Folate Derived from Cecal Bacterial Fermentation Does Not Increase Liver Folate Stores in 28-d Folate-Depleted Male Sprague-Dawley Rats[1]

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ABSTRACT This study assessed the ability of rats to absorb and store the folate synthesized by cecal bacteria. Male weanling Sprague-Dawley rats were folate depleted by feeding a low folacin AIN93G formulated basal diet for 28 d; they were then fed repletion diets containing folate (0.25–1.0 mg/kg diet), dietary fiber (DF; wheat bran, oat bran, ground corn, wheat germ) or undigested and fermented dietary material (UFDM; polydextrose, inulin) in the presence and absence of an antibiotic (succinylsulfathiazole). Fermentation was stimulated by DF and UFDM and reduced by the antibiotic. In the absence of succinylsulfathiazole, the increase in liver folate (during the repletion phase) was proportional only to the folate content of the diet and did not vary with added DF or UFDM. Adding succinylsulfathiazole lowered total folate excretion from 13.8 ± 8.2 to 4.8 ± 2.9 nmol/d (pooled diets, $P < 0.00001$) in agreement with its role in inhibiting bacterial folate synthesis. In addition, succinylsulfathiazole lowered liver folate in rats fed control and test diets approximately equally with a mean decrease from 11.6 ± 2.5 to 7.5 ± 2.5 nmol/g wet liver (pooled diets, $P < 0.00001$), suggesting that the antibiotic also affected rat folate absorption and/or metabolism. Increased bacterial fermentation and excretion as well as increased bacterial folate production in the presence of added DF and UFDM were demonstrated by increased volatile fatty acid content in cecal and fecal samples ($P < 0.000001$) and increased diaminopimelic acid, muramic acid and folate in feces ($P < 0.00001$). The magnitude of these changes depended on the type of DF and UFDM. These results show that bacterially synthesized folate is not substantially absorbed and stored in the liver of Sprague-Dawley male rats. J. Nutr. 133: 1347–1354, 2003.

KEY WORDS: • *folate bioavailability* • *dietary fiber* • *inulin* • *polydextrose* • *fermentation* • *rats*

Folate is a generic term that refers to compounds based on the simplest form of folic acid [pteroylglutamic acid; (1)]. Although humans can synthesize all components of folate, they lack the conjugase enzyme that condenses them. Folate thus remains an essential human dietary component (2). Both deficiency and excess folate intake are physiologically important. Folate deficiency is associated with anemia and neural tube defects, which constitute important public health problems. To address this problem, folate fortification of cereal-based products is mandatory in Canada (3). Excess folate intake has been associated with both detrimental and beneficial effects. On the one hand, it may confound diagnosis of vitamin B-12 deficiency, a common problem in the elderly (4), whereas on the other hand, it may attenuate hyperhomocysteinemia, an independent risk factor for cardiovascular disease (5). If dietary fiber (DF)[3] does enhance production of bioavailable folates by human gut bacteria, fortification requirements could be decreased, thus limiting potential long-term increased exposure to folates by groups such as the elderly.

Work with rats suggests that dietary folate is not the only source of biologically available folate and that folate synthesized by cecal bacteria may also be absorbed and stored in the liver (6–9). This suggests that diets containing DF and undigested and fermented dietary material (UFDM) provide more folate than diets low in DF because of the increased bacterial fermentation associated with DF and UDFM. A demonstration of this effect was provided by experiments with folate-depleted/replenished rats fed diets high in xylan or California small white beans (6) as well as in rats fed wheat germ or Brewers yeast (7). The contribution of bacterially derived folate to liver stores has also been inferred from increased cecal and fecal folate after feeding rats human milk, which is partly fermented by these animals (8). This increase was not observed when rats were fed milk plus succinylsulfathiazole in an attempt to inhibit cecal bacteria growth (9). In humans, higher urinary excretion of specific folacin isomers was observed after feeding diets high in California small white beans (10). Direct absorption of bacterially synthesized folate has been confirmed by radioactively labeling the bacterial folate pool (11) although an estimation of the absolute absorption suggested that this effect was small. The argument that colonic

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[3] Abbreviations used: DAPA, diaminopimelic acid; DF, dietary fiber; MA, muramic acid; UFDM, undigested and fermented dietary material.

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