Moderate Folate Deficiency Influences Polyamine Synthesis in Rats¹,²

Dongmei Sun, Armin Wollin* and Alison M. Stephen³,⁴

Division of Nutrition and Dietetics, College of Pharmacy and Nutrition and *Department of Physiology, College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada S7N 5C9

ABSTRACT Spermidine, spermine and putrescine are polyamines, essential growth factors in mammalian cells. Decarboxylated S-adenosylmethionione (SAM) is an essential precursor in the formation of both spermidine and spermine. SAM is formed from methionine through the addition of adenosine. Because 5-methyltetrahydrofolate donates a methyl group to homocysteine to produce methionine, folate deficiency may decrease polyamine synthesis. Weanling male Sprague-Dawley rats were fed an amino acid–defined diet with 2 mg folic acid/kg diet (control) or no added folic acid (test). Blood, liver, brain, jejunum, ileum and colon samples were collected at the end of 5 wk. Compared with controls, rats fed the test diet had a 72% reduction in plasma folate (123.6 ± 13.1 vs. 34.6 ± 2.2 nmol/L, P < 0.001) and a 42% reduction in RBC folate (2834.4 ± 218.3 vs. 1651.8 ± 75.9 nmol/L, P < 0.001). Hepatic spermidine and spermine in folate-depleted rats were 58% (P < 0.001) and 67% (P < 0.01) higher, respectively, than in controls. Plasma putrescine was 27% higher (P < 0.05) than in controls. The polyamine concentrations of the jejunum, ileum, colon and brain did not differ. This study suggests that mild folate deficiency influences polyamine synthesis, but contrary to our hypothesis, hepatic spermidine and spermine were increased, as was circulating putrescine. This may have occurred for a number of reasons including increased enzyme activity or overcompensation by the betaine-homocysteine transmethylation pathway in the liver. Further study is necessary to clarify interactions between folate and polyamine metabolism and to determine whether polyamines are involved in the damaging effects of folate deficiency. J. Nutr. 132: 2632–2637, 2002.

KEY WORDS: • folate deficiency • methionine • polyamine synthesis • rats • gastrointestinal tract

Substantial evidence exists for relationships between folate deficiency and neural tube defects (NTD)³ (1–3), and there is growing evidence of a relationship between folate deficiency and the development of cardiovascular disease and cancer, particularly colon cancer (4–6). Intervention studies have indicated that increased folate intake decreases the incidence of NTD, although the mechanism remains unclear (1–3). In relation to cancer, the epidemiologic evidence linking a diminished folate status with an increased risk of colorectal cancer is impressive (4–7), and in rats treated with the carcinogen dimethylhydrazine, low folate diets and folate deficiency were shown to promote the development of colonic neoplasia (8).

Several potential mechanisms for the damaging effects of a low folate diet have been proposed. Folate is essential in purine synthesis and thymidylic acid synthesis, and it has been suggested that in folate deficiency, cellular concentrations of these essential components of DNA and RNA would be altered and nucleic acid metabolism impaired (9). As shown in Figure 1, folate also plays an important role in the synthesis of S-adenosylmethionine (SAM), the methyl donor in a number of essential biological reactions, including DNA methylation (9,10). Homocysteine receives a methyl group from 5-methyltetrahydrofolate to be converted to methionine, or in the liver from betaine, derived from choline (11). Folate deficiency has been proposed to lead to reduced synthesis of SAM (12), and may result in DNA hypomethylation, leading to the activation of genes normally quiescent when methylation is adequate (13).

In addition to its role as a methyl donor, a proportion of SAM is decarboxylated, providing the aminopropyl group for the synthesis of the polyamines, spermidine and spermine (14,15). Spermidine, spermine and putrescine play important roles in the proliferation and development of living cells (15). Studies have shown that the concentration of SAM in the liver is significantly decreased in rats during folate deficiency (16). Therefore, we hypothesized that during folate deficiency, spermidine and spermine synthesis would be decreased, providing an underlying mechanism for the damaging effects of folate deficiency, particularly in relation to NTD, in which closure of the neural tube is impaired. To our knowledge, there have not been any previous studies that have investigated the effect of folate deficiency on polyamine synthesis.
MATERIALS AND METHODS

Animals and diet. Male weanling Sprague-Dawley rats (n = 20) with weights ranging from 51.5 to 66.3 g were purchased from Charles River Canada, St. Constant, Canada. They were randomly assigned to control or folate-deficient groups of 10. Rats were housed individually in wire-bottomed stainless steel cages with controlled temperature and humidity and a 12-h light:dark cycle. Throughout a 5-wk feeding period, rats had free access to food and tap water. Food consumption and weight were measured daily. The use of animals in this experimental protocol was approved before commencement by the University of Saskatchewan Protocol Review Committee for Animal Care and Supply. Both the control and the deficient diets were based on a L-amino acid purified diet, whereas the folate-deficient diet was identical except the folate was omitted (Dyets, Bethlehem, PA). A moderate folate deficiency was sought to resemble the likely degree of deficiency that might be encountered in a free-living population. The reason for the omission of a sulfa drug in these rats was used as a quality control sample for the folate assay. Plasma was diluted 1:4 or 1:7 before the folate assay. For the polyamine analysis, 0.5-mL aliquots of plasma were mixed with 1 mol/L PCA solution to achieve a final concentration of 0.5 mol/L and were stored at −20°C until analysis.

Analytical methods. Plasma and RBC folate were determined using a procedure based on the microbiological method developed by Tamura (18). Samples were added to 96-well tissue culture plates, and serial dilutions were made with ascorbate phosphate buffer (1 g/100 g sodium ascorbate, 0.1 mol/L KH2PO4, pH 6.3). Glycerol-protected Lactobacillus casei (ATCC 7469; American Type Culture Collection, Rockville, MD) was mixed with the assay medium to a concentration of 1% (150 μL glycerol-protected bacteria in 15 mL of medium) and then 150 μL was added to each well. The plate was sealed in a plastic bag, incubated at 37°C for 18 h and read on a Titertek Multiskan microplate reader (Flow Laboratories, Mississauga, Canada) at a wavelength of 492 nm. The folate concentration of the pooled rat plasma sample was determined with each assay, with a CV of 10%. Polyamines were measured by an modified HPLC method based on the procedure of Verkoelen et al. (19) with 1,7-heptanediamine as the internal standard, made up as a stock solution of 0.15 g in 50 mL doubly distilled water, and diluted 1:3 as a working standard before use. The working internal standard (10 μL) was added to plasma and tissue homogenates, which were then centrifuged at 10,000 × g for 10-30 min, and the supernatant of 200-400 μL was added to a final volume of 0.5 mL with 0.5 mol/L PCA before derivatization. Derivatives were obtained by adding 2 mL of 2 mol/L NaOH and 20 μL benzyl chloride to each tube and mixing vigorously for 30 min at room temperature, after which 3.5 mL of chloroform was added. After centrifugation at 350 × g for 10 min and removal of the top alkaline phase, the chloroform layer was washed with doubly distilled water 3 times; a 3-mL aliquot was removed and evaporated completely at 80°C.

For analysis, the residue was reconstituted with the mobile phase methanol:H2O (58:42 v/v). Each sample or standard (100 μL) was injected onto a 3.2 × 250 mm LiChrospher RP-18 analytical column (Supelco, Oakville, Canada) on a Waters 710B HPLC system (Waters Associates, Milford, MA), with a 490 Programmable Multiswavelength detector. Samples were analyzed at a flow rate of 0.6 mL/min at room temperature, with UV detection at 229 nm. Each run was completed in 40 min. Quality control samples, containing putrescine, spermidine and spermine were prepared, 2 at low concentration, 2 at

Plasma and RBC folate were determined using a procedure based on the microbiological method developed by Tamura (18). Samples were added to 96-well tissue culture plates, and serial dilutions were made with ascorbate phosphate buffer (1 g/100 g sodium ascorbate, 0.1 mol/L KH2PO4, pH 6.3). Glycerol-protected Lactobacillus casei (ATCC 7469; American Type Culture Collection, Rockville, MD) was mixed with the assay medium to a concentration of 1% (150 μL glycerol-protected bacteria in 15 mL of medium) and then 150 μL was added to each well. The plate was sealed in a plastic bag, incubated at 37°C for 18 h and read on a Titertek Multiskan microplate reader (Flow Laboratories, Mississauga, Canada) at a wavelength of 492 nm. The folate concentration of the pooled rat plasma sample was determined with each assay, with a CV of 10%. Polyamines were measured by an modified HPLC method based on the procedure of Verkoelen et al. (19) with 1,7-heptanediamine as the internal standard, made up as a stock solution of 0.15 g in 50 mL doubly distilled water, and diluted 1:3 as a working standard before use. The working internal standard (10 μL) was added to plasma and tissue homogenates, which were then centrifuged at 10,000 × g for 10-30 min, and the supernatant of 200-400 μL was added to a final volume of 0.5 mL with 0.5 mol/L PCA before derivatization. Derivatives were obtained by adding 2 mL of 2 mol/L NaOH and 20 μL benzyl chloride to each tube and mixing vigorously for 30 min at room temperature, after which 3.5 mL of chloroform was added. After centrifugation at 350 × g for 10 min and removal of the top alkaline phase, the chloroform layer was washed with doubly distilled water 3 times; a 3-mL aliquot was removed and evaporated completely at 80°C.

For analysis, the residue was reconstituted with the mobile phase methanol:H2O (58:42 v/v). Each sample or standard (100 μL) was injected onto a 3.2 × 250 mm LiChrospher RP-18 analytical column (Supelco, Oakville, Canada) on a Waters 710B HPLC system (Waters Associates, Milford, MA), with a 490 Programmable Multiswavelength detector. Samples were analyzed at a flow rate of 0.6 mL/min at room temperature, with UV detection at 229 nm. Each run was completed in 40 min. Quality control samples, containing putrescine, spermidine and spermine were prepared, 2 at low concentration, 2 at

FIGURE 1 Polyamine biosynthetic pathway. Enzymes: 1, methylenetetrahydrofolate homocysteine methyltransferase (methionine synthase); 2, betaine-homocysteine methyltransferase; 3, S-adenosylmethionine synthetase; 4, S-adenosylhomocysteine de-carboxylase; 5, spermidine synthase; 6, spermine synthase; 7, arginase; 8, ornithine decarboxylase; 9, polyamine oxidase; 10, acetyl CoA:spermidine N'-acetyltransferase. SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; CH3, methyl group
a middle concentration and 2 at a high concentration and were analyzed with each run. These quality control concentrations varied with the tissue being analyzed, e.g., for the colon, the low standard contained $4 \times 10^{-7}$ mol/L putrescine, $6 \times 10^{-6}$ mol/L spermidine, and $3 \times 10^{-6}$ mol/L spermine; for the brain, the low standard contained $4 \times 10^{-7}$ mol/L putrescine, $30 \times 10^{-6}$ mol/L spermidine and $14 \times 10^{-6}$ mol/L spermine. The protein concentration of tissue samples was used to normalize the polyamine values by expressing these as nmol polyamine/mg protein.

Protein was measured using the method of Lowry et al. (20) with albumin as the standard. Tissue homogenates were diluted with 1 mol/L NaOH (100–250 μL); aliquots of these mixtures (15–30 μL) were added to SDS (1 g/100 g) to a total volume of 200 μL for analysis. Hemoglobin and hematocrit of rats were measured at the Veterinary Diagnostic Service, College of Veterinary Medicine, University of Saskatchewan.

**Statistical methods.** Results are expressed as means ± SEM. Statistical significance of differences was evaluated using unpaired t tests. A probability of $<0.05$ was used to indicate significance. All statistical analyses were performed using Microsoft Excel 97 (Microsoft, Redmond, WA).

**RESULTS**

All of the rats remained healthy during the 5-wk feeding period. They did not exhibit any signs of anemia or any other abnormal behavior. However, both the control and deficient groups had one rat that developed ulcerative dermatitis on the shoulder, neck and tail end during the feeding period. The infection was cured by applying Bactroban (SmithKline Beecham, Oakville, Canada) twice a day for a week. During the treatment, the rats did not change their eating habits or food consumption.

There were no differences in the initial body weights of rats between the control group and the deficient group (Table 1). Total food consumption for the 5-wk feeding period for the deficient group tended to be greater ($P = 0.08$) than for the control. The gain:feed ratio did not differ between groups. Total weight gain of the rats and final body weight were greater in the deficient group than in the control group ($P < 0.05$).

**Folate status.** Compared with the controls, rats fed the folate-deficient diet had a 72% reduction in plasma folate (P < 0.001) and a 42% reduction in RBC folate (P < 0.001; Table 2). The precision around the mean estimated by calculating interday relative standard deviations based on the results of the quality control plasma sample was 10%.

**TABLE 1**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control diet</th>
<th>Folate-deficient diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight, g</td>
<td>59.7 ± 1.4</td>
<td>59.8 ± 1.2</td>
</tr>
<tr>
<td>Total food consumption, g</td>
<td>638 ± 15.9</td>
<td>678 ± 13.9</td>
</tr>
<tr>
<td>Total weight gain, g</td>
<td>261 ± 6.0</td>
<td>280 ± 5.9 *</td>
</tr>
<tr>
<td>Final weight, g</td>
<td>320 ± 5.8</td>
<td>340 ± 6.9 *</td>
</tr>
<tr>
<td>Gain:Feed ratio, g/g</td>
<td>0.4 ± 0.004</td>
<td>0.4 ± 0.01</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.4 ± 0.005</td>
<td>0.4 ± 0.01</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>132 ± 1.8</td>
<td>131 ± 2.3</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 10. *Different from control, $P < 0.05$.

**TABLE 2**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control diet</th>
<th>Folate-deficient diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma, nmol/L</td>
<td>123.6 ± 13.1</td>
<td>34.6 ± 2.2 ***</td>
</tr>
<tr>
<td>RBC</td>
<td>2834.4 ± 218.3</td>
<td>1651.8 ± 75.9 ***</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 10. *Different from control, $P < 0.05$.

**Polyamine concentrations.** Spermine concentration in rat plasma was below the limit of detection of the HPLC method (Table 3). Compared with the controls, rats fed the folate-deficient diet had a 27% greater plasma putrescine concentration (P < 0.05). The folate-deficient rats had a 58% greater hepatic spermidine concentration (P < 0.001) and a 67% greater hepatic spermine concentration than controls (P < 0.01). Polyamine concentrations did not differ between the two dietary treatment groups in the brain, jejunum, ileum or colon.

**DISCUSSION**

Inverse relationships between folate intake and the incidence of cancer, heart disease and NTD are now recognized (1–7). Because folate is essential in purine and thymidylate synthesis and DNA methylation, folate deficiency has been thought to interrupt nucleic acid synthesis or alter the expression of certain genes resulting in damaging consequences (21–

**TABLE 3**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Folate-deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Putrescine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain, nmol/mg protein</td>
<td>0.1 ± 0.01</td>
<td>0.1 ± 0.01</td>
</tr>
<tr>
<td>Liver, nmol/mg protein</td>
<td>0.1 ± 0.01</td>
<td>0.2 ± 0.03</td>
</tr>
<tr>
<td>Jejunum, nmol/mg protein</td>
<td>2.8 ± 0.3</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>Ileum, nmol/mg protein</td>
<td>2.1 ± 0.2</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>Colon, nmol/mg protein</td>
<td>0.4 ± 0.02</td>
<td>0.5 ± 0.03</td>
</tr>
<tr>
<td>Plasma, μmol/L</td>
<td>2.6 ± 0.1</td>
<td>3.3 ± 0.3 *</td>
</tr>
<tr>
<td>Spermidine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain, nmol/mg protein</td>
<td>18.1 ± 1.0</td>
<td>18.1 ± 0.6</td>
</tr>
<tr>
<td>Liver, nmol/mg protein</td>
<td>6.5 ± 0.5</td>
<td>10.3 ± 0.8 ***</td>
</tr>
<tr>
<td>Jejunum, nmol/mg protein</td>
<td>35.0 ± 1.5</td>
<td>31.6 ± 2.0</td>
</tr>
<tr>
<td>Ileum, nmol/mg protein</td>
<td>29.6 ± 1.0</td>
<td>28.1 ± 1.7</td>
</tr>
<tr>
<td>Colon, nmol/mg protein</td>
<td>18.9 ± 0.9</td>
<td>18.9 ± 0.6</td>
</tr>
<tr>
<td>Plasma, μmol/L</td>
<td>8.4 ± 0.8</td>
<td>10.7 ± 1.2</td>
</tr>
<tr>
<td>Spermine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain, nmol/mg protein</td>
<td>6.6 ± 0.4</td>
<td>6.6 ± 0.2</td>
</tr>
<tr>
<td>Liver, nmol/mg protein</td>
<td>3.0 ± 0.4</td>
<td>5.0 ± 0.6 ***</td>
</tr>
<tr>
<td>Jejunum, nmol/mg protein</td>
<td>16.9 ± 0.8</td>
<td>16.2 ± 0.7</td>
</tr>
<tr>
<td>Ileum, nmol/mg protein</td>
<td>18.5 ± 0.6</td>
<td>17.5 ± 1.0</td>
</tr>
<tr>
<td>Colon, nmol/mg protein</td>
<td>19.9 ± 1.1</td>
<td>19.8 ± 0.9</td>
</tr>
<tr>
<td>Plasma, μmol/L</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 10. Different from control, *$P < 0.05$.
2 $P < 0.01$, ***$P < 0.001$. 

Downloaded from https://academic.oup.com/jn/article-abstract/132/9/2632/4687770 by guest on 11 April 2019
24). However, folate is also involved in the synthesis of poly-
amines, which play important roles in the proliferation and development of living cells (10,15). In this study, the effects of folate deficiency on polyamine synthesis in rats were exam-
ined.

Over the 5-wk feeding period, total food consumption of the deficient group tended to be greater than that of the control group and the total weight gain of the folate-deficient rats was significantly greater than that of controls despite their having almost the same initial weights. The gain:feed ratios did not differ between groups, indicating that the greater weight gain of the folate-deficient group was due to increased food consumption. Semchuk et al. (25) did not observe signi-

ficant differences in food consumption and weight gain between folate-deficient and control rats fed for 5 wk, and in a study of 25 wk, rats appeared healthy and no significant differences in weight gain between folate-deficient and control rats were observed (26). Our finding that folate-deficient rats gained more weight than controls was unexpected. However, the pellets of the folate-deficient diet were broken into small pieces when they arrived and may have been easier to eat than the control diet. We contend that this was the reason for the significantly greater weight gain of the folate-deficient rats.

Contrary to our hypothesis, hepatic spermidine and sperm-
ine concentrations in the folate-depleted rats were signifi-
cantly higher than those of the control group. Although SAM concentrations were not measured in our study, lower hepatic SAM concentrations have been observed in folate-deficient rats at both 15 and 24 wk of feeding compared with controls (16). A decrease in cellular SAM level could have resulted in increases in ornithine decarboxylase (ODC) and S-adenosyl-

methionine decarboxylase (SAMDC) activity (27). In cell cultures, it has been found that even when SAM was depleted to nearly undetectable levels, polyamine pools were only min-

imally affected because of increased activity of key enzymes in polyamine synthesis (28).

In most tissues, 5-methyltetrahydrofolate donates a methyl group to combine with homocysteine to produce methionine, which then forms SAM (Fig. 1). However, in the rat liver and perhaps also the kidney, betaine, derived from choline, can also act as a methyl donor in methionine synthesis (11). Moreover, a choline-deficient diet has been shown to result in decreased hepatic concentrations of betaine, methionine and SAM (29), indicating that the betaine pathway is important in methionine metabolism, and that the remethylation of homocysteine using 5-methyltetrahydrofolate alone cannot meet the total requirement.

It has been suggested that betaine can be used by the rat liver to compensate for inadequate folate and can maintain remethylation of homocysteine (29–31). In studying treat-

ment with methotrexate (MTX) (31), a folate antagonist that decreases tetrahydrofolate supply by inhibiting dihydrofolate reductase, Barak et al. (32) found that hepatic betaine concentra-

tions were significantly lower than in controls, and concluded that betaine compensated for 5-methyltetrahydrofolate as a methylating agent during MTX treatment. The betaine pathway has also been shown to be able to compensate for impaired folate-dependent methionine synthesis caused by a prolonged ethanol diet. More recently, Kim et al. (33) examined the effect of folate deficiency on hepatic choline, the precursor of betaine. In weanling rats fed a diet severely depleted in folate, hepatic SAM concentration was only 20% of control and was accompanied by significantly decreased choline levels. Secondary depletion of hepatic choline may have been caused by increased utilization to compensate for the reduced capacity of the folate-dependent transmethylation pathway in methionine synthesis. In moderate folate defi-
ciency, hepatic SAM concentrations were 35% lower and choline concentrations 36% lower than controls, although the choline differences were not significant. Betaine and the choline-dependent remethylation pathway may not have been sufficient to maintain hepatic SAM concentrations for the extended feeding period used in that experiment (24 wk). In our study of only 5 wk, it is possible that the betaine-homo-
cysteine transmethylation pathway was fully able to compen-
sate in methionine metabolism for the inadequate folate sup-
ply, and that significantly increased hepatic spermidine and spermine concentrations in folate-depleted rats were part of an overcompensation of the betaine-homocysteine pathway.

There were no significant differences in polyamine concen-

trations in brain between folate-deficient rats and controls. However, limited transport of polyamines has been seen across the blood-brain barrier (34); thus, it would not be surprising that brain polyamines were unchanged, whereas hepatic sper-

midine and spermine and plasma putrescine levels were ele-

vated. Folate conservation in brain may also be different from other tissues (26). Rats fed a folate-deficient diet for 25 wk showed significantly lower folate concentration in the liver, kidney and spleen, but no changes in the brain. Therefore, brain folate levels were unlikely to have been depleted and polyamine metabolism in brain was unlikely to have been affected in our study.

Polyamine concentrations in intestinal tissues were similar to those in other studies (34–36), and no significant differences in polyamine concentrations were seen in jejunum, ileum and colon between folate-deficient and control rats. Colonic SAM levels were unchanged in rats fed moderately folate-deficient diets for 15 or 24 wk, in spite of lower colonic folate concen-

trations (16), suggesting that more severe folate deficiency must be present to deplete colonic SAM. Compensation by the betaine-homocysteine transmethylation pathway may have maintained colonic mucosal SAM concentrations during folate deficiency, although it remains uncertain whether the betaine pathway is functional in the colon. Alternatively, bacteria resident in the large intestine may synthesize folate (37), and SAM levels may have been maintained in the colon through the supply of folate from this source.

Contrary to our hypothesis, rats fed the folate-deficient diet had 30% greater plasma putrescine concentrations compared with controls, with no significant differences in plasma sper-

midine. The origins of plasma polyamines are not certain (38). Food, bacterial synthesis in the gastrointestinal tract and release from cells may all contribute to plasma polyamine concen-

trations (38,39). Peripheral blood cells transport most of the circulating polyamines; erythrocytes transport the majority of spermidine and spermine, and platelets transport most of the putrescine (39). Polyamine uptake into blood cells de-

pends on plasma polyamine concentrations (40).

Elevated total plasma putrescine levels have been found in mice bearing Lewis lung carcinoma (41), and rats grafted with tumors have enhanced intestinal absorption of putrescine compared with controls (42). In these animals, polyamine concentrations and ODC activity in intestinal mucosa were the same in tumor-bearing rats and controls, yet tumors were found to have the ability to retain putrescine from the diet (42). The growth rate of Lewis lung carcinoma in mice has been found to be significantly reduced by inhibitors of ODC and polyamine oxidase as well as by a polyamine-deficient diet (15). In the present study, there may have been increased putrescine release into the circulation of the folate-depleted
rats, resulting in significantly higher levels of plasma putrescine compared with controls. The origin of this increased putrescine is uncertain, although it is possible that more plasma putrescine was released from the liver or other tissues as a result of increased ODC activity during folate deficiency. Putrescine is the direct product of the ODC reaction, and ODC activity is thought to be critically involved in malignant transformation (43). Tumor-bearing mice have higher plasma putrescine levels and tumor cells have the ability to capture dietary putrescine at the expense of other tissues, such as liver and intestine (41,42). In vitro, oncogene transcription in hepatoma cells is accompanied by a rapid increase in putrescine concentration (44). In human breast cancer, ODC activity in tumor tissue is a negative independent prognostic factor (45), and ODC activity in colon cancer tissue has been found to be higher than in the normal mucosa from the same patient (46). There has also been impressive evidence linking a diminished folate status with an increased risk of colorectal cancer in humans (5,7). Therefore, the duration of our study might have been insufficient to show the effects of folate deficiency in some tissues. These results should therefore be regarded as preliminary, and future investigations could be done to further characterize polyamine metabolism during folate deficiency. Levels of SAM in various tissues, activity of key enzymes in polyamine synthesis and the betaine methylation pathway, and the uptake of circulating polyamines by liver cells could be examined to explain the increased hepatic spermidine and spermine. Meanwhile, the uptake of polyamines from the gastrointestinal tract of folate-deficient rats could be examined to explain the origin of elevated plasma putrescine in folate deficiency. Finally, it would be worthwhile to investigate polyamine metabolism in folate-depleted rats with chemically induced carcinogenesis, as well as folate depletion over the long term.

ACKNOWLEDGMENTS

We thank Joan Bobyn, Weiwei Tan and Courteney Magnuson for their technical assistance.

LITERATURE CITED


35. Porter, C. W., Dworaczyk, D., Ganis, B. & Weiser, M. M. (1980) Poly- 
amines and biosynthetic enzymes in the rat intestinal mucosa and the influence of 
Lepoint, P. (1986) Spermine and spermidine induce intestinal maturation in the 
synthesized folate in rat large intestine is incorporated into host tissue folyl 
vitro studies on the entry of polyamines into normal red blood cells. Biochimie 66: 
385–393.
41. Catros-Quemener, V., Leray, G., Moulinoux, J. P., Havouis, R., de 
polyamine transport by plasma lipoproteins in the mouse. Biochim. Biophys. Acta 
134: 30–37.
42. Brachet, P., Quemener, V., Havouis, R., Tomé, D. & Moulinoux, J. 
(1994) Alterations in intestinal uptake of putrescine and tissue polyamine concentration 
thine decarboxylase activity is critical for cell transformation. Nature (Lond.) 360: 
355–358.
44. Lafarge-Frayssinet, C., Cassingena, R., Frayssinet, C., Estrade, S., 
Havouis, R. & Moulinoux, J. Ph. (1993) Polyamine content and oncogene 
expression in hepatoma cells in culture during methionine deprivation and refeed- 
ing. Anticancer Res. 13: 491–496.
influence on survival of increased ornithine decarboxylase activity in human 