A REVIEW OF FOLATE INTAKE, METHODOLOGY, AND STATUS

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ABSTRACT

Folacin, one of the B complex vitamins, is a coenzyme for certain reactions involving transfer of one-carbon units in nucleic and amino acid metabolism. An estimate of the folate available from principal folate-containing foods indicates that the amount available in the daily diet of the United States population is about 225 µg. However, the usefulness of such estimates is limited by deficits in the amount and accuracy of available data on the folate content of foods and on the bioavailability of various forms of food folates. In this report, frank folate deficiency is defined as a concentration of less than 3 ng/ml serum and less than 140 ng/ml red cells. Although dietary intake is the main determinant of folate status in healthy persons, other factors that influence folate status include alcohol, drugs, inhibitors of intestinal folate conjugases, diseases of the bowel, and deficiencies of iron, zinc, or vitamin B₁₂. Population groups that may be at risk of developing folate deficiency include premature infants, adolescents, pregnant or lactating women, women during the first 2 to 3 years postpartum, alcoholics, and the elderly. Substantial percentages of such groups have blood folate values at deficiency levels, but no causally related illnesses have been reported. Available evidence of compromised folate status based solely on mean values of serum and red cell folate derived from large-scale surveys is insufficient to determine whether a problem of folate deficiency of public health significance exists in this country.

INDEXING KEY WORDS

folic acid; folic acid deficiency; food analysis; food, fortified; nutrition; nutrition surveys
INTRODUCTION

Folacin, one of the essential nutrients in the B complex of vitamins, occurs naturally in many common foods in the form of folates. Folic acid consists of a pteridine nucleus joined by a methylene bridge to p-aminobenzoic acid which, in turn, is linked to glutamic acid by a peptide-like bond. This oxidized parent form of the vitamin is found in foods and in mammalian tissues only as a product of oxidation of the naturally occurring reduced forms. Tetrahydrofolic acid, unsubstituted or with one of several possible single carbon substituents and bearing a poly-γ-glutamyl chain, is the active coenzyme form of the vitamin. In mammalian systems, folate derivatives function as coenzymes for the transfer of one-carbon units, i.e., formyl, methyl, or formimino groups. Such transfers occur in de novo purine synthesis, thymidylate synthesis, certain pathways of amino acid metabolism, generation and utilization of formate, and, in some bacteria, methylation of transfer RNA (9, 48).

Recent data suggest that dietary intakes of folate in certain North American population groups such as adolescents, pregnant women, and the elderly may be insufficient to maintain health. Conversely, possible excessive intakes of folate as a result of consumption of folate-containing foods and supplements containing folic acid cause concern because of the risk of temporary masking of pernicious anemia by intakes of folate in amounts not strikingly greater than those in the diet that are necessary to maintain health.

In view of the question of possible deficiencies or excesses of folate consumption by individuals in this country, the Food and Drug Administration (FDA) requested the Life Sciences Research Office (LSRO) of PASEB to provide comprehensive information and scientific opinion on folates in the food supply,
methodology for determining folate levels in foods and body tissues, and folate status of the United States population. This paper summarizes the resulting report (3) which was based on a scientific literature review and information presented informally at an ad hoc review group meeting held at FASEB headquarters on March 23-24, 1981.

The LSRO report (3) analyzes available data on folate nutriture, bioavailability and stability of food folates, biomedical aspects of folate deficiency or excess, and the influence of drugs, alcohol, and various nutrients on folate availability and metabolism. In addition, the study focuses on current and developmental analytic methods for folates, and the clinical and biochemical characterization of compromised folate status. Critical issues are identified and suggestions for further research are provided.

CURRENT STATUS OF FOLATE METHODOLOGY

Folates are present in foods and tissues in numerous forms having nutritional and metabolic significance for man. This heterogeneity leads to considerable difficulty in analyzing the folate content of foods and biological materials. The most widely accepted method currently available for determination of total folate concentrations in foods and biological materials is a microbiological assay utilizing Lactobacillus casei. This microorganism is responsive to the many biologically active forms of folate (8, 84) and, the assay, when coupled with a conjugase treatment to hydrolyze the polyglutamyl chain (15), is considered a measure of biologically active folate. A modification of this assay,
in which $^{14}\text{CO}_2$ produced by oxidation of gluconate is measured, overcomes some of the problems of the turbidimetric assay (23) but, currently, cost is a disadvantage.

An alternate method used widely in clinical laboratories, the radiometric binding assay, is based on the principle of binding of radiolabeled folate by relatively specific protein binders (102). This method is well-suited for serum samples (101) and preliminary reports (39, 88) indicate reasonably good correlation between analyses of food and tissue samples by the radiometric binding assay and the *L. casei* microbiological assay.

High-performance liquid chromatography (HPLC) techniques show promise as a sensitive method for separation and quantification of oxidized and reduced one-carbon-substituted folate monoglutamates (2, 33, 76) and separation and identification of folyl polyglutamates (17). Profiles of folates in foods have been determined by HPLC (25, 40) and this information may be essential for interpretation of studies of folate intake and bioavailability. Further modifications in technique (33, 36, 59) may increase the sensitivity of HPLC to permit analysis of samples having low folate concentrations, such as sera.

**FOOD FOLATES**

The folate values of foods included in U.S. Department of Agriculture (USDA) tables of food composition (72, 73, 89-95) appear to be the most accurate values available. Total folate content of foods appears to be the best index of available folate; however, these values should be used with caution since
preferential utilization of different forms of folate may occur (35). Although not complete, the most extensive compilations of total food folates are the USDA Agricultural Handbooks 8-1 through 8-7 (89-95), the USDA provisional table for food folate (73), the supplement to the provisional table (72), and the Canadian (51, 52) and British (71) tables of food folates.

Problems with methodology have been a major deterrent to wide acceptance of the folate values in tables of food composition (14). Values currently supplied to USDA by university, government, and industry laboratories are based on the L. casei assay with conjugase treatment for total folate content. Large variations among samples and lack of agreement among laboratories have been experienced repeatedly. Nevertheless, these values may be correct and may merely reflect large variations in folate content among food samples.

Food folates differ in their stability to conditions encountered during storage and processing such as changes in pH, presence of oxidizing agents, and exposure to heat and light (30, 34, 70). In general, total food folate content and polyglutamate chain length decrease under the test conditions (4, 58, 60, 75), but analyses of changes in specific one-carbon-substituted folate monoglutamates and polyglutamates were not consistent among studies. Hurdle et al. (54) reported that as much as 50-95% of folate activity may be lost during food processing and home preparation. Thermal stability of folacin derivatives is strongly influenced by dissolved oxygen concentration (24) and, when foods are processed under conditions of sufficiently low oxygen content, destruction of labile reduced folates may be greatly decreased (34). Such unknown and uncontrollable differences in storage and processing of foods constitute confounding factors for studies relying on tables of food composition for estimating dietary folate intakes.
Availability of folates in various foods has been estimated from hematologic responses, changes in blood folate levels, urinary folate excretion, intestinal perfusion studies, and growth responses (21). It is difficult to compare results among studies utilizing these techniques; however, they do provide valuable indications of absorption of, and physiologic responses to, the complex mixtures of folates in foods.

Studies with single folate compounds indicate that bioavailability depends on such factors as the specific forms of folate (10, 12, 38, 42) and the pH in the small intestine (62, 83). Although such studies have provided valuable knowledge of folate bioavailability, much remains to be learned about the availability of folate in foods consumed under normal dietary conditions.

IDENTIFICATION OF COMPROMISED FOLATE STATUS

Biochemical and clinical indicators may be used to differentiate between individuals with frank folate deficiency and those at risk of the deficiency whose marginal reserves of the vitamin may be depleted by intervening events such as pregnancy, poverty-induced undernutrition, alcoholism, or development of malabsorptive disorders. Assessment of folate status in clinical studies and nutrition surveys is limited by the available methodology, and correlation between estimated folate intake and overall body status is tenuous. Problems of determining folate status may be further compounded by malabsorptive syndromes, folate-drug interactions, or masking of folate deficiency by concomitant nutrient deficiencies. Despite these limitations, compromised folate status may be determined by a number of methods.
Blood and its components are considered the best tissues available for
determination of folate status in humans, although it is recognized that folate
deficiencies in select cell lines, for example, cervical cells and lymphocytes
(13, 32, 103), may not always be detected by analysis of blood. Several
definitions of compromised folate status exist in terms of blood values, but
commonly accepted biochemical criteria are <6 ng/ml for serum and <160 ng/ml
for red cell folate concentrations for persons considered to have marginal
status and <3 ng/ml and <140 ng/ml respectively, for persons having folate
deficiency (82). Serum folate levels are considered indicative of immediate
past intake of the vitamin, but red cell folate concentrations, which represent
liver reserves more closely than serum folate values (105), are thought to pro-
vide a more reliable measure of folate status over longer periods of time.

In clinical or survey protocols, hematologic measures such as neutrophil
hypersegmentation, red cell volume, and hemoglobin estimation may suggest the
need for consideration of low folate status. Suggestion of compromised status
is likely to arise from a traditional diagnostic approach, i.e., a careful
medical and dietary history, physical examination, and routine laboratory
procedures including hemoglobin estimation, complete blood count, and examina-
tion of a blood smear. From these procedures, the presence may be established
of macrocytosis, hypersegmented polymorphonuclear neutrophils, and pancytopenia.
The foregoing findings should suggest the need for serum and red cell folate
and serum vitamin B<sub>12</sub> determinations for the biochemical confirmation of folate
deficiency.

Additional laboratory procedures useful in differential diagnosis of folate
deficiency are the deoxyuridine suppression of incorporation of radiolabeled
thymidine into DNA in bone marrow cells or transformed lymphocytes (29), and
elevated urinary excretion of methylmalonic acid which occurs in vitamin B₁₂ deficiency but not in folate deficiency (16). Urinary excretion of formimino-glutamic acid (FIGLU) also indicates the possibility of folate deficiency, but does not differentiate between folate and vitamin B₁₂ deficiencies (20, 61). These procedures are not presently considered useful for routine determinations of folate status in surveys.

FACTORS AFFECTING FOLATE STATUS

Dietary intake is the main determinant of folate status in most healthy individuals. However, accurate estimates of dietary intakes of folates for large numbers of individuals are limited. A WHO study group (56) estimated the per capita free folate intake to be 37 to 279 µg/d in the United States. More recently, folate availability was calculated on per capita disappearance of principal foods in the United States to be about 225 µg/d (85). These estimates are likely to be high since they do not account for food wastage or nutrient losses from deterioration during storage. It should be noted that these estimates of per capita folate consumption fall short of the RDA for adults, 400 µg/d (69). Determinations of folate intakes by calculation from tables of folate content of foods or by analysis of dietaries (15, 31, 63, 68, 80, 98) suggest that total folate intakes may range from 0 to 3200 µg/d. These data yield only crude comparisons because of differences in data collection and assay methods; however, it is of interest that in most studies the lower limit of the range was considerably lower than the RDA for the group studied (69) and in several studies did not reach the minimum dietary requirement for the adult, 50 µg/d (47).
Dietary analyses in large-scale nutrition surveys in the United States (1, 97) have not included calculation of folacin intakes from dietary recalls of the participants because of incomplete data in food folate tables and because of problems concerning the microbiological assay for folate. Similarly, the U.S. Department of Agriculture Nationwide Food Consumption Survey (96) and the Food and Drug Administration Total Diet Study (37) have omitted analysis of folates.

Factors other than dietary intake may substantially influence folate status of individuals in certain conditions. These factors include impairments in absorption or metabolism and increases in requirements, body losses and destruction (48). Situations in which these conditions may occur are described in the report.

EVIDENCE OF COMPROMISED FOLATE STATUS

Epidemiological Studies

Herbert (46) estimated that up to one-third of all pregnant women in the world experience folate deficiency. Chanarin (19) ranked folate deficiency with iron deficiency as one of the common nutritional deficiencies seen in clinical practice. Few cases of frank folate deficiency, as defined by morphologic changes in blood and bone marrow, have been documented in the general population. However, in some populations such as hospitalized patients in London (50), folate deficiency manifesting macroovalocytosis and megaloblastic bone marrow is reportedly not uncommon.
The prevalence of folic acid deficiency and associated megaloblastic anemia, on a global basis, has probably been underestimated. Among pregnant women in developed countries, the estimated frequency of megaloblastic anemia resulting from folate deficiency has ranged from 2.5 to 5%, with a higher prevalence among pregnant women in developing countries (81). Colman et al. (27) reported that megaloblastic anemia of pregnancy is common among South African blacks and listed economic status, adverse diet, poverty, and prolonged breast feeding as contributory factors. Examples of folate deficiency in developed countries presumably resulting from inadequate dietary intake involved primarily the elderly, but also younger persons at increased risk because of pregnancy, illness, or other cause of elevated metabolic demand (5, 6, 7, 74, 99).

Limited data suggest that folate status is suboptimal in certain population groups in the United States such as adolescents, poorly nourished pregnant women, the elderly, and users of such commonly prescribed drugs as oral contraceptives, anticonvulsants, and sulfasalazine (6, 7, 18, 49, 100). Inadequate dietary folate is considered a key etiologic factor. Data from the Health and Nutrition Examination Survey II (HANES II)* should provide more extensive information on the frequency of marginal and deficient folate status in segments of the population. If data from HANES II and other sources support the observations of earlier surveys with respect to folate nutrure, the aggregate evidence may indicate that compromised folate status is sufficiently widespread to constitute a public health issue.

* These data will be available in late 1982.
Attempts to reach firm conclusions on the folate status of populations from mean values of serum and red cell folate levels such as reported in the Ten-State Nutrition Survey (97) and the Nutrition Canada Survey (43) have been criticized (11, 49, 87). For example, the Ten-State Nutrition Survey suggested that folate nutriture in the tested population was adequate based on the mean serum and red cell data. However, Herbert et al. (49) cautioned that "mean values may obscure the existence of a substantial number of actual values which are sufficiently below the mean as to suggest widespread folic acid deficiency," and noted that the Massachusetts section of the sample yielded an incidence of folate deficiency of 25% among pregnant women.

Clinical Studies

Rosenberg and Dyer (79) noted that four target clinic populations at risk of developing folate deficiency are pregnant women; alcoholics; patients with gastrointestinal diseases, particularly those on restrictive diets or with malabsorption; and, chronic users of drugs such as anticonvulsants and sulfa-salazine. Lactating women, premature infants, persons with hemolytic anemias, and persons undergoing dialysis may represent additional subgroups of the population at risk.

Studies of low-income pregnant women in New York and Florida indicate that about 30% (49) and 40% (5), respectively, had red cell folacin levels in the marginal or deficient ranges. In both studies, diets contained few green, leafy vegetables and the foods tended to be overcooked, thereby decreasing the folate content.
Alcohol abusers are estimated to exceed ten million in the United States (79) and chronic alcoholism is probably the leading cause of folate deficiency in this country (41). Persons diagnosed as being alcoholics, but who otherwise consume an adequate diet, are not likely to become folate deficient. However, it is estimated (41) that about 40% of derelict alcoholics are likely to have megaloblastosis, with higher percentages manifesting folate deficiency, based on serum and red cell folate concentrations.

Patients with gastrointestinal tract diseases such as gluten sensitive enteropathy, Crohn's disease or ulcerative colitis are also at risk of developing folate deficiency. Drug treatment (sulfasalazine) for the latter two conditions is often associated with development of folate deficiency as is anticonvulsant therapy for treatment of recurrent seizures (77). Folate deficiency has also been reported in women taking oral contraceptives; however, this effect has not been confirmed.

As indicated by the studies cited, folate deficiencies, as measured by serum and red cell folate levels, have been identified in several population groups in the United States. However, most subjects with serum folate levels in the range defined as deficient exhibit no clinical manifestations, and folate deficiency has not been clearly identified as a medical problem in the general population.

**EVIDENCE OF EXCESSIVE INTAKES**

A few reports claim that folic acid taken in pharmacologic or massive doses as supplements may produce adverse effects (22, 53, 65, 67, 104) but
double-blind controlled studies (44, 78) failed to support such claims. Large numbers of patients have received similar doses of folic acid without apparent ill effects (55, 66). A 1981 FDA survey of nutrient supplement intakes may provide information concerning use of dietary supplements containing folic acid. Little is known about possible subclinical effects of long-term, high dietary intakes of the vitamin.

The most often noted effect of large doses of folic acid is the masking of pernicious anemia, thereby delaying diagnosis of the disease and increasing the risk of neurological degeneration. The smallest oral dosage of folic acid causing a hematologic response in one pernicious anemia patient was 400 μg/d (45). While doses of folic acid of 400 μg/d to some individuals might mask pernicious anemia, it is not known whether long-term consumption of this level in foods would have a similar effect.

ADDITION OF FOLATES TO FOODS

Addition of folic acid to staple foods in South Africa (26, 27, 28, 64) and to wine in the United States (57) has proved an effective means of increasing serum and red cell folate in certain groups at risk of developing folate deficiency. Calculation of the folate intake possible by consumption of selected foods containing added folic acid, such as cereals now available in the United States, also suggests that such foods can contribute significantly to folate intake (86). However, the actual patterns of use of such products by individuals in population groups considered at risk of folate deficiency have yet to be determined. These data are essential to any consideration of folate fortification of foods in this country.
SUGGESTIONS FOR RESEARCH

A number of suggestions for future consideration were included in the LSRO report (3). Among those suggestions were:

- More reliable information is needed for the folate content of foods and the bioavailability of the several forms of the vitamin. Development of newer technologies to measure folate concentrations, bioavailability, and status should be encouraged.

- Better estimates of folacin intakes and correlation of these intakes with biochemical indicators of folate status are needed.

- Valuable data might be obtained from analysis of the folate status of persons consuming foods already fortified with folate compared with the status of persons not consuming these products.

- The HANES II data on folate status should be analyzed to determine whether data reported from less extensive nutrition surveys can be confirmed.

- In view of the evidence that some apparently healthy people have low or borderline folate status, more data are needed to characterize the condition of being at risk of folate deficiency, to refine norms, and to account for the seeming lack of impairment among such individuals. This kind of information would assist, as well, in efforts to define the daily folate requirements for various segments of the population.
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