FOLIC ACID REVISITED

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Folic acid, or pteroylglutamic acid, is a well-known water soluble vitamin of the B-complex group. It is necessary for DNA synthesis and normal erythropoiesis. Tetrahydrofolate, the active form of this vitamin, functions as a coenzyme in various metabolic reactions involving transfer of one-carbon moieties. Folate and vitamin B12 metabolic pathways intersect at the conversion of homocysteine to methionine. Human beings cannot synthesize this vitamin and must obtain preformed folate through dietary sources like green leafy vegetables, cereals, fruits, organ meats and yeast. Synthetic folic acid is more bioavailable than food folate. Absorption is predominantly from the upper small intestine and elimination predominantly renal, with modest hepatic storage. The daily requirement varies by age and is greater during pregnancy and lactation. Apart from increased demand, folate deficiency can occur in malnutrition, malabsorption, chronic hemolytic anemias, chronic alcoholism, repeated hemodialysis, and unusual dietary situations like total parenteral nutrition. The use of certain antiepileptic, antimalarial, antimicrobial and anticancer drugs may interfere with the absorption, conversion or utilization of folate leading to megaloblastic anemia. The primary therapeutic indication is in the prophylaxis and treatment of deficiency states. Pharmacological supplementation is recommended in situations like pregnancy (for preventing macrocytic anemia, occurrence or recurrence of neural tube defects, counteracting teratogenic effect of anticonvulsants, etc.), malnutrition, malabsorption and chronic hemodialysis. It may be supplemented during lactation, in infants, the elderly, alcoholics, and in renal failure patients. Folic acid may also reduce orofacial clefting and ameliorate methotrexate toxicity in rheumatoid arthritis. Pharmacological doses are well-tolerated but folate supplementation alone in megaloblastic anemia primarily due to vitamin B12 deficiency can worsen the neurological condition. Of late, interest in folic acid has grown with the realization that modest folate supplementation can prevent hyperhomocysteinemia, which is an independent graded risk factor for atherosclerotic cardiovascular disease, and is possibly beneficial in certain cancers. The recent stipulation for mandatory folate fortification of cereal products in USA and the inclusion of folic acid in the WHO model list of essential drugs recognize the increasing importance of folate in human nutrition. Indeed, folic acid has revisited with new therapeutic applications and its mysteries are still unfolding more than 7 decades after its discovery.

INTRODUCTION

Folic acid is a water soluble vitamin of the B-complex group. Its discovery dates back to the early 1930s when Wills and Talpade of the Haffkine Institute in Bombay reported that undernourished mothers of premature babies were frequently consuming diets deficient in vitamin B complex. Subsequently Wills and coworkers described a form of macrocytic anemia in Indian women (‘tropical macrocytic anemia’), resembling pernicious anemia, that responded to a factor present in yeast extracts but not in the purified fractions known to be effective in pernicious anemia1. The significance of Wills’ anti-anemia factor was not immediately realized. Researchers in Europe and America continued studies on anemias of nutritional deficiency and the same factor came to be known under different names by its curative effect in different deficiency states in man, monkey, chick, rat, and guinea pig, and also by its growth promoting effect on certain microorganisms. Thus, names such as vitamin M, factor U,
vitamin B<sub>6</sub>, vitamin B<sub>9</sub>, eluate factor, L-casei factor, etc., can be found in old literature. Eventually, the present day accepted term 'folic acid' was coined by Mitchell, Snell and Williams in 1941 after reporting the presence of a factor in spinach leaves essential for the growth of the bacterium *Lactobacillus casei*. They also isolated folic acid to a high degree of purity in 1944. In August 1945, the synthesis of folic acid was accomplished in the United States of America (USA) by a team of researchers from the Lederle Laboratories at Pearl River and from the Bound Brook, New Jersey laboratories of American Cyanamid Company. Man cannot synthesize folic acid and hence must necessarily obtain it from food. The human body requires only small quantities of folate, but this is vital to various metabolic pathways, including DNA synthesis and normal erythropoiesis. As a pharmaceutical product, folic acid is categorized as a vitamin, a nutritional supplement and as a diagnostic aid (in folate deficiency).

**Chemistry, natural sources and physiological role**

Folic acid, or pteroylglutamic acid, consists of the base pteridine linked to para-aminobenzoic acid (PABA) and glutamic acid (Figure 1A). The full chemical name is $N\{4-[(2\text{-amino-1,4-dihydro-4-oxo-6-pteridinyl}-methyl)\text{amino}]\text{benzoyl}\}-L\text{-glutamic acid}$ ($C_{19}H_{19}N_7O_6$, molecular weight 441.41). In the pure state, it is a yellow to orange brown, almost odorless crystalline powder, very slightly soluble in water and insoluble in most organic solvents. However, it
Figure 2. The interconversion of folate coenzymes and their involvement in various metabolic pathways.
dissolves readily in dilute solutions of alkali hydroxides and carbonates. Solutions are inactivated by ultraviolet light and oxidation. Injections have an alkaline pH and require protection from light and freezing. Folic acid may precipitate in some proprietary amino acid solutions and in the presence of high concentrations of calcium ions, but it appears to be stable if the pH remains above 5.6. Animals are incapable of synthesizing PABA or of attaching glutamate to pteroic acid. They thus require preformed folate in their diet. Good dietary sources of folic acid include vegetables (especially green vegetables), potatoes, cereals and cereal products, fruits, and organ meats such as liver and kidney. In plants, folic acid is present as a polyglutamate conjugate consisting of a γ-linked polypeptide chain of 7 glutamate residues. In the liver, the principal storage site, the major folate is a pentaglutamyl conjugate. Yeast is also a good source. For vegetarians, milk and dairy products like buttermilk, yogurt and ripened soft cheeses, constitute a fair source. N5-methyl-tetrahydrofolate is the major folate form in milk and perhaps also in fermented dairy products. The folate content of various Indian foodstuffs has been documented by the Indian Council of Medical Research (ICMR). It is to be noted that heat and cooking can destroy up to 90% of the folates in food. Folic acid itself does not occur in nature; the pharmaceutical product is chemically synthesized. The L-enantiomer is biologically active.

Folate derivatives in the diet are cleaved by specific intestinal enzymes to monoglutamyl folate for absorption. Most of this is reduced to tetrahydrofolate (H4folate) in the intestinal cell in a two-step reaction catalyzed by the enzyme folate reductase (Figure 1B). Inhibitors of the folate reductase enzyme act as antifolates. Tetrahydrofolate polyglutamates are probably the functional coenzymes in tissues. H4folate is thus regarded as the active form of folate which serves as the carrier of various activated one-carbon units in metabolic reactions; these units represent a series in various redox states, viz., methyl, methylene, methenyl, formyl, and formimino. All are metabolically interconvertible [Figure 2]. H4folate
The methionine synthase catalyzed reaction as an intersecting point between folate and cobalamin metabolism.

Figure 4. The methionine synthase catalyzed reaction as an intersecting point between folate and cobalamin metabolism.

Coenzymes are involved in the synthesis of purines and thymidylate, and hence in the synthesis of DNA and normal erythropoiesis. They are also involved in the metabolism of some amino acids such as glycine, methionine and histidine, and in the formation and utilization of formate.

The amino acid serine is the major source of a one-carbon unit in the form of a methylene group, which it transfers reversibly to $H_4$ folate to form glycine and $N^5,N^{10}$-methylene-$H_4$ folate in a reaction catalyzed by serine hydroxymethylase. The latter derivative plays a central role in one-carbon unit metabolism. It can be reduced to $N^6$-methyl-$H_4$ folate. Alternatively, it can be oxidized to $N^5,N^{10}$-methylnyl-$H_4$ folate which can then be hydrated to either $N^6$-formyl-$H_4$ folate or to $N^5$-formyl-$H_4$ folate. The latter is also known as folic acid, a stable form that can be used for administration of reduced folate.

Formiminoglutamate (Figlu), a catabolite of histidine, transfers its formimino group to $H_4$ folate to form $N^5$-formimino-$H_4$ folate. In folate deficiency Figlu will accumulate after oral challenge with histidine. Formate...
can be converted to \(N^\text{10}\text{-formyl}-H_4\text{folate}\) in an ATP-dependent reaction catalyzed by formyl synthetase.

The enzyme methylenetetrahydrofolate reductase (MTHFR) plays a central role in the folate cycle and contributes to the metabolism of the amino acid homocysteine. It catalyzes the reduction of \(N^5,N^\text{10}\text{-methylene}-H_4\text{folate}\) to \(N^5\text{-methyl}-H_4\text{folate}\), thus generating the active form of folate required for remethylation of homocysteine to methionine. Deficiency of MTHFR may be associated with an increase in plasma homocysteine. Genetic polymorphisms of this enzyme are known to occur. This is the subject of intense study as it may affect folate metabolism and requirements\(^9,10\). \(N^\text{10}\text{-formyl}-H_4\text{folate}\) reacts with 5-amino-4-imidazole carboxamide ribonucleotide (AICAR) to generate the purine inosine monophosphate. This reaction is catalyzed by AICAR transformylase and regenerates \(H_4\text{folate}\).

\(N^\text{5},N^\text{10}\text{-methylene}-H_4\text{folate}\) provides the methyl group in the formation of thymidylate, a necessary precursor of DNA synthesis (Figure 3) and hence erythrocyte development. Concomitant with the reduction of the methylene to the methyl group, there is oxidation of \(H_4\text{folate}\) to dihydrofolate, which must be reconverted to \(H_4\text{folate}\) for further use. Therefore, cells that synthesize thymidylate (for DNA) are particularly vulnerable to deficiency of folic acid and to inhibitors of folate reductase such as methotrexate.

The complexities of the interaction of vitamin \(B_{12}\) and folate are a consequence of their common participation in the methionine synthase reaction (Figure 4) which converts homocysteine to methionine using \(N^5\text{-methyl}-H_4\text{folate}\) as the methyl donor and methylcobalamin as coenzyme. The methionine synthase reaction is largely responsible for the control of the recycling of folate cofactors, maintenance of intracellular concentrations of folylpolyglutamates, and, through the synthesis of methionine and its derivative S-adenosylmethionine, the maintenance of a number of methylation reactions\(^11\). The megaloblastic anemia of vitamin \(B_{12}\) deficiency may be ameliorated by extra folate in the diet, but this treatment will not cure homocystinuria, methylmalonic aciduria or the neurologic component of vitamin \(B_{12}\) deficiency.

### Human folate requirements and deficiency

The body store of folate in healthy adults is about 5 to 10 mg. Estimates of folate requirements have been based on intakes associated with maintenance of normal plasma and erythrocyte folate concentrations and functional tests that reflect abnormalities in folate-dependent reactions. Dietary reference intakes that have been developed recently are based primarily on metabolic studies in which erythrocyte folate concentration is considered the major indicator of adequacy\(^12\). Differing amounts are recommended by official committees, for infants and children of varying ages, for adult males and females, and for pregnant and lactating women. Folate requirements are higher to protect against neural tube defects (NTDs)\(^13\). This is discussed later.

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**Table 1.** Recommended daily folic acid intakes in USA and Canada.

<table>
<thead>
<tr>
<th>Individuals / Age group</th>
<th>USA (mcg)</th>
<th>Canada (mcg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants and children</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth to 3 years of age</td>
<td>25-50</td>
<td>50-80</td>
</tr>
<tr>
<td>4 to 6 years of age</td>
<td>75</td>
<td>90</td>
</tr>
<tr>
<td>7 to 10 years of age</td>
<td>100</td>
<td>125-180</td>
</tr>
<tr>
<td>Adolescent and adult males</td>
<td>150-200</td>
<td>150-220</td>
</tr>
<tr>
<td>Pregnant females</td>
<td>150-180</td>
<td>145-190</td>
</tr>
<tr>
<td>Breast-feeding females</td>
<td>400</td>
<td>445-475</td>
</tr>
</tbody>
</table>

**Table 2.** FAO/WHO and ICMR recommendations on daily free folate intake.

<table>
<thead>
<tr>
<th>Individuals / Age group</th>
<th>FAO/WHO (mcg)</th>
<th>ICMR (mcg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants and children</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td>0 - 6 months</td>
<td>60</td>
<td>25</td>
</tr>
<tr>
<td>1 - 12 months</td>
<td>100</td>
<td>30-70</td>
</tr>
<tr>
<td>To 18 years and over</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Lactation</td>
<td>300</td>
<td>150</td>
</tr>
</tbody>
</table>
In the United Kingdom (UK) the reference nutrient intake (RNI) for adult males and non-pregnant females is 200 mcg daily and the estimated average requirement (EAR) is 150 mcg daily. As summarized in Table 1, in USA the recommended dietary allowance (RDA) has been set at 200 mcg for adult males and 180 mcg for adult non-pregnant females. Canadian recommended nutrient intakes (RNI) are higher for infants and children. The joint United Nations Food and Agricultural Organization (FAO) and World Health Organization (WHO) Expert Group on vitamin requirements and the ICMR Expert Group in India have recommended folic acid intakes in terms of free folate as given in Table 2.

Folate requirements are increased during pregnancy, apparently due to increased metabolic breakdown of folate in addition to fetal transfer. RNI of 300 mcg daily has been suggested during pregnancy in the UK and a RDA of 400 mcg in the USA, so far.

Interestingly, in the light of recent confirmation of the value of folate in preventing NTDs, reports suggest that the traditionally recommended intakes during pregnancy may be lower than actual requirements. Synthetic folic acid is more bioavailable than natural food folate and the concept of dietary folate equivalent (DFE) has been used to interpret studies in which folate is provided as a combination of the two. Many population-based studies confirm that approximately 300 to 400 mcg of folic acid per day, consumed in conjunction with a low-folate diet, prevents folate deficiency in pregnant women. Data from metabolic studies support a RDA of 600 mcg DFE per day on the basis of the maintenance of normal red cell folate concentrations and agree with the findings from population studies. It is now being recommended that women planning a pregnancy should have a total daily intake (food plus supplement) of about 600 mcg, before conception and during the first trimester.

A number of authorities in the UK and USA have advocated folic acid supplementation of bread or flour to increase the intake in women of childbearing age. Since toxicity is minimal, everyone can potentially benefit from increased folate consumption. Indeed, the United States Food and Drug Administration has recently mandated that cereal grains and products be fortified with 140 micrograms of folic acid per 100 g, which will add approximately 100 mcg of folic acid daily to the average diet. Current studies indicate 200 mcg/day may be the minimum effective amount of fortification needed for normalizing homocysteine concentrations and preventing a significant number of NTDs. Thus, a even higher level of food fortification may be warranted. On the other hand, there is also debate over the appropriateness of such action, including the risk of masking underlying vitamin B12 deficiency. For instance, fortification of foods with folate is not recommended in Norway on the ground that some groups may then exceed the recommended upper level of intake.

Although folates are widely distributed in foods, folate deficiency may be more frequent than expected because the actual availability may be affected by cooking and various food processing conditions. Apart from pregnant women, deficiency is frequently observed in elderly people, smokers, alcoholics and oral contraceptive users. It is also associated with the mutation leading to the thermolabile variant of MTHFR, which is prevalent in about 10% of the population.

In clinical practice, folate or cobalamin deficiencies are usually suspected from hematologic abnormalities, such as a macrocytic anemia, or often milder signs, such as hypersegmented neutrophils. However, deficiency may be associated with clinical conditions in which anemia and/or macrocytosis are absent, such as inborn errors of folate or cobalamin metabolism. In advanced settings, a battery of sensitive tests, including plasma and red cell folate assays, serum methylmalonic acid and homocysteine assays, and the deoxyuridine suppression test in the bone marrow, allows early detection of deficiency. Additional tests may identify the underlying cause, such as the Schilling test for showing food cobalamin malabsorption. The diagnostic strategy differs according to the clinical and hematologic presentations.

Pharmacokinetics

Absorption and distribution: Folic acid is rapidly absorbed from normal diets, mainly from the proximal part of the small intestine, and distributes to body tissues. The naturally occurring folyl polyglutamates are largely deconjugated and reduced prior to absorption. Once absorbed, folic acid is converted to tetrahydrofolate and reconjugated within the cells to form active polyglutamates. It is the
N\textsuperscript{5}-methyl-H\textsubscript{4}folate which appears in the portal circulation, where it is extensively bound to plasma proteins. Commercially available crystalline folic acid is stated to be better absorbed than dietary folate and its availability is less affected by malabsorption syndromes such as tropical sprue. Peak plasma concentration is attained in 30 to 60 minutes. However, there is no relation between the cellular effects of folic acid and its plasma concentration.

The principal storage site for folic acid is the liver. It is also actively concentrated in the cerebrospinal fluid. Folate also distributes into breast milk.

**Metabolism and elimination:** Folic acid is converted, in the presence of ascorbic acid, in the liver and plasma to its metabolically active form (tetrahydrofolic acid) by dihydrofolate reductase. A large proportion of the biotransformation is hepatic. Folate deficiency can be linked to scurvy.

There is an enterohepatic circulation for folate but the elimination is predominantly renal, and almost entirely as metabolites. About 4 to 5 mcg is excreted in the urine daily. Excess quantities beyond daily needs will be excreted in urine. Thus, administration of larger doses of folic acid leads to proportionately greater urinary excretion. Folic acid is removed by hemodialysis. Dialysis patients therefore require supplementation - an estimated 100 to 300% of the recommended daily allowance.

**Assay:** Folic acid is assayed by saturation analysis or a microbiological assay method involving *Lactobacillus casei*. The microbial assay is quite sensitive and detects all folate analogs with one glutamic acid residue.

**Intracellular penetration of folates:** Folic acid enters cells either through receptor-mediated endocytosis facilitated by the folate receptor, or with the help of carrier proteins, such as the reduced folate carrier (RFC). Multiple carrier-mediated mechanisms have been identified that can fulfill this role in a variety of mammalian cell types, including neoplastic cells. The absorption of dietary folates also relies on the function of a carrier-mediated system in mature luminal epithelium. The various carrier-mediated systems can be distinguished by their preferences for various folate compounds as well as by differences in temperature and pH dependence. The widely studied one-carbon, RFC system is mediated by a transporter encoded by the newly discovered RFC-1 gene. Gene expression appears to regulate luminal epithelial cell folate absorption in the small intestine as well as penetration into various nonabsorptive cell types. However, there may be subtle molecular differences in RFC-1-mediated folate transport in absorptive and nonabsorptive cell types. The RFC system can also mediate internalization of antifolates into cells. This anion exchanging concentrative process is opposed by independent exit pumps that are directly coupled to energy metabolism. The balance of these processes governs the free intracellular folate level.

The folic acid receptor is overexpressed on a number of human tumors, including cancers of the ovary, kidney, uterus, testis, brain, colon, lung, and myelocytic blood cells. Conjugates of folic are not substrates of carrier-mediated folate transport but penetrate cells exclusively via receptor mediated endocytosis. Folic acid, linked via its gamma-carboxyl to either a single conjugate molecule or assembly of molecules, can thus enter receptor-expressing cancer cells via folate receptor-mediated endocytosis. Because the affinity of folate conjugates for cell surface folate receptors is high, folic acid derivatization allows the selective delivery of diagnostic and therapeutic agents to cancer cells. This raises the interesting possibility of folate-mediated targeting of a variety of entities (imaging agents, low molecular weight chemotherapeutic drugs, protein toxins, antisense oligonucleotides, ribozymes, genes, liposomes with entrapped antineoplastic drugs, immunotherapeutic agents, etc.) specifically to cancer cells. In most cases, *in vitro* studies demonstrate a significant improvement in potency and/or cancer-cell specificity over the nontargeted form of the same pharmaceutical agent. *In vivo* animal studies are also encouraging.

**Therapeutic indications**

The primary indication for folic acid is the prophylaxis and treatment of folate deficiency. Deficiency is unlikely in healthy men and non-pregnant women receiving an average balanced diet. Simple nutritional deficiency of individual B vitamins is rare since dietary inadequacy usually results in multiple deficiencies. Therefore, for prophylaxis of simple folic acid deficiency, dietary improvement, rather than supplementation, may be advisable. For treatment of folic
during pregnancy are iron deficiency anemia (approximately 75%) and folate deficiency macrocytic anemia, both of which are more common in women on inadequate diets and not receiving prenatal iron and folate supplements.

A mild to moderate maternal anemia does not appear to significantly affect fetal hemoglobin concentration. However, severe anemia may have adverse effects on both the mother and the fetus. A hemoglobin level less than 6 g/dL is generally associated with poor pregnancy outcome and may be complicated by prematurity, spontaneous abortions, low birth weight, and fetal deaths.

After iron, the next most common nutritional deficiency contributing to anemia in pregnancy is that of folate. Women from communities with high prevalence of adverse pregnancy outcome, often consume diets poor in vitamins and minerals, including folate. A central feature of embryonic and fetal development is widespread cell division; folate is vital because of its role in nucleic acid synthesis. During gestation, marginal folate nutriture can impair cellular growth and replication in the fetus or placenta. Low concentrations of dietary and circulating folate are associated with increased risks of NTDs, fetal growth retardation, preterm delivery and low birth weight infants. Further, high maternal homocysteine concentrations, a potential metabolic effect of folate deficiency, has been associated with habitual abortion, placental abruption and pre-eclampsia, which further increase the risk of poor pregnancy outcome and of low birth weight. Affected offspring may continue to have problems in later life.

Recent systematic reviews of the Cochrane database have attempted to assess the effects of iron and folate supplementation on hematological and biochemical parameters and on outcome in pregnancy. They conclude that routine supplementation raises or maintains the serum iron and ferritin levels and serum and red cell folate levels and results in a substantial reduction of women with hemoglobin below 10 g/dL in late pregnancy. Unfortunately, it is difficult to draw conclusions on other outcomes for either mother or baby. Folate supplementation alone is associated with a reduction in the proportion of women with low hemoglobin in late pregnancy and megaloblastic erythropoiesis, with a possible reduction in the incidence of low birthweight. It should be...
noted that these reviews included little data from developing countries where iron and folate deficiency in pregnancy is much more common and anemia is a serious health problem.

Overall, it is desirable that folate supplementation should be routine in pregnancy in order to maintain satisfactory hemoglobin and folate status at delivery. During multiple gestation, additional folic acid supplementation of 1 mg per fetus has been recommended\(^4\). In addition, as discussed in the following section, the periconceptional use of folic acid containing supplements reduces the first occurrence, as well as the recurrence, of NTDs. Folate intake if sustained after complete closure of the neural tube may decrease the risk of other poor pregnancy outcomes. Further randomized controlled trials are necessary to clarify this last issue.

**Neural tube defects (NTDs)**

Failure of the fetal neural tube to fuse normally along with its protective bony shield during the first 4 weeks of gestation may result in one of several congenital defects. These include anencephaly, encephalocoele, cephalocele and spina bifida\(^9\). In spina bifida, failure of vertebral fusion may produce defects ranging from spina bifida occulta, where neurological abnormalities are rare, to meningocele or meningo(myelo)cele, which carry the risk of associated hydrocephalus and paralysis of the lower limbs and sphincters. The reasons for failure of normal development are not well understood but appear to include both environmental and genetic factors. The risk is increased in certain geographical areas, and in the offspring of parents with previous affected children, or of parents who themselves suffer from the condition.

Since the 1960s there has been some evidence that the mother’s folate status is significant, and since the early 1980s, evidence has been accumulating that folic acid supplementation in the period around conception, with or without other vitamins, reduces the incidence of NTDs in the offspring of mothers with previously affected children\(^10\). A large multicenter study initiated by the Medical Research Council of UK was terminated early because of overwhelming evidence that folic acid 4 mg daily, taken from before conception till the twelfth week of gestation, by women with a history of past pregnancies affected by NTDs, reduced the incidence of such defects by about two-thirds. Multivitamins alone (A, D, B\(_1\), B\(_2\), nicotinamide, B\(_6\), and C) did not demonstrate a similar benefit\(^11\). A recent systematic review of the Cochrane database reinforces the hypothesis\(^12\). It covered various approaches - randomized and quasi-randomized trials comparing periconceptional supplementation by multivitamins with placebo, folate with placebo, or multivitamins with folate; different dosages of multivitamins or folate; pre-pregnancy dietary advice and counseling to increase the consumption of folate-rich foods, or folate-fortified foods, with standard care; increased intensity of information provision with standard public health dissemination. The review concluded that periconceptional folate supplementation has a strong protective effect and that women who have had offspring with NTDs should be offered continuing folate supplementation.

NTDs are common congenital malformations in humans. For the most part they are multifactorial in their pathogenesis, having both genetic and environmental components. While a great deal of epidemiologic evidence demonstrating the importance of folate supplements in preventing these malformations has accumulated, it is unfortunate that the mechanisms underlying the benefit are not well understood\(^13\). Animal studies suggest that folate alters head mesenchyme, allowing closure of the forebrain-midbrain boundary to be initiated\(^14\). This boundary is the site of the developmental block in NTDs. In mice, maternal treatment with folic acid appeared to promote full formation of the cranium in offspring mice with a mutant Cart 1 gene (which encodes cartilage homoeoprotein 1); such a genetic mutation would otherwise cause severe cranial defects and partial anencephaly. Folic acid is also necessary for normal brain development and a deficiency results in delayed maturation of the basic electroencephalographic patterns\(^15\).

First occurrences of NTDs account for about 95% of cases, and there are obvious public health implications if the benefits of folate in mothers known to be at risk can be extended to the general pregnant population. Committees worldwide have set almost identical folate recommendations for the prevention of the first occurrence of NTDs. Three options are suggested to achieve the extra 400 mcg folic acid per day being recommended by the official committees - increased intake of folate-rich foods, folic acid...
supplementation, and folic acid fortification of food. A significant increase in dietary consumption of folate-rich foods was shown to be a relatively ineffective means of improving red blood cell folate status in women, compared with equivalent intakes of folate-fortified food, presumably because the synthetic form of the vitamin is more stable and bioavailable. Although folic acid supplements are highly effective in optimizing folate status, supplementation may not be an effective strategy for the primary prevention of NTDs because of poor compliance. Thus, food fortification is seen by many as the only option likely to succeed. The mandatory folic acid fortification of grain products introduced recently in USA is projected to provide an additional mean intake of 100 mcg folic acid per day. This is in addition to the earlier recommendation from the US Public Health Service that all women of childbearing age who are aiming at pregnancy should receive folic acid 400 mcg/day, although care should be taken to keep folate consumption below 1 mg daily without medical supervision. Admittedly, this is not an ideal solution; by aggressively fortifying food, a potential hazard is created for those who do not need extra folate and may have some problems with excess of it. In the UK, the current recommendation is that all women planning a pregnancy should take an extra 400 mcg of folic acid daily before conception and during the first trimester, bringing the average folate intake to about 600 mcg daily. As in the USA food fortification is being considered and some food is already fortified.

For prevention of recurrence, it is recommended in the UK that all women with spina bifida or with previous offspring with NTDs, should receive folic acid 5 mg daily when they plan to become pregnant, until the twelfth week of pregnancy. In the USA, the recommendation is 4 mg daily from at least four weeks before conception through the first 3 months of pregnancy. It must be borne in mind that only about 60 to 70% of NTDs appear to be folate-sensitive, and parents should be counseled appropriately. Investigators acknowledge that the 4 or 5 mg dose may not be optimal, and there are both early and more recent studies to imply that much lower doses of folic acid may be sufficient to reduce the risk of recurrence, but this is yet to be clearly demonstrated. Furthermore, the optimum length of time that supplements should be given to these women before conception, always a matter of practical difficulty, is unknown.

Some authors have even recommended that physicians should seriously consider screening women who are planning to get pregnant for red cell folate status - they anticipate that the identification and supplementation of women with red cell folate levels < 840 nmol/L would reduce the risk of NTDs in these women by over 85%. Inadequate intake of folic acid is also connected with preterm delivery, intrauterine growth retardation and placental abruption and infarction. Supplementation can thus bring other health benefits as well. Interestingly, recent results from the Hungarian program suggest that multivitamin supplements (including folate) may also reduce the occurrence of other congenital abnormalities.

Orofacial clefting

Orofacial clefting (OC) is one of the most frequent congenital anomalies, with a higher birth prevalence that Down’s syndrome or NTDs, but lower than cardiovascular malformations. Babies with OC require a multidisciplinary approach for management, including surgery and rehabilitative treatments over time. This implies an elaborate demand in terms of social organization and healthcare costs. The etiology of OC is complex and heterogeneous. Environment and genetic influences are known. Much still remains unknown, specially regarding the role of genetics in producing susceptibility to the environment. Among the categories of genes that may confer genetic susceptibility to OC are those having biological activities linked to OC pathogenesis without direct involvement, such as genes coding for MTHFR and the folic acid receptor. Among the environmental factors implicated, folic acid deficiency may be responsible for OC malformations, in addition to NTDs, through a common mechanism that interferes with embryonic development, depending on the maternal or embryo genotype. There is some evidence that folic acid supplementation may reduce the incidence of oral and facial clefting. Further research with multidisciplinary approaches in biochemistry, genetics, gene/environment interactions, and embryology is indicated.

US researchers observed that dietary intake of folic acid in multivitamin preparations may decrease the risk of cleft palate in neonates born to women who take such preparations periconceptionally. They obtained data from a population-based case-control study involving 731 mothers of fetuses and neonates
with orofacial abnormalities and 734 mothers of neonates without malformations. The risk of cleft palate was reduced by 20-35% in infants born to women who used multivitamin supplements containing folic acid. However, it is possible that other multivitamin components may also have contributed to this effect.

**Pregnant women receiving anticonvulsants**

Pregnant women with epilepsy constitute 0.5% of all pregnancies and proper seizure control is a prime goal in these subjects. Psychological, hormonal and pharmacokinetic changes in pregnancy may escalate seizure activity. Unfortunately, the commonly used anticonvulsants are established human teratogens. Factors such as anticonvulsant-induced teratogenicity, patient's genetic predisposition and the severity of the convulsive disorder determine outcome for children of epileptic mothers. Anticonvulsant interaction with folic acid may lead to an increased risk for NTDs\(^65\). In addition to counseling and other measures, preventive folic acid medication may ameliorate some of this risk\(^65,66\). Folic acid 4 to 5 mg/day has been recommended for 3 months before conception and during the first trimester to prevent folic acid deficiency-induced malformations\(^67,68\). This is at the same level recommended to prevent recurrence of NTDs in general.

**The elderly**

Although a notion is prevalent that folate deficiency is uncommon in the elderly, the use of the plasma total homocysteine concentration as a metabolic marker of folate status is changing this attitude. Plasma homocysteine measurements in epidemiological studies suggest that subclinical folate deficiencies are common in various populations, including the elderly. The clinical consequences of a compromised folate status may include increased risk of coronary heart disease and cancer, and a possible association between folate deficiency and neuropsychiatric illness has been suggested\(^69\).

However, the available evidence is not strong enough to recommend folate supplements routinely to all elderly people. There is no evidence that a suboptimal folate status is causally involved in the pathogenesis of the above mentioned disorders. Furthermore, cancer and the cardiovascular diseases are chronic conditions and it may be too late to use folate supplementation as preventive measures in elderly persons. Nevertheless, folate supplementation should be considered in elderly people with elevated plasma total homocysteine concentrations and proven cardiovascular disease and in elderly patients treated with drugs known to induce folate deficiency. The daily folate supplement should be at least 500 mcg/day but it should never be used as a substitute for a diet rich in fruits and vegetables\(^69\).

Both folate and vitamin B\(_{12}\) are essential to normal brain function in the elderly\(^70\). Administration of folate supplementation alone may accelerate vitamin B\(_{12}\) associated neuropathy. The biochemical basis of the interdependence between folate and cobalamin is the maintenance of two functions, nucleic acid synthesis and the methylation reactions. The latter is particularly important in the brain and relies especially on maintaining the concentration of S-adenosylmethionine which, in turn, maintains the methylation reactions whose inhibition is considered to cause cobalamin deficiency associated neuropathy. S-adenosylmethionine mediated methylation reactions are inhibited by its product S-adenosylhomocysteine (SAH). This occurs when cobalamin is deficient and, as a result, methionine synthase is inhibited causing a rise of both homocysteine and SAH. Other potential pathogenic processes related to the toxic effects of homocysteine are direct damage to the vascular endothelium and inhibition of N-methyl-D-aspartate receptors. Thus, vitamin B\(_{12}\) deficiencies must be excluded before folate supplementation is commenced. If in doubt, it may be safer to supplement folate and vitamin B\(_{12}\) together.

**Chronic alcoholism**

Chronic alcoholics frequently suffer from micronutrient deficiencies, including vitamins involved in one carbon metabolism, i.e., folate, pyridoxine and vitamin B\(_{12}\)\(^71\). Metabolism of the amino acid homocysteine is closely linked to the metabolism of these three vitamins. In fact, homocysteine stands at the intersection of two pathways: methylation and transsulfuration. In methylation, homocysteine acquires a methyl group from N\(^5\)-methyl-H\(_4\)folate in a vitamin B\(_{12}\) dependent reaction, whereas in the transsulfuration pathway, homocysteine condenses with serine to form cystathionine in an irreversible
reaction catalyzed by the pyridoxal-5'-phosphate-containing enzyme, cystathionine-beta-synthase. Due to these relationships, nutritional deficiency of one of these vitamins, as a consequence of chronic alcohol intake, could lead to metabolic disruption and potentially to hyperhomocysteinemia. Studies in chronic alcoholics have revealed hyperhomocysteinemia along with disturbed vitamin status and DNA hypomethylation in peripheral lymphocytes. It is also possible that these metabolic abnormalities could be involved in the pathogenesis of organic diseases associated with chronic alcoholism. Thus alcoholics may benefit from a supplementation of folic acid and other B-vitamins. Interestingly, beer drinkers may escape folic acid deficiency due to relatively high content of folates in beer.

Renal failure patients

In renal failure, four factors - restricted diet, uremic toxins, drug-nutrient interactions, and, in end-stage renal disease (ESRD), the dialysis process - affect the normal absorption, retention and activity of various micronutrients. Studies have shown that the typical renal failure diet is low in B vitamins, that uremic factors affect folate and pyridoxine activities, and that many B vitamins are lost on dialysis. Ongoing research suggests that some B-vitamins, such as folic acid and pyridoxine, if provided in higher than normal amounts, may reduce the risk of some aspects of renal cardiovascular disease. This raises the question of supplementing B vitamins in renal failure and ESRD patients.

The hormone erythropoietin is now frequently used to counteract the anemia associated with chronic renal disease. Concomitant use of iron enhances response to erythropoietin, and more recently other adjuvant therapies such as ascorbic acid, L-carnitine, folic acid, vitamin D, androgens, cytokines and growth factors have been investigated. Folic acid has been used as an adjuvant during erythropoietin therapy but there is, as yet, no consensus on its use. Possibly, supplementation would not be of benefit unless there is underlying deficiency.

Hyperhomocysteinemia and atherosclerotic cardiovascular pathology

N5-methyl-H4folate, is required for the methylation of the sulfhydryl amino acid homocysteine to methionine. By restricting methylation, folate deficiency induces homocysteine efflux into the circulation leading to hyperhomocysteinemia. Many studies have demonstrated a negative correlation between plasma folate, particularly N5-methyl-H4folate, and circulating homocysteine levels. Other acquired causes of hyperhomocysteinemia include other B-vitamin deficiencies and renal insufficiency. The most important inherited cause is a point mutation in the MTHFR gene.

There is now overwhelming epidemiological and experimental evidence that hyperhomocysteinemia is a graded cardiovascular risk factor, independent of the traditional risk factors, although a cause-and-effect relationship is still unproven. Studies of patients with cerebrovascular disease reveal elevated homocysteine concentrations in 30-40% of cases. Many studies demonstrate a correlation between elevated homocysteine concentrations, risk of myocardial infarction, and mortality. The relationship between serum homocysteine level and coronary artery disease (CAD) seems to be stronger in younger than in older persons. In addition, hyperhomocysteinemia and decreased folic acid concentrations have been identified in ESRD and type 2 diabetic patients, while both concentrations remained normal in healthy controls. A methionine loading test identifies substantially more subjects with hyperhomocysteinemia compared to fasting homocysteine determination alone. Repeated blood sampling is necessary due to intra-individual variability in homocysteine concentrations to the tune of up to 25%. A conservative reference value for fasting homocysteine is 15 micromol/L, although there seems to be no definite threshold in the presumed linear relation between homocysteine concentration and cardiovascular risk. The pathophysiological mechanism of homocysteine-induced cardiovascular disease is still not elucidated. Homocysteine seems to cause endothelial dysfunction, increase oxidant stress, and promote vascular smooth muscle growth. Recent human studies using methionine loading to experimentally induce moderate hyperhomocysteinemia have demonstrated rapid and profound impairment of resistance and conduit artery endothelial function.

Since folic acid deficiency might increase the risk of cardiovascular disease by increasing circulating homocysteine levels, it stands to logic that this risk
may be reduced by increasing folate intake. Indeed, folate acid supplementation can be considered the optimum homocysteine lowering therapy, except in renal failure patients. A recent meta-analysis has concluded that, in western populations, daily supplementation with 0.5-5 mg folic acid and about 0.5 mg vitamin B<sub>12</sub> would be expected to reduce blood homocysteine concentrations by about a quarter to a third. Studies using folic acid 650 mcg/day have reduced homocysteine concentrations to within normal range after two weeks of treatment. Use of vitamin B<sub>6</sub> or B<sub>12</sub> alone is ineffective, but when administered along with folic acid, they may help lower homocysteine concentrations further. All therapies must be given for the lifetime of the patient. In addition, patients must use discretion in their diet, as common beverages, such as coffee, have a strong correlation with hyperhomocysteinemia, while foods high in folic acid, vitamin B<sub>6</sub> and vitamin B<sub>12</sub> may reduce homocysteine concentrations. A meta-analysis had concluded that fortification of foodgrains with folic acid could have a substantial impact in reducing hyperhomocysteinemia-related CAD deaths. However, to date, no clinical trials have actually assessed the effects, on overall morbidity and mortality, of treating high homocysteine concentrations in atherosclerotic patients. Thus, while there may already be sufficient evidence to prescribe homocysteine-lowering therapy in subjects deemed to be at high risk of cardiovascular disease, definite recommendations regarding primary prevention strategies will have to await the completion of long-term, randomized, prospective studies.

**Anticancer effects**

Collectively, the evidence from epidemiologic, animal and human studies strongly suggests that folate status modulates the risk of developing cancers in selected tissues, the most notable of which is the colorectum. Folate deficiency, by reducing intracellular S-adenosylmethionine, can alter cytosine methylation in DNA, leading to inappropriate activation of proto-oncogenes, altered expression of critical tumor suppressor genes and eventual induction of malignant transformation. Alternatively, since folic acid is crucial for normal DNA synthesis and repair, folate deficiency may cause an imbalance in DNA precursors, uracil misincorporation into DNA, and chromosome breakage. Evidence has been obtained from cellular, animal and human studies that folic acid can modulate DNA by such mechanisms.

If folate depletion enhances the risk of carcinogenesis, it is plausible that folate supplementation above what is presently considered to be the basal requirement may confer a protective effect. Pilot studies show favorable effect on colorectal cell proliferation from supplementation with calcium; vitamins A, C, and E; omega-3 fatty acids; and folic acid. Intravenous calcium leucovorin, the N<sub>5</sub>-formyl derivative of tetrahydrofolate, has shown an advantage when combined with 5-fluorouracil (5-FU), over 5-FU alone, in the treatment of advanced colorectal carcinoma, in terms of objective tumor response. This regimen is less toxic and less expensive than the combination of 5-FU and interferon-alfa. However, the increase in response does not seem to result in discernable improvement in overall survival. Furthermore, results do not support the routine use of 5-FU plus leucovorin in all patients with colon cancer. Hopefully, further attempts to elucidate the complex mechanisms of this potentially synergistic drug combination may allow the rational design of regimens with a superior therapeutic result.

Two recently published large, prospective epidemiologic studies suggest that maintaining adequate levels of serum folate or moderately increasing folate intakes from dietary sources and vitamin supplements can significantly reduce the risk of pancreatic and breast cancer, respectively. This protective effect of folate appears to be operative in subjects at risk for developing these cancers, namely, male smokers for pancreatic cancer, and women regularly consuming alcohol for breast cancer.

Micronutrient deficiency is not an uncommon phenomenon. For instance it has been estimated that even in a highly developed country like USA, folate deficiency occurs in approximately 10% of the average population, and in a much higher percentage of the poor. However, such deficiencies can be remedied at low cost. The expanding role of folate nutrition in cancer prevention has major public health implications. Research needs to be continued on relationship of folate to carcinogenesis.

**Ameliorating methotrexate toxicity in rheumatoid arthritis patients**

The use of weekly low-dose methotrexate for treating rheumatoid arthritis is well documented. Efficacy
and adverse events are both dose dependent, and adverse drug reactions, rather than the lack of response, is the main reason for discontinuing therapy. Several adverse reactions appear to be related to folate deficiencies, and are largely due to the antifolate properties of methotrexate. In order to diminish side effects without compromising drug efficacy, clinical trials have been performed using folic acid concomitantly with methotrexate. This is a better option than reducing the dose of methotrexate. Important to this achievement are both the timing of folate supplementation and the weekly folate-to-methotrexate ratio, otherwise methotrexate activity may be compromised. Calcium folinate is also effective for this indication, but suffers the disadvantage of higher cost. A recent meta-analysis of selected double blind randomized controlled trials, in which adult patients with rheumatoid arthritis were treated with low dose methotrexate (< 20 mg/week) concurrently with folic or folinic acid, has concluded that folate supplementation does indeed reduce methotrexate toxicity related to the oral and gastrointestinal systems without compromising efficacy. However, an individually adjusted supply of folic acid has been proposed for this indication. For several patients a properly balanced diet is sufficient; they do not need additional folate supplements when using methotrexate.

Other indications

Fragile X syndrome: A common cause of inherited mental retardation, the fragile X syndrome is characterized by subtle phenotypic abnormalities, cognitive impairment, the presence of a fragile site (gap) detectable in folate-free culture medium on the short arm of X-chromosome called FRA X A, and transcriptional inhibition, through overmethylation, of an mRNA protein-binding gene called FMR-1. Inheritance is in an atypical X-linked dominant mode. Despite hopes of improvement in the sensory-motor impairment in this disorder through folate supplementation, more recent reports indicate that the benefits of both folic acid and folinic acid are equivocal.

Infants fed on goat’s milk: Goat’s milk is poor in folate and infants reared largely on goat’s milk tend to develop a megaloblastic tendency within 4 to 5 months. Folic acid supplementation may be needed in such cases while improving the diet.

Chronic myelofibrosis: This is associated with a high degree of ineffective erythropoiesis that tends to increase folate requirement. About a third of patients with this disease develop a megaloblastic anemia at some stage and twice that number show poor red cell folate status. Folate supplementation may thus be required. However, as this is a disorder of the elderly it is necessary to rule out vitamin B12 deficiency before instituting folic acid.

Dosage and administration

The usual oral dose of folic acid is 200 to 500 mcg daily in the prophylaxis of megaloblastic anemia of pregnancy. For women at high risk of having a pregnancy affected by NTD, the dose is 4 to 5 mg daily starting before pregnancy (in the USA the recommendation is 4 weeks before) and continued through the first trimester. In case of unplanned pregnancy, supplementation should start as soon as pregnancy is diagnosed. Lower supplemental doses suffice for breast-feeding women.

For prophylaxis of deficiency in children, proportionately lower doses are recommended based on normal recommended daily intakes.

Prophylactic administration of folic acid 5 mg daily or weekly by mouth may be necessary in chronic hemolytic states such as thalassemia major or sickle-cell anemia, depending on the diet and rate of hemolysis; similar doses may be necessary in some patients receiving hemodialysis in order to prevent deficiency.

For deficiency treatment, the dose is best individualized based on severity of deficiency till an adequate hemopoietic response has been obtained. Doses of 5 mg daily may be given by mouth for 4 months or longer. Up to 15 mg daily may sometimes be necessary in malabsorption states. Maintenance treatment with lower doses may be required.

As a diagnostic aid in folate deficiency, the dose is 100 to 200 mcg daily for ten days by oral or intramuscular route plus low dietary folic acid and vitamin B12.

Parenteral administration of folic acid (intravenous, intramuscular or subcutaneous injection as the sodium salt) is indicated only when oral administration
is not acceptable or possible. The usual adult and adolescent dose for deficiency treatment is 250 mcg to 1 mg daily till a hematologic response occurs.

For deficiency prophylaxis by the parenteral route, folic acid is administered by intravenous infusion, as part of total parenteral nutrition solutions, the specific amount determined by individual patient needs.

**Adverse drug reactions, cautions and drug interactions**

Adverse reactions to folic acid are rare at usual supplemental doses of up to 5 mg/day. Gastrointestinal disturbances may occur. Hypersensitivity reactions, such as bronchospasm, erythema, skin rash or itching, have been reported rarely but may require medical attention.

Other potential adverse reactions which have raised concern include:

- neurotoxicity, including seizures
- stimulation of malignant neoplasms
- increased susceptibility to malaria

However, in a recent review of these concerns, it was concluded that the information is inconclusive, and consists mainly of case reports and uncontrolled studies; these problems have not surfaced in larger trials.

The only absolute contraindication to folic acid supplementation is known hypersensitivity to folate or to excipients in the preparation.

Folic acid should never be given alone or in conjunction with inadequate amounts of vitamin B₁₂ for the treatment of undiagnosed megaloblastic anemia, since it may produce a hemopoietic response in vitamin B₁₂-deficient megaloblastic anemia (e.g. pernicious anemia) without ameliorating the neurological component. Furthermore, reversal of the red cell abnormalities may affect the identification of cobalamin deficiency. The masking of the true deficiency state can lead to serious neurological damage, such as subacute combined degeneration of the cord. Thus, doses of folic acid greater than 400 mcg per day are not recommended until pernicious anemia has been ruled out, except during pregnancy and lactation. The danger of vitamin B₁₂ deficiency associated neuropathy is perhaps greater in the elderly. The biochemical basis of this toxicity has been discussed earlier.

Caution is also advised in the presence of folate-dependent tumors. Otherwise, no problems have been documented in use of folic acid supplements in pregnant and lactating women, in children and the elderly. Folic acid injection that contains benzyl alcohol as a preservative should not be used in newborn and immature babies. The use of benzyl alcohol in neonates has been associated with a fatal toxic syndrome consisting of metabolic acidosis and central nervous system, respiratory, circulatory, and renal impairment.

Folate status may be affected by a number of drugs. Alcohol, anticonvulsants, oral contraceptives, antitubercular drugs, and folic acid antagonists (e.g. methotrexate, pyrimethamine, sulfasalazine, sulfonamides, triamterene, trimethoprim and trimetrexate) have all been reported to produce folate deficiency states and thus increase the requirement for this vitamin. Although folic acid 4 to 5 mg/day has been recommended for 3 months before conception and during the first trimester to prevent folic acid deficiency-induced malformations in pregnant women receiving phenytoin, there is current rethinking on this aspect since it has been found that folic acid doses as low as 1 mg/day may perturb phenytoin metabolism leading to loss of seizure control.

During methotrexate use, as previously mentioned, carefully timed replacement therapy may be necessary to prevent megaloblastic anemia developing. In case of high dose methotrexate for malignancy, intravenous leucovorin calcium is used for folate rescue. Folic acid itself cannot be used because of the blockade of its enzymatic activation.

Folic acid may reportedly interfere with the action of pyrimethamine in toxoplasmosis. The triazine antimalarials, like proguanil and cycloguanil, inhibit dihydrofolate reductase in sensitive plasmodia, depleting folate cofactors and inhibiting DNA synthesis. There is theoretical possibility of antagonism of their action by folic acid. In vitro studies have found that the minimum inhibitory concentrations of cycloguanil and analogs are not affected by physiological concentrations of folic or folinic acids in...
Table 3. Miscellaneous drug-interactions with folic acid of potential clinical significance.

- Antacids - May reduce folate absorption; patients should be advised to take antacids at least 2 hours after folic acid\(^\text{109}\).
- Antibiotics - May interfere with the microbiologic assays for serum and erythrocyte folic acid concentrations and cause falsely low results\(^\text{110}\). Concurrent use of chloramphenicol may attenuate the hematologic response to folic acid. Monitoring is necessary.
- Cholestyramine - May interfere with absorption of folic acid; folate supplementation should be taken at least 1 hour before or 4 to 6 hours after cholestyramine\(^\text{111}\).
- Estrogens or oral contraceptives - Requirements for folic acid may be increased in patients receiving these medications\(^\text{112}\).
- Zinc supplements - Some studies have reported that folate may decrease the absorption of zinc, but not in the presence of excessive zinc; other studies have found no inhibition\(^\text{113,114}\).

human serum\(^\text{108}\). With *Plasmodium falciparum* growing in erythrocytic culture, the antimalarial effect of cycloguanil was readily antagonized by folinic acid\(^\text{108}\). The parasite exposed to cycloguanil showed depressed levels of thymidine 5'-triphosphate (dTTP) attributable to inhibition of dihydrofolate reductase. However, the addition of folinic acid did not restore dTTP levels in the parasites, suggesting that the drugs may have additional or different mechanisms of toxicity.

Other interactions of potential clinical significance are given in Table 3.

**CONCLUSION**

The importance of folic acid in human nutrition is still unfolding more than half-a-century after its identification and isolation. The realization that proven benefits can be extended to the community at large, at minimal costs, should lend urgency to the exploration of grey areas. Further developments in the field are eagerly awaited.

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AVIJIT HAZRA AND S.K. TRIPATHI


INVITATION TO REGISTER FOR THE SHORT COURSE ON “RECEPTORS, ION CHANNELS, AND SIGNAL TRANSDUCTION”

The 53rd Indian Pharmaceutical Congress has organized one day pre-conference workshop (short course) on the topic “Receptors, ion channels, and signal transduction” on 20th December 2001 at the venue of the conference (IARI Campus, New Delhi-12). The workshop would contain an in-depth study of topics related to receptors, ion channels, cell signaling, and second messenger system. These topics are of great relevance to pharmacologists, biochemists, molecular biologists and pharmaceutical scientists engaged in teaching, drug discovery, and drug research.

**December 20, 9 AM to 5 PM**

Any suggestions on the short course and the symposium may kindly be addressed to: jagadeeshg@cdr.fda.gov.

Registration information (both courses and the main conference): Please note that there is limited number of seats available for the short course. The selection would be made on a first come first served basis. Interested persons should see the registration brochure issued by the: Office of the secretariat, 53rd IPC, Apothecaries Pvt. Ltd., Majeeida hospital, Jamia Hamdard, Hamdard Nagar, New Delhi.

**Delegate fee : (Delegate fee includes course materials, working lunch and tea for the day of the workshop only)**

Rs. 500/- for delegates from Industries; Rs. 350/- for teachers and research scholars.

**For more information, please write to:**

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