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Folate, cancer risk, and the greek god, Proteus: a tale of two chameleons

Joel B. Mason, M.D.

Associate Professor of Medicine and Nutrition, Tufts University, U.S.D.A. Human Nutrition Research Center on Aging at Tufts University, 711 Washington St, Boston, MA 02111, joel.mason@tufts.edu, Phone: +1-617-556-3194/

Abstract

Evidence indicates that an abundant intake of foodstuffs rich in folate conveys protection against the development of colorectal cancer, and perhaps some other common cancers as well. The issue is a complex one however, since some observations in animal and human studies demonstrate that an overly abundant intake of folate among those who harbor existing foci of neoplasia might instead produce a paradoxical promotion of tumorigenesis. The pharmaceutical form of the vitamin, folic acid, might affect the process in a manner that is distinct from natural forms of the vitamin, although this remains a speculative concept. Our limited understanding of this complex relationship is unfortunately impeding efforts to move ahead with widespread folic acid fortification, but this may be necessary to ensure that such programs are instituted in a safe manner.

Introduction

The greek god, Proteus, was heavily pursued by mortals and gods alike, interested in exploiting his special powers of clairvoyance. In order to elude his pursuers, he often tricked them by assuming the form of an animal or sometimes even an inanimate object such as an ocean wave. Proteus was truly a chameleon, albeit a mythological one.

This review focuses on a more modern-day and tangible chameleon: the B-vitamin folate. For the past several decades the collective evidence from both pre-clinical and clinical studies has indicated that greater dietary intake of this vitamin conveys protection against the development of certain common cancers. The evidence has been most compelling for colorectal cancer, with lesser degrees of evidence to support such a connection for cancers of the breast, lung, pancreas and others. reviewed in 1·2 However, folate's central role as a co-factor in nucleotide synthesis also means that abundant availability of the vitamin can facilitate the proliferation of rapidly dividing cells, and hyperproliferation is a feature of most dysplastic ('pre-cancerous') and malignant ('cancerous') neoplasms. Evidence indicative of this latter effect began to emerge in the 1940s,3·4 and subsequent observations from both animal and human studies continue to support this concept. Thus, under most circumstances adequate availability of folate appears to assume the role of a cancer protective agent, presumably by enhancing genetic stability.5 However, in select circumstances in which an individual who harbors a neoplasm (or perhaps even a microscopic clone of dysplastic cells) consumes an overly abundant quantity of the vitamin, folate may instead facilitate the expansion of neoplastic cells and thereby metamorphose

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into an agent producing the opposite effect: one of cancer promotion. Like Proteus, folate appears to assume different guises depending on the circumstances.

This 'dual effect of folate', as it pertains to its effects on cancer risk (a term coined by Kim⁶), is creating considerable consternation for those who are trying to establish guidelines for the healthful intake of folate, particularly since it underscores the point that the level of intake of a micronutrient that is safe and healthful for one person may be potentially harmful to another. The issue is a particularly poignant one at present because many countries are debating as to whether to institute, or increase, mandatory folic acid fortification of grains, the latter being an effective means of diminishing the occurrence of births complicated by a neural tube defect (NTD). There is a great sense of urgency to complete such deliberations and act since the institution of national fortification programs has generally decreased NTD events by up to 25–50%, although the magnitude of reduction varies tremendously⁷. The design of cogent public health policies that effectively optimize health for many, while presenting no or minimal risk to others, must often occur in the absence of complete information, but we are nevertheless obliged to deliberate with as much an in-depth understanding as the existing science allows. This review is intended to provide an in-depth appreciation of the topic.

What does the science in this field actually tell us?

Although a few dissenting studies exist, the consensus of over 30 case-control and prospective cohort studies indicate that individuals who habitually consume the greatest quantities of dietary folate or who have the highest concentrations of blood folate, have a 40–60% reduction in the risk of developing colorectal cancer as well as a similar reduction in the risk of having the precursor lesion, the adenomatous polyp.¹ Moreover, the protective effect is more pronounced among those who are moderate-to-heavy consumers of alcohol,⁸ a known inhibitor of folate metabolism.⁹ Not surprisingly, a meta-analysis of the prospective cohort studies has confirmed the protective effect of higher folate status against the development of colorectal cancer.¹⁰

Several lines of evidence support a true mechanistic role for folate in the prevention of cancer. First, controlled studies in a number of animal models have demonstrated a dose-dependent protective effect of folate.^{reviewed in 11} Moreover, the fact that a common polymorphism in a key folate-dependent enzyme, methylenetetrahydrofolate reductase, is associated with a modulation in the risk of developing colorectal cancer also supports a mechanistic role for folate availability in carcinogenesis.^{reviewed in 12} Finally, the likelihood of a true cause and effect relationship between inadequate folate intake and an increased risk of cancer is strongly supported by the biological plausibility of the concept since folate is a critical co-factor in both biological methylation and nucleotide synthesis,¹³ and aberrancies of each of these two processes are thought to be among the most common mechanisms leading to cancer. In further support of the mechanistic roles played by folate depletion in carcinogenesis, recent studies in animal models have identified the *Wnt* cascade and *p53*-dependent control of the cell cycle in the colonic epithelium as vulnerable signaling pathways that develop pro-transformational aberrations as a result of mild decreases in the availability of dietary folate and other related 1-carbon vitamins.^{14•15}

The evidence implicating a protective role for high folate status in other cancers is not as compelling, in part because the issue has not yet been as extensively examined, and because there is not a consistency of results among the studies. Nevertheless, observations implicating a protective role for folate in human cancer do exist for the oropharynx, esophagus, stomach, pancreas, lungs, cervix, ovary, breast, neuroblastoma and leukemia. Sufficient numbers of good quality observational studies have been performed in some of

these cancers to conduct meta-analyses. For instance, a meta-analysis supports a protective effect for the three non-colonic GI cancers: esophagus, stomach and pancreas.¹⁶ Two recent meta-analyses of the large number of studies done in breast cancer suggest that a protective effect of folate may exist, although they suggest that the protection is confined to those women who are moderate-to-high imbibers of alcohol,^{17,18} a feature that is reminiscent of some of the observations in CRC.

The necessity of integrating other '1-carbon nutrients' as determinants of cancer risk

However, it is both myopic and misleading to attempt to understand the cellular functions of 1-carbon metabolism by merely assessing the nutritional status of folate since several other dietary micronutrients, including vitamins B2, B6 and B12, serve as critical co-factors for several pivotal enzymes as well (figure 1).

Vitamin B2 is a co-factor for methylenetetrahydrofolate reductase, which catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate; vitamin B12 is a co-factor for methionine synthase, a reaction in which 5-methyltetrahydrofolate participates as a substrate in the remethylation of homocysteine to form methionine; and vitamin B6 is a necessary co-factor for interconversion of tetrahydrofolate and its 5,10 methylene-substituted form as well as for the disposal of homocysteine through the transsulfuration pathway. It is therefore very uncommon for a biochemical function of 1-carbon metabolism to be influenced solely by the availability of one of its dietary co-factors. For instance, the disposal of homocysteine (which is ultimately the determinant of plasma homocysteine concentrations) reflects a complex 3-way interaction between folate, B12, and B6 status.¹⁹ Moreover, since riboflavin is a co-factor for MTHFR, plasma riboflavin is also a determinant of plasma homocysteine among those who carry the C677T MTHFR polymorphism.²⁰ Mathematical modeling of the known enzyme kinetics of 1-carbon metabolism reveals that critical functions of the pathway, such as nucleotide synthesis, are a complex function of the availability of all four of these vitamins.²¹ The inter-connectedness of these four vitamins in regard to metabolic outcomes that are relevant to carcinogenesis is further supported by observations that demonstrated that limiting B12 availability alone in a rodent model alters uracil incorporation in colonic DNA, a function of 1-carbon metabolism that is 'distant' from the methionine synthase reaction that B12 participates in.²² In addition, other observations in a rodent model demonstrate that many of the pro-transformational molecular events that occur as a result of altered 1-carbon metabolism, such as upregulation of Wnt signaling, are greatly magnified when inadequacies of multiple 1-carbon micronutrients are present compared to folate depletion alone.¹⁴ Thus, as we pursue a mechanistic understanding of how folate modulates carcinogenesis, it would be wise to examine all four of the 1-carbon vitamins.

Two further clinical observations provide a compelling argument for why all of these 1-carbon micronutrients should be examined in combination in regard to their impact on cancer risk. First, in the past 5 years there have been an increasing number of epidemiological studies that have observed an association between low vitamin B6 and B12 status with an increased risk of common neoplasms.^{23–25} It is also important to note that these relationships persist when the effects of folate blood levels or intake are regressed out of the relationship. Secondly, the study of these inadequacies is entirely relevant to the adult U.S. population and those of other industrialized nations: although flagrant deficiencies of these vitamins are uncommon in industrialized societies, marginal status, which is to say biochemical measures of vitamin status below the accepted normative range but in the absence of signs or symptoms of the classical deficiency syndromes, are rather common. Population-based studies in industrialized nations have reported that 18–25% of adults have

low B6 status and 10–20% of healthy elders are thought to have ‘subclinical’ B12 deficiency.^{26,27} Moreover, the most recent nationally-representative nutrition survey of the United Kingdom reveals that over 50% of adults have subnormal levels of the most accurate blood marker of riboflavin status.²⁸

The potential for cancer promotion with abundant intake of folate

The initial evidence that folate intake at levels achievable through the use of supplements and/or fortification might cause a paradoxical acceleration of carcinogenesis in individuals who harbor neoplastic lesions dates back to the 1940s when two groups of investigators gave large doses of folic acid to patients with acute leukemia and observed what Sidney Farber politely termed “the acceleration phenomenon”, whereby the rate of expansion of the leukemic clone increased tremendously.^{3,4} This is almost certainly due to the fact that neoplastic cells have a high rate of proliferation and therefore require large quantities of folate to maintain thymidine synthesis at a pace that is necessary to meet the heightened needs for DNA synthesis.²⁹ Controlled studies in a variety of animal models of colon cancer have provided similar findings: in settings where there is a particularly strong underlying predisposition to colon cancer or in a setting where neoplastic tumors are already established, supplemental folic acid is protective only before neoplastic foci appear in the intestine. Once such foci are established, the more folic acid that is given, the faster microscopic foci and macroscopic tumors arise.^{30–34} The results of a recent randomized trial of folic acid in the prevention of colorectal adenomas are consistent with such an effect as well.³⁵ This study, which enrolled subjects with a history of prior adenomas, did not observe a modulation of recurrence rates after the first follow-up colonoscopy at 3 years. However, at the time of the second follow-up, 6–8 years after initial randomization, a significant 2.3-fold increase in the multiplicity of recurrent adenomas occurred among those receiving folic acid supplements (as well as a marginally significant 1.7-fold increase in so-called ‘high risk’ adenomas). These results are consistent with the concept that the abundant intake of folic acid may accelerate tumorigenesis among those who harbor existing foci of neoplasia. Recent data indicating that the ‘miss rate’ for advanced adenomas is ~10%³⁶ and that ‘flat adenomas’, which are difficult to detect by conventional colonoscopy, are common and much more likely to contain foci of carcinoma than polypoid adenomas³⁷ underscore the plausibility that trials such as this contain sizeable numbers of individuals with high risk neoplasms in spite of undergoing a baseline ‘cleansing’ colonoscopy. To date, three other folic acid supplementation trials that have used adenoma recurrence as an endpoint have been reported^{38–40} and none have observed a similar increase in tumorigenesis, but importantly, none of these other three have had the extended follow-up over 6–8 years that was provided for in the *Cole et al* trial. Moreover, in an ecological study recently reported by myself and colleagues, we observed an increase of ~4 cases/100,000 individuals in the nationwide rates of colorectal cancer diagnoses in conjunction with the institution of mandatory folic acid fortification of cereal grains in both the U.S.A. and Canada in the 1990s.⁴¹ Studies of an ecological nature have inherent weaknesses; moreover, the question arises as to whether colorectal cancer rates could feasibly increase within the first year after the institution of fortification, as was observed to occur. Nevertheless, data from the abovementioned studies collectively raise a concern: 1) that is consistent with the known biochemical functions of folate in DNA synthesis, 2) which can be readily replicated in animal models of colorectal carcinogenesis, and 3) which is a phenomenon that has been observed to occur in small, inadvertent studies of human subjects with hematologic malignancies.

The possibility of a paradoxical promoting effect of excessive quantities of folate may be operable in human breast cancer as well: in a recent prospective cohort study among ~25,000 postmenopausal women the risk of developing breast cancer was significantly

increased among those taking folic acid supplements;⁴² a group among whom consumption was ≥ 853 mcg of folate per day. Like colorectal cancer, the promoting effect of folic acid has also been observed to occur in an animal model of breast carcinogenesis.⁴³

The concept has therefore evolved that higher intakes of folate are cancer-protective in nearly all circumstances except among those individuals who have existing neoplastic lesions and who are consuming exceptionally large quantities of the vitamin, in which case the folate presents additional risk for tumorigenesis. Cancers that slowly evolve over years through an indolent dysplastic phase, such as the colorectal adenoma or a dysplastic nodule of the prostate, are exceedingly common in the general population of healthy, elder adults and would be particularly prone to such an effect.

The fact that folic acid, which is not a naturally-occurring form of the vitamin, is used by food and pharmaceutical industries for fortification and supplementation is potentially of importance. Folic acid is converted to a natural biological form of the vitamin as it passes through the intestinal wall, with enzymatic reduction and methylation resulting in the circulating form of the vitamin, 5-methyltetrahydrofolate. Nevertheless, it has been known for some years that oral doses of folic acid in physiologic quantities can saturate this conversion mechanism, resulting in detectable levels of circulating folic acid, and there has been some concern that this oxidized, nonsubstituted form of folate might feasibly be detrimental because it is not a naturally occurring coenzymatic form of the vitamin. Several studies have shown that as little as 200 mcg of oral folic acid may produce transiently detectable levels of folic acid in the bloodstream,^{44–46} and more recently, it has been shown that the daily ingestion of 400 mcg produces a sustained appearance of folic acid in the blood.⁴⁷ In the U.S., 40% of adults 60 years and older are thought to regularly consume a supplement containing folic acid, most of which contain 400 mcg per pill.⁴⁸ When this folic acid intake is added to that which appears in voluntarily fortified foods such as ready-to-eat breakfast cereals plus the additional quantity that is now consumed in the form of mandatory cereal grain fortification, it should come as no surprise that a large percentage of the population has detectable quantities of unmetabolised folic acid chronically circulating in the bloodstream. Consistent with these projections are recent observations from the Framingham study that demonstrate that those who are regular users of vitamin supplements have a mean concentration of unmetabolised folic acid in fasting plasma that is ~40% higher than that of non-users; moreover, in this era of mandatory fortification, 81% of vitamin users have detectable levels of unmetabolised folic acid in their plasma.⁴⁹ Of additional concern are recent observations of Troen et al,⁵⁰ who observed that increasing concentrations of plasma folic acid among elder women who took folic acid-containing supplements were inversely associated with decreases in the cytotoxicity of circulating natural killer cells. Natural killer cells are a population of lymphocytes thought to play a role in the destruction of arising clones of neoplastic cells. Thus, the coenzymatic form of folate used for fortification is perhaps relevant in determining whether a detrimental effect is produced from excessive intake.

The intersection between the dual effect of folate and public health in the 21st century: a source of concern

By integrating what we know about the cellular functions of folate with what has been observed in cell culture, animal studies and humans, there is compelling evidence that *under select circumstances* an overly abundant intake of folate promotes the proliferation of cells in a neoplasm. The essential question that needs to be resolved is whether such conditions have inadvertently been recreated among certain segments of our society from the various sources of folic acid that are presently available in foodstuffs and supplements. On the one hand, observations suggesting that a cancer-promoting effect has been created among

segments of the population are rather weak, but on the other hand the biological plausibility of the effect can not be denied and the large-scale ramifications of such an effect, if indeed it exists, are not ones that we can afford to overlook in this era of folic acid fortification.

In theory, a partial answer to this question could be provided by observations arising from the numerous homocysteine-lowering trials that have been conducted over the past several years. The daily intervention in these cardiovascular disease prevention studies has included ≥ 600 mcg of folic acid and in several of these trials data on the incidence of cancer was collected as a secondary endpoint. Some have reported numerical, but not statistically significant, increases in cancer incidence among those receiving folic acid supplements⁵¹⁻⁵² whereas others have reported a null effect⁵³⁻⁵⁴ but the reality is that all of them are terribly underpowered to detect a cancer-promoting effect since the trials were designed with other primary endpoints in mind.

The question of whether cancer promotion can feasibly be occurring in the population as a result of excess folic acid is a germane one on a worldwide basis. Clearly, ample evidence now exists to indicate that increasing the folic acid intake of women in the periconceptional period substantially diminishes the risk of pregnancies complicated by a neural tube defect.⁵⁵ As a result many countries have already mandated folic acid fortification of their foodstream and most others that have not yet done so have the matter under active deliberation. In some countries such an enrichment of the food supply probably does not present any problems. However, in many countries there already exist a plethora of other sources of folic acid, either incorporated into foodstuffs or in the form of supplements. In the U.S., for instance, a sizeable segment of the population avidly seeks out such sources and as a result develops quite high concentrations of plasma folate as well as readily detectable levels of circulating folic acid, even in the fasting state.⁴⁹ In the U.S.A., individuals in the uppermost quintile of serum folates (≥ 18 ng/mL) have a geometric mean serum folate concentration of 24.6 ng/mL. Among such individuals, it is estimated that the mean daily intake of folic acid alone--not including natural sources of dietary folate--is ~ 660 mcg.⁵⁶ Supplements and, to a lesser degree, ready-to-eat cereals account for approximately 80% of the folic acid intake among these individuals. Demographically, this quintile of 'high folic acid consumers' is significantly older than the lower four quintiles, with a mean age of 56, which is of concern since elder age is such a strong determinant of colorectal cancer risk.⁵⁶ Although the proportion of daily folic acid intake derived from mandatory fortification of cereal grains among high folic acid consumers is only $\sim 20\%$, data from the Framingham Study nevertheless revealed that the institution of mandatory fortification still produced a 62% rise in serum folate levels among those who regularly ingest vitamin supplements.⁵⁷

Summary and Conclusions

And so an agonizing dilemma has arisen that is impeding the institution of folic acid fortification programs in many countries and thereby contributing to the ongoing occurrence of babies born with neural tube defects. One potential solution, proposed by the Food Standards Board of the United Kingdom, is to institute mandatory folic acid fortification of cereal grains and concurrently curtail other widespread sources of folic acid presently available in the form of supplements and voluntarily fortified food products (presented at *Folic Acid: a Scientific Update*, a meeting of the European Food Safety Authority and Swedish National Food Administration, Uppsala, Sweden, January 21–22, 2009), and by means of this latter strategy restrict the major sources of folic acid by which the 'high folic acid consumers' develop such high blood folate levels.

This author does not have a definitive solution to this dilemma; moreover, it is worth recognizing that the correct course to pursue for one country may differ from what is correct

for another. From this dilemma nevertheless emerge a number of realities that should be taken into consideration as deliberations regarding mandatory fortification move forward: 1) the compelling and irrefutable benefits that mandatory fortification offer in regard to reducing the occurrence of anemias and NTDs dictate that decisions regarding fortification will likely be made in the near future in the absence of adequate information regarding the potential risk of cancer promotion, 2) if a country decides to proceed with mandatory fortification, it should ensure that adequate monitoring systems are in place to provide accurate estimates of cancer rates before, and following the institution of fortification. It is essential that data can be retrieved and analyzed quickly from the monitoring system so that prompt action can be taken in response to unexpected shifts in cancer rates. At the very minimum such a monitoring system should be able to assess colorectal cancers, and preferably others as well, and 3) vigorous research efforts need to proceed without delay to better define the relationships between folate intake and cancer development and to determine whether folic acid, as opposed to the natural forms of the vitamin, possesses unique properties in this regard.

There is little question that folate may act as a chameleon under certain experimental conditions. The challenge before us is to determine whether we have inadvertently enabled it to play its paradoxical role as a cancer-promoting agent on a population-wide basis. The evidence to support such a contention is sparse, but it is not a possibility that we should cast aside without careful consideration.

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Figure 1. 1-carbon metabolism in cytosol of most mammalian cells, emphasizing inter-conversion of different forms of folate (simplified)

DHF, Dihydrofolate; dmG, dimethylglycine; S-AdoHcyst, S-adenosylhomocysteine; SAdoMet, S-adenosylmethionine; THF, tetrahydrofolate; 1, methionine synthase; 2, betaine:homocysteine methyltransferase; 3, methylenetetrahydrofolate reductase; 4, serine hydroxymethyltransferase; 5, cystathionine β -synthase