of intracellular folate. Enhanced activity of lysosomal γ-glutamyl hydrolase has been observed in human hepatoma (53) and sarcoma (54) cells and enhanced activity of membrane-bound γ-glutamyl hydrolase has been observed in human prostate carcinoma cells (55). Furthermore, the cellular retention of folate antagonists has been shown to be modulated by the activities of folylpolygluta-
mate synthase and γ-glutamyl hydrolase (53, 56, 57). It is not known whether there is decreased activity of poly(γ-glutamyl) synthase or increased activity of γ-glutamyl hydrolase in the colonic mucosa of persons with adenomatous polyps, although this remains an interesting possibility. Future studies are therefore warranted to examine whether abnormal metabolic pathways in the colonic epithelial cells are responsible for the localized folate depletion observed in patients with polyps.

The utility of blood measurements of folate in predicting colorectal cancer risk was not conclusively shown in previous studies. In 3 case-control studies (2, 6, 7) and 1 prospectively conducted clinical study (5) in which blood folate concentrations were measured, RBC folate concentrations were lower in persons with neoplastic lesions than in control subjects but were still well within conventionally accepted ranges of normality (2, 5, 6). Additionally, sex specificity was shown in one study (6). In several studies, serum or plasma folate concentrations in persons with neoplastic lesions were not significantly different from concentrations in healthy control subjects (2, 5–7). The relative insensitivity of blood measurements of folate in ascertaining colorectal cancer risk may be related to the observation in epidemiologic (2, 5–7) and animal (18, 19) studies that mild folate depletion, rather than the development of overt, systemic folate deficiency, is sufficient for enhancing the risk of colorectal neoplasia. In fact, in some epidemiologic studies, the risk of developing colorectal neoplasms was observed to be inversely proportional to dietary folate intake in a dose-responsive fashion, even over a range of folate intakes that exceeded the amount necessary to prevent overt folate deficiency (16, 17). One corollary of our results, therefore, is that blood measurements of folate are less sensitive than colonic concentrations of folate in defining those persons with colorectal neoplasms. Our data suggest that, among the commonly available blood measurements of folate status, serum homocysteine concentrations might be the most sensitive reflection of folate concentrations in the colonic mucosa.

Although the mean difference in serum homocysteine concentrations between subjects with adenomatous polyps and those with hyperplastic polyps appeared to be small (1.7 μmol/L), it was significant when multivitamin users were included (P = 0.04; Table 2) and fell just short of significance when multivitamin users were excluded (P = 0.06; Table 3). The use of homocysteine as an indicator of folate status in clinical studies is still in its infancy; what constitutes an optimal range of homocysteine values, as well as the implications of minor increases in homocysteine concentrations, remains to be clearly elucidated. However, in the field of homocysteine and cardiovascular disease, a comparably small increase in the homocysteine concentration is often associated with significant increases in the risk of disease. For instance, a meta-analysis of observational studies of total homocysteine concentrations and vascular disease indicated that a decrease of only 1 μmol/L in the total homocysteine concentration was associated with a 10% reduction in risk of coronary artery disease (58). The significance of our observations of homocysteine concentrations in the 2 groups is not currently known.

In summary, this study indicated that serum and RBC folate and serum homocysteine concentrations accurately reflect colonic mucosal folate concentrations, and that serum homocysteine appears to be the best indicator. This study showed diminished concentrations of folate in the normal rectosigmoid mucosa of persons with adenomas compared with those with hyperplastic polyps. Conventional measures of systemic folate status were not significantly diminished, although a more sensitive indicator of systemic folate status, serum homocysteine, was mildly elevated in the adenoma group. The latter observation suggests that a systemic impairment in folate metabolism may be present in these individuals. Future studies of the ability of folate to modulate colorectal carcinogenesis may benefit from the measurement of colonic folate and serum homocysteine because systemic measures of folate may be inadequate in some respects.

We thank Shahin Sarkarati and Scott Valliere of the Nutrition Evaluation Laboratory for technical assistance; Ann Marie Brown, Marie Carten, and Helen Murray in the Clinical Study Unit for processing blood samples; and Tamara Levesque for data collection. We also thank the gastroenterology fellows, endoscopy suite nurses, and endoscopy suite secretaries for their considerable patience and generous devotion of the time involved in the scheduling of subjects and performance of colonoscopies.

REFERENCES