# ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY

VOLUME 206

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# THE ROLE OF VITAMIN $B_{12}$ AND FOLATE IN CARCINOGENESIS*

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## ABSTRACT

The roles of vitamin $B_{12}$ and folate in carcinogenesis are largely extensions of and linked to their roles in normal metabolism, particularly 1-carbon unit metabolism. A possible key area may be hypomethylation to "switch on" genes and methylation to "switch them off." Some vitamin analogues may act as antivitamins in these reactions, as may some vitamin-binding proteins. Others may act as specific delivery proteins. Using appropriate radioactive substrates and suspensions of vitamin-dependent normal and malignant cells, it may be possible to work out their positive and negative control of DNA synthesis.

## INTRODUCTION

The roles of vitamin $B_{12}$ and folate in carcinogenesis are largely extensions of and linked to their roles in normal metabolism. One key area is the conversion of homocysteine to methionine (methyl homocysteine). This process is dependent on folate delivering its 1-carbon unit to vitamin $B_{12}$, which then becomes methyl-$B_{12}$ and transfers that methyl unit to homocysteine (Fig. 1). Newberne et al.[1] recently reviewed the role of the lipotropes choline and methionine and related factors in oncogenesis, including the impaired hormonal and cell-mediated immunity in folate-deficient humans and animals, and they pointed out the synergism between high fat diets and methyl deprivation.[2] Poirier[3] reviewed the protective effect of methionine against hepatocarcinogenesis, and Farber[4] discussed the carcinogenesis promotion effect of the ethyl analogue of methionine, ethionine.

ABBREVIATIONS: azaC = 5-azacytidine; TC II = transcobalamin II; PGA = pteroglutamic acid; SAM = S-adenosylmethionine; dU = deoxyuridine; AIDS = acquired immunodeficiency syndrome; dThd = thymidine; PHA = phytohemagglutinin A.

*Supported in part by the Research Service of the U.S. Veterans Administration and U.S. Public Health Service Grant AM35709.

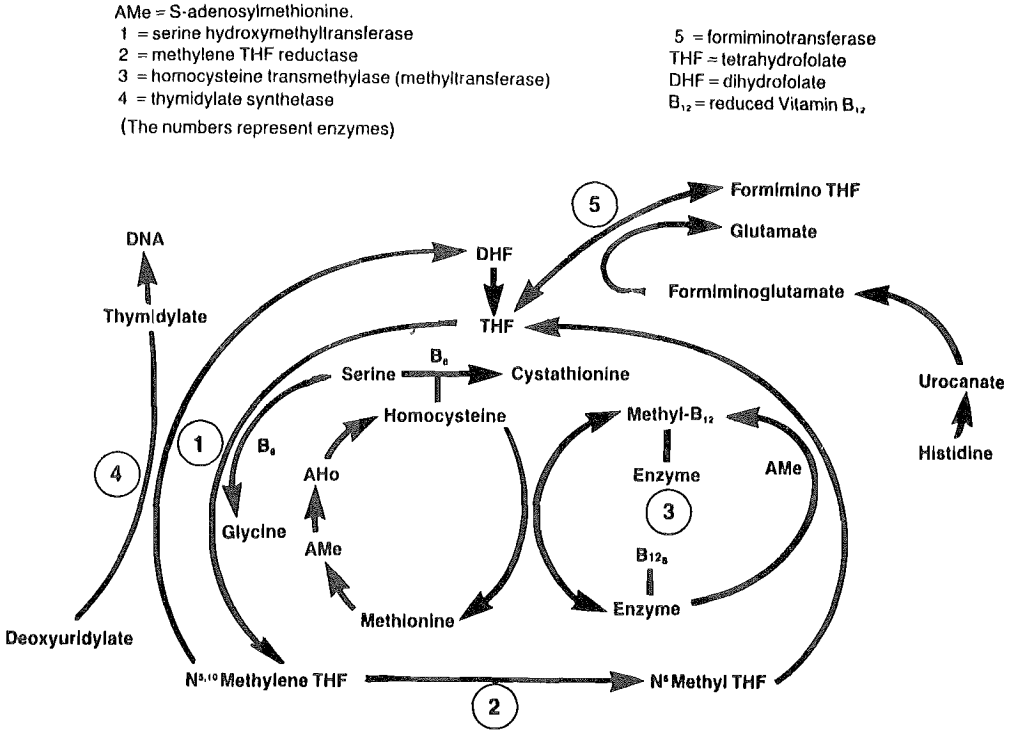


Fig. 1. Biochemical interrelationships between vitamin $B_{12}$ and folate in human metabolism.

In a series of painstaking studies, Poirier's group[5] determined that, over a 76-week period, dietary methyl deficiency markedly promoted liver carcinogenesis and exhibited complete carcinogenic activity in this organ in the rat. They showed this in rats fed methyl-deficient, amino acid-deficient diets. When the diets were also devoid of folic acid and vitamin $B_{12}$, the diethylnitrosamine-initiated rats died within 23 experimental weeks, before developing hepatocellular carcinoma, but all had livers containing hepatocytes of atypical appearance and, particularly at the 2 higher dosages of diethylnitrosamine, a cirrhotic pseudonodular architecture. They also found neoplastic conversion of rat liver epithelial cells in culture by ethionine and S-adenosylethionine.[6]

Krumdieck[7] reviewed the literature pertaining to the role of folate deficiency in facilitating carcinogenesis through 1982 and Eto[8] has carried the subject through the beginning of 1985. Folate is essential in the biosynthesis of both purines and pyrimidines[9,10] and therefore is required by all dividing cells. The conversion of deoxyuridylate to thymidylate (methyldeoxyuridylate) is folate- and $B_{12}$-dependent,[9-11] involving these 2 vitamins in a key step in DNA synthesis (Fig. 1). These biochemical facts underlie the chromosomal abnormalities[9] that characterize human clinical deficiency of vitamin $B_{12}$ and/or folate.[12,13] A wide range of chemical carcinogens inhibit DNA methylation in vitro.[14] It has been suggested that deficiency of folate or vitamin $B_{12}$ or any cause of failure to methylate DNA and/or RNA can activate malignancy by hypomethylating oncogenes, leading to such gene expression and/or gene amplications, and that methylating oncogenes can inhibit malignancy by making them

dormant.[3,15] This is similar to the concept of "relaxed control" of RNA synthesis, discussed 3 decades ago by Borek and co-workers.[16] They noted that when an organism auxotrophic for methionine is deprived of methionine, it loses its ability to suppress synthesis of RNA, which is then synthesized more rapidly; they tied that observation to methylation of RNA. We speculated that vitamin $B_{12}$ or folate deficiency could produce such "relaxed control,"[17] and we noted more recently[15] that folate, vitamin $B_{12}$, and their antagonists could be involved in the control of normal gene expression if in fact hypomethylation of DNA "switches on" normal genes and methylation "switches them off."[18] Although the evidence of this process is significant but inconclusive, one would expect that hypomethylation of the DNA or RNA of oncogenes would activate them and methylation would inactivate them. Perhaps some of the second cancers that develop after successful antimetabolic chemotherapy are due to the same chemotherapy that directly destroys an active cancer, demethylating an oncogene of a dormant cancer.

Gene amplification is a mechanism for tumor resistance to antimetabolites.[19] One can speculate that it may also be a mechanism to aid in tumor proliferation by, for example, producing gene amplification of the hepatic Phase I enzymes that activate carcinogens.[20]

Gautsch and Wilson[21] found that de novo methylation of the input provirus occurs in embryonal carcinoma cells but not in permissive, differentiated teratocarcinoma. Harrison et al.[22] demonstrated a 3-way correlation between tumorigenicity, trisomy for 3q, and specific demethylation, suggesting that decreased DNA methylation may be involved both in differentiation and in tumorigenicity and that the antileukemia drug azaC may induce chromosomal aberrations as well as altering DNA methylation. Altering DNA methylation is just one of the varied effects of azaC on cellular metabolism.[23-25] The drug reduces DNA methylation and induces theoretically therapeutically valuable differentiation of human promyelocytic leukemia cells (HL-60) in culture, although this induction is less effective than that brought about in these cells by dimethylsulfoxide and L-ethionine.[26] Anderson and colleagues[25] found that azaC selectively hypomethylates fetal globin genes, supporting work by Ley et al.[23] and Charache and associates.[27]

Patients with neoplasms excrete elevated levels of certain methylated bases in their urine, and Borek's group[28-30] has been attempting to correlate the quantity of such excretion with the degree of tumor activity. Gross's group, in collaboration with our group,[15,31,32] were unable to show any reproducible inhibitory effect of 5-methylcytidine on the development of presumably RNA virus-induced transplanted L2C leukemia-lymphoma in guinea pigs; this appears to be an animal analogue to human leukemia-lymphoma of RNA virus etiology.[33,34] After we switched to 5-iodocytidine, which seemed more promising, both groups unsuccessfully sought funding targeted to continue this work. Gross's[35] recent dramatic report of reduction in the incidence (i.e., the initial development) of radiation-induced tumors in rats after restriction of caloric food intake has been associated with renewed funding. His group previously noted that restriction of food intake will not significantly influence the growth or progress of established tumors in mice.[32] American Cancer Society statistics suggest an increased frequency of malignancy in obese persons (L. Gross, personal communication).

The roles of vitamin $B_{12}$ and folate in carcinogenesis are not at the simple level at which serum vitamin levels correlate with extent of disease. No correlation has been found between serum folate and vitamin $B_{12}$ levels and the extent of small cell lung cancer.[36] However, a correlation may exist between levels of certain naturally occurring folate and

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