Folate was isolated in 1941 from spinach and named after the Latin word *folium* (= leaf; ref. 1). Although the initial impetus for research on folate metabolism was to find a cure for anemia, it was soon recognized that the administration of folate enhanced the growth of existing tumors and that folate metabolism may be a promising target for anticancer drug design (2). Already developed by the late 1940s, folate antagonists, such as methotrexate, have rapidly become a mainstay of cancer chemotherapy (3). Because of the role of folate in nucleotide synthesis, deficiency affects primarily rapidly dividing tissues, such as the epithelium of the gastrointestinal tract, hematopoietic cells, and tumors. This increased need of proliferating tissues for folate explains why macrocytic anemia is one of the clinical manifestations of folate deficiency. Similarly, the crucial role of folate in preventing neural tube defects may be attributed to the high fetal requirement for folate to sustain rapid cell division; clinical trials have shown a clear reduction in neural tube defects with periconceptional folic acid administration (4-6). This success story led policy makers, including the USPHS in 1992 and the Institute of Medicine in 1998, to recommend that all women of reproductive age consume 400 μg folic acid daily from supplements or fortified foods (7, 8). Unfortunately, health promotion efforts targeting childbearing women have been largely unsuccessful in achieving this level of intake. Therefore, policy makers opted for generalized folic acid fortification largely unsuccessful in achieving this level of intake. There-
unclear; even more unclear is whether such high intakes prevent cancer, especially among the elderly.

**A Dual Role for Folate in Carcinogenesis?**

An increasing body of evidence suggests that folate plays a dual role in carcinogenesis, involving both the prevention of early lesions and potential harm once preneoplastic lesions have developed. Observational studies suggest that high folate intakes reduce cancer risk, particularly for malignancies of the colon, pancreas, esophagus, stomach, and possibly cervix and breast. However, folate can promote the growth of existing cancers and, as noted above, antifolate drugs are potent chemotherapeutic agents. Initial experimental studies have suggested that folate supplementation can enhance the carcinogenic progression of mammary tumors (25). Where is the line between benefit and possible harm? Elegant work by Kim et al. (26) has shown that the dose and timing of folate interventions may be critical. For example, in two colorectal cancer mouse models, modest doses of folate supplementation suppressed the development and progression of colorectal cancer. However, this beneficial effect was observed only for folate given before the establishment of neoplastic foci in the intestine (27, 28). Once preneoplastic lesions were present, dietary folate enhanced the development and progression of colorectal cancer (27, 28). Similar results in a rat model of breast cancer suggest that folate deficiency inhibited, rather than enhanced, cancer development (25, 29). Preliminary results from the first randomized-controlled trial of folic acid for chemoprevention of colorectal adenomas (25) and greater adenoma multiplicity, with a significant increase (rate ratio, 1.04). However, participants in the folic acid group tended to not prevent the recurrence of colorectal adenomas (rate ratio, 0.83). Follow-up colonoscopies were scheduled years after the initial endoscopy and supplementation continued or without aspirin. The role of folate in fostering the progression of premalignant lesions. Although participants had the initial polyp removed before study entry, the increased risk of later multiple metachronous polyps suggests that, among a subgroup of individuals who had multiple preneoplastic lesions, folic acid supplementation may have promoted their growth. The results may also suggest that this effect may be modest at supplementation below 1,000 μg/d, but do not provide any information about the effects on growth of existing polyps that are not detected during a colonoscopy.

What are the possible mechanisms of a dual role for folate in carcinogenesis that depends on timing and perhaps dose? What is the function of folate in nucleotide synthesis may be central. Folate is essential for the synthesis of thymidine via thymidylate synthase, and of purines, as illustrated in Fig. 1. Folate deficiency results in a reduced production of thymidine and misincorporation of uracil into DNA (31, 32). During the excision of uracil, single-strand breaks and, eventually, double-strand breaks can occur (32). Because of the lack of reliable biomarkers, the effects of folate deficiency on purine synthesis and apurinic sites in DNA are less well established. However, some epidemiologic studies suggest that this common form of DNA damage may be a critical mechanism linking folate to cancer risk (33, 34). If we assume that folate deficiency in a rapidly proliferating tissue (such as the colon) enhances genomic instability, then the probability of a loss of function of genes that prevent adenoma formation increases. Although an initial animal study did not observe an effect of folate deficiency on the rate of APC mutations, the sample size may have been too small to obtain reliable results (35). After a small tumor or microadenoma has been established, this tumor may grow more rapidly with folate supplementation, due to the greater provision of nucleotides:

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How could one estimate the net effect of these two potentially competing mechanisms of folate supplementation? Figure 1. Overview of folate metabolism, entry of folic acid and physiologic folates into the cell, and links to methylation reactions and nucleotide synthesis (modified with permission from ref. 3). THF, tetrahydrofolate; DHF, dihydrofolate; RFC, reduced folate carrier; hFR, human folate receptor; MTHFR, 5,10-methylenetetrahydrofolate reductase; DHFR, dihydrofolate reductase; GART, glycaminide ribonucleotide transformylase; AICARFT, 5-aminomimidazole-4-carboxamide ribonucleotide transformylase; AICAR, 5-aminomimidazole-4-carboxamide ribonucleotide; GAR, glycaminide ribonucleotide; SAM (AdoMet), S-adenosylmethionine; SAH (AdoHcy), S-adenosylhomocysteine; SHMT, serine-hydroxy-methyltransferase; MS, methionine synthase; TS, thymidylate synthase; MT, methyltransferases.
on carcinogenesis? The epidemiologic evidence to date suggests that higher folate intakes generally correlate with a reduced risk, thus implying an overall reduction in risk. (Nonetheless, it needs to be remembered that folate intake is highly correlated with supplementary vitamin intake in general, which raises the potential for confounding.) Building on a previously developed mathematical model of colorectal carcinogenesis (37), we have investigated the question of a “net effect” of the putative opposing mechanisms by simulating the effect of a folic acid intervention on reduction of mutations and increase in tumor growth simultaneously. Initial results suggest that the net effect of folic acid fortification on individual colon cancer risk is modified by age: Whereas children may be more likely to experience reduced colorectal cancer rates in the future, rates among middle-aged adults are likely to increase. The findings of this mathematical model clearly illustrate the need for a better quantification of the molecular effects of folate (see below).

**Folate and Carcinogenesis—Yet Another Discrepancy between Observational Studies and Clinical Trials?**

When the results from the folate polyp-prevention trial (30) are published, it will be important to interpret them carefully and to avoid rejecting folate as a chemoprevention agent altogether. The discrepancy between results from observational studies and randomized controlled studies has recently been discussed in this journal (38). The story of folate adds yet another possible explanation for such disparate findings: differences in biological effects of potent agents depending on the stage of carcinogenesis. Almost all chemoprevention trials aim at prevention among those with a history of earlier lesions (precancer or cancer), largely because of the higher risk of subsequent tumors in such patients, and thus reduced cost and time and increased power. However, we should not lose sight of the limitations of this approach: As discussed above, for folate, the experimental evidence suggests that administration is beneficial before the appearance of preneoplastic lesions, but potentially harmful after. Whereas a randomized trial that evaluates the influence of folic acid administration on recurrent polyps after a primary diagnosis will be able to answer exactly that question, it is unlikely to provide relevant information regarding folic acid supplementation early in life. Primary prevention trials of folate (intervention before early lesions) would be expensive and of long duration. We may need to continue to rely on less explicit evidence, including that from ecologic studies of folate acid fortification by age cohort, observational epidemiologic studies, and animal experiments.

**Folate and Cardiovascular Disease**

The discrepancy between findings from observational studies and randomized controlled trials is now also apparent for another possible health benefit attributed to folate. Because of the unequivocal homocysteine-lowering effects of folic acid supplementation and experimental data suggesting a role of homocysteine in endothelial damage, the prevention of cardiovascular disease was assumed to be another health benefit of increased folate intakes (39). Unfortunately, the first results from randomized controlled trials with actual disease outcomes provide no evidence for such an effect (40). We may need to be more cautious of biomarkers of disease until they are established as being in the causal pathway.

**Other Potential Deleterious Effects of High Folic Acid**

Two recent studies raise concern about the implications of very high folic acid intakes on other health outcomes. A prospective cohort of 3,718 elderly individuals (>65 years) who participated in the Chicago Health and Aging Project investigated cognitive decline over the course of 6 years in relation to dietary intakes of folate and vitamin B12. Unexpectedly, high folate intake was associated with a faster rate of cognitive decline. Those in the highest quintile of folate intake (comprised largely of supplement users with a mean intake of 742 µg/d) had a statistically significantly more pronounced decline, and this association was particularly strong for supplement use in excess of 400 µg/d. Limitations of the study were its lack of biomarker assessments and the potential for confounding by indication. Nevertheless, these unexpected findings urge us toward further research on the cognitive implications of high levels of folic acid in older individuals.

An additional concern has arisen in relation to immune function: In a preliminary cross-sectional study of 104 postmenopausal women, we reported an inverse U-shaped relationship between folate from dietary sources and supplements and natural killer cell cytotoxicity (41). Natural killer cells are part of the innate immune response and low cytotoxicity may increase cancer risk (42). Unmetabolized folic acid was detected in 78% of fasting plasma samples from the participants. This is the first study reporting the presence of this compound in healthy individuals who are not subjected to pharmacologic doses of folic acid. The presence of unmetabolized folic acid was associated with decreased natural killer cytotoxicity, and a trend toward lower natural killer cytotoxicity with greater amounts of folic acid in plasma was observed among older women (>60 years). Although the study should be considered preliminary, it highlights the need for a better understanding of the relation of folate to immune function.

**Future Research Needs**

It is clear that the relationships between folate and health outcomes are complex; further, we need a better understanding of the relevant biological mechanisms to avoid misinterpretation. For solid tumor carcinogenesis, we need experimental studies that quantify possible effects of folate on growth of aberrant crypt foci, polyps, and similar early lesions in other tissues. The role of folate in hematopoietic malignancies also needs further research: Hematopoietic cells are particularly sensitive to folate deficiency and pediatric leukemias and
lymphomas are commonly treated with antifolate drugs (47). For malignancies treated with antifolates, we also need to understand better which treatment efficacy is altered by excessive supplement use.

Folate not only plays a role in nucleotide synthesis but is also critical for the provision of S-adenosylmethionine (Fig. 1), the universal methyl donor. DNA methylation of promoter regions has been established as one mechanism of gene regulation (48). A recent study of agouti mice shows that methyl supplementation (including folic acid) can alter the epigenetic gene regulation of offspring (49). Pennisi (50) subsequently reported on folate supplementation as a means of modifying morphology in tails of mice with a transposable element in the atrans gene. Whether there are other effects of excessive perinatal folic acid supplementation on epigenetic mechanisms, less benign than changes in coat color or unkinking of tails, defines another area of research need.

We also need more research on the safety of folic acid per se. Because this compound is now found in the bloodstream, data are needed on whether there are implications for the transport of natural folates. Human studies of folic acid at multiple levels, lasting several months or even years, and monitoring all beneficial and adverse effects described to date are a critical step. To understand the health effects of more chronic long-term intakes of doses at the upper intake level, studies ancillary to the recently completed randomized controlled trials of cancer chemoprevention are needed, targeting the specific outcomes of the animal and human studies described above, particularly cognitive and immune function. This is perhaps a unique opportunity to settle the issue of safety of long-term high intakes.

Epidemiologic studies should expand their investigations toward the high end of folate intakes and carefully evaluate the potential for confounding in the interpretation of results. Finally, there are multiple genetic polymorphisms in folate metabolism that result in interindividual differences in response (51). A full genetic screen of mutations in this biological pathway has not yet been undertaken. Exploration of the effect of multiple genetic variants, under different dietary conditions, on critical biomarkers relevant to carcinogenesis and other health outcomes is essential.

It remains unclear whether the possible deleterious effects of high folic acid outweigh the known and potential benefits. Further, this balance may differ across individuals and populations, by genetic characteristics and by life stage. Because of the high intake of folic acid from supplements and fortified foods in a large fraction of the population, these populations, by genetic characteristics and by life stage. Further, this balance may differ across individuals and populations, by genetic characteristics and by life stage.

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