CHAPTER I

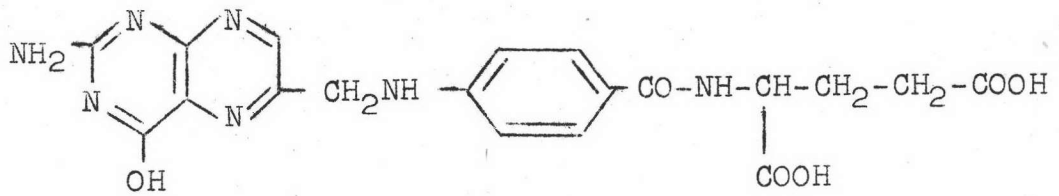
INTRODUCTION

Folic acid is one of the B group of vitamin. It derives its name from the Latin word folium (leaf) because it was first isolated from spinach leaves. Nowaday it is known to be occurred in relatively minute amounts, compared to other food sources. Various investigators succeeded in isolating this potent nutritional factor from other different natural sources (Osol & Hoover, 1970). The best food sources of folic acid are liver,kidney, meat, green leafy vegetable, dry beans, asparagus, mushrooms, offal, cereal, broccoli, and collards. Other good sources include spinach, peanuts, lima beans, cabbage, sweetcorn, chard, turnip greens, lettuce, milk and whole wheat products (B.P.C. 1973; Krantz & Carr, 1967).

Folic acid apparently is identical with a compound essential for the growth of Lactobacillus casei (the L. casei factor). It is composed of a number of related substances, containing glutamic acid and pteroyl groups, joined together by para-aminobenzoic acid, and known collectively as pteroyl-glutamates. Several apparently unrelated factors had been isolated in various laboratories before realization that they had in common the same parent compound i.e.,pteroyl-L-glutamic acid. These factors were factor U (a chick growth factor).

vitamin M (a factor for monkeys), vitamin B_c (a chick anti-anemia factor), liver and yeast L. casei factors (bacterial growth factors), and others (Osol & Hoover, 1970).

The term "pteroyl-glutamic acid" has been chosen as a suitable chemical name for folic acid. Its molecular weight is 441.4 and its structure formula is :-



N-p-(2-amino-4-hydroxypterid-6-yl methylamino)benzoyl-L(+)-glutamic acid. or Pteroyl(mono)glutamic acid (PGA ; formerly called folic acid or folacin).

It may be observed that the molecule contains 3 distinct grouping :

(a). glutamic acid

(b). para-amino benzoic acid and

(c). a pteridyl ring

The folic acid in food is composed of conjugates of this structure with additional molecules of glutamic acid and hence of higher molecular weight. The vitamin conjugate has 7 molecules of glutamic acid in the molecule and has a molecular weight of 2.8 times that of folic acid (Krantz & Carr, 1967). A series of folic acid with several molecules of glutamic acid

attached to the first glutamic acid radical in peptide linkage have been synthesized. Compounds with one, two, three and seven glutamic acid groups have been isolated. The latter three presumably are known as conjugates. Some animals and man can utilize them as a source of pteroyl-glutamic acid, because appropriate digestive enzymes can hydrolyze them. Microorganisms can use them to only a variable and limited extent, unless they are first hydrolyzed to the free form with liver, kidney, or pancreatic enzymes, called conjugases. The functional form of this vitamin group called folic acid is basically the 5,6,7,8-tetrahydro PGA in which a formyl group (-CHO), when present, is attached at either or both the N^5 or N^{10} position. The hydrogenated N^5-formyl compound is named folinic acid,or leucovorin, which is available, as the monosodium salt of PGA and as a discrete pharmaceutical preparation. Separately, the three moieties which make up the PGA molecule (pteroic acid, p-aminobenzoic acid and glutamic acid) have no vitamin activity (Osol & Hoover,1970).

Folic acid is itself inactive (Laurence, 1965). It is readily and completely absorbed by the proximal third of the small intestine. Low concentrations probably utilize an active transport mechanism, but at high concentrations folic acid is probably also absorbed by diffusion (Meyer et al., 1972). It is converted into the biologically active coenzyme tetrahydrofolic acid which is important in the biosynthesis

of amino acid and nucleic acids, and therefore in cell division. Absorption of orally administered supplemental folic acid is usually satisfactory even in the presence of disorders of the small bowel which have produced a deficiency state (Meyer et al., 1972). Conjugated folate in food is less absorbed, dietary correction of the deficiency is usually impractical in the presence of intestinal disease. Folic acid is widely distributed to all tissues and concentrated in the cerebrospinal fluid. Only small amounts of folic acid appear in the urine of subjects on normal diets, but excretion by this route is high following large doses (Meyers et al., 1972). The degree of absorption and utilization of folate may vary depending on the degree of liberation of folic acid by conjugase enzyme present in the jejunum. Without folic acid the living cell cannot divide but is halted in metaphase, this property underlies the use of folic acid antagonists in the treatment of neoplastic disease. The formyl derivative of tetrahydrofolic acid or citrovorum factor (leucovorin) and this can be used where the body fails to effect the conversion of folic acid (Laurence, 1966). It is probable that cyanocobalamin, folic acid and folinic acid all play some essential but little understood role in the metabolism of nucleic acid and that all these factors are required for the continuation of normoblastic blood formation. They play an important part in many other

metabolic processes in the body (Davidson, 1958).

Folic acid appear to be essential nutrient for the functioning of the bone marrow. It deprivation in the diets of chicks and monkeys has been shown to produce characteristic anemias. In man, folic acid deprivation leads to macrocytic anemia. The role played by folic acid as an essential nutrient to the bone marrow apparently occurs after the conversion of folic acid to leucovorin or folinic acid. In this conversion reduced diphosphopyridine nucleotide, vitamin B_{12} and ascorbic acid play an important function. Folinic acid is 5-formyl-5,6,7,8-tetrahydropteroyl-glutamic acid. In cases of macrocytic anemia refractory to folic acid, it may be valuable. For example, in the macrocytic anemia accompanying scurvy, Castle (1951) found folinic acid effective, but folic acid was effective only when administered with adequate ascorbic acid therapy. Folinic acid is also quite effective in combating the effects of folic acid antagonist (Krantz & Carr, 1967).

Folic acid is one of the important hematopoietic agents necessary for proper regeneration of the blood-forming elements and their functioning. Although the mechanism whereby PGA performs this vital role is not understood, much is known about the involvement of folic acid as a coenzyme in intermediary metabolic reactions in which one-carbon units are transferred. These reactions are important in inter-

conversions of various amino acids and in purine and pyrimidine synthesis. This role is in contrast to that of choline in furnishing and transferring so-called labile methyl groups in transmethylation reactions. The biosynthesis of purines and pyrimidines is ultimately linked with that of nucleotides and ribo- and deoxyribo-nucleic acids, functional elements of all cell (Osol & Hoover, 1970).

The concept of antivitamins and vitamin antagonists is exemplified in a particular aspect of folic acid metabolism. By virtue of its structural similarity, sulfanilamide competes with p-aminobenzoic acid in the biological synthesis of folic acid. The organism is thus deprived of needed folic acid. Sulfonamides act, therefore, as growth inhibitors of certain pathogenic organisms, a competitive antagonism which is responsible for the antibacterial action of sulfa drugs. Since mammals use preformed folic acid, sulfonamides do not disrupt the host metabolism (Osol & Hoover, 1970).

Numerous analogs of pteroylglutamic acid have been prepared which exhibit potent anti-folic acid activity. Several compounds, notably aminopterin (4-aminopteroyl-glutamic acid) and methotrexate (4-amino-N^{10}-methyl pteroyl-glutamic acid), compete with PGA in nucleic acid synthesis and have been used in the treatment of leukemia and other cancer (Osol & Hoover, 1970).

Folate deficiency can be diagnosed on clinical grounds

-i.e., the appearance of macrocytosis or an overt megaloblastic anemia or by one of several laboratory procedures. A deficiency is best diagnosed by the demonstration of low levels of the vitamin in serum or blood by microbiological assay, by the hematological response to a physiological dose of folic acid, 50 to 200 mcg. intramuscularly per day for 10 days or by the urinary excretion of formiminoglutamic acid (FIGLU) measured following the administration of histidine. Histidine is enzymatically converted to FIGLU and reacts with tetrahydrofolic acid to form fomiminotetrahydrofolic acid and glutamic acid. In folic acid deficiency this reaction can not occur, and excess FIGLU accumulates and is excreted in the urine. Both the FIGLU test and serum folate levels have proved to be unreliable in some cases of undisputed folic acid deficiency occurring in pregnancy (Meyers et al., 1972).

Folic acid deficiency results in megaloblastic haematopoiesis, glossitis, diarrhea and weight loss. A deficiency may occur in pregnancy, in the malabsorption syndrome, after continuous administration of large doses of pyrimethamine, and in epileptics on continuous anticonvulsive treatment. In this last condition the folic acid deficiency may lead to mental deterioration, which can be prevented by giving folic acid and vitamin B_{12}. Minor variations of folic acid metabolism may occur in patients with cardiac failure

and rheumatoid arthritis (B.P.C. 1973).

Folic acid and vitamin B_{12} separately and spectacularly stimulate the hematopoietic system, causing a return to normal of the blood picture. Vitamin B_{12} checks and often mitigates the neurologic symptoms. Under folic acid therapy these symptoms progress. It is evident, therefore, that folic acid may be considered possibly as only an adjunct to vitamin B_{12} in the treatment of pernicious anemia and never substitute for it. The metabolic activities of folic acid and vitamin B_{12} do not appear to be limited to hematopoiesis. They are likely involved in the synthesis of all body proteins. Perhaps their deficiency is manifested first in red cell formation, owing to the enormous amount of protein synthesis involved in red cell production (Krantz & Carr, 1967).

Many tropical diseases may alter the serum folate level and folic acid absorption. Very few studies on these aspects in patients with malaria, hookworm and opisthorchiasis infection have been reported.

Malaria is characterized clinically by fever, which is often periodic ; varying degrees of anemia ; spleenic enlargement ; and various syndromes resulting from the physiological and pathological involvement of certain organs including the brain, the liver and the kidney (Adams and Maegraith, 1953). In malaria anemia, there is polychromasia,

basophilia, poikilocytosis and anisocytosis. Normoblast and megaloblast may also appear in the blood in severe cases (Manson - Bahr, 1960). Morphological changes of megaloblastosis suggesting a deficiency of folic acid may be seen in patients with malaria infection (Manson - Bahr, 1960). Minimal to moderate megaloblastic changes observed in the aspirated bone marrow, with low serum folate levels have been reported recently in American soldiers with Plasmodium falciparum malaria in Vietnam (Strickland and Kostinas, 1970).

Hookworm infection is the gastro-intestinal tract infection of man. The main pathological effects of hookworm arise primarily from blood loss and the consequent production and development of anemia (Adams and Maegraith, 1953). Foster and Landsberg (1934), demonstrated that hookworm disease was caused by the continuous mechanical withdrawal of blood from the patient by the worms attached to the intestinal wall. In hookworm disease, the anemia was predominantly of the iron-deficiency type and was often associated with degenerative changes in the epithelial tissue e.g. koilonydria, angular stomatitis, glossitis, hypochlorhydria or achlorhydria with chronic gastritis or gastric mucosa atrophy in some hookworm patients (Davidson and Markson, 1955; Badenoch et al., 1957). Changes in the duodenal appearance characteristic of an ulcer and disorder of motor function, characterized by excessive peristaltic and segmental contraction with distortion of the

mucosal pattern, have also been reported in association with hookworm infection (Yenikomshian and Shehadi, 1943; Kause and Crilly, 1943; Hodes and Keefer, 1945). The morphological and functional changes of the small intestine may be the possible cause of the malabsorption of fat and nitrogen and other nutrients (Sheehy et al.,1962; Floch and Thanassen, 1963).

Opisthorchiasis is a common disease of the people resided in the Northeast Thailand. A recent survey showed that in fifteen provinces of Northeast Thailand 78.9 per cent of the people showed positive opisthorchis infection(Harinasuta, et al., 1965). Haematological and biochemical changes with signs of diarrhea loose stools occurred 3-4 times a day and dyspeptic flatulence have been reported (Harinasuta and Vajrasthira, 1960). It is possible but not proved, that the malfunction of the gastro-intestinal tract as described, can cause impaired absorption of nutrients in these patients.

No experiments in evaluating the normal serum folate levels in Thais have been recorded. This may be due to the fact that the method is complicated and time consuming for culture the L. casei for used in bioassay. The purposes of these studies are to determine the normal serum folate levels in Thai blood donors and compared with those of patients of tropical diseases. Furthermore folic acid absorption in these patients were also studied as well as in normal subjects.