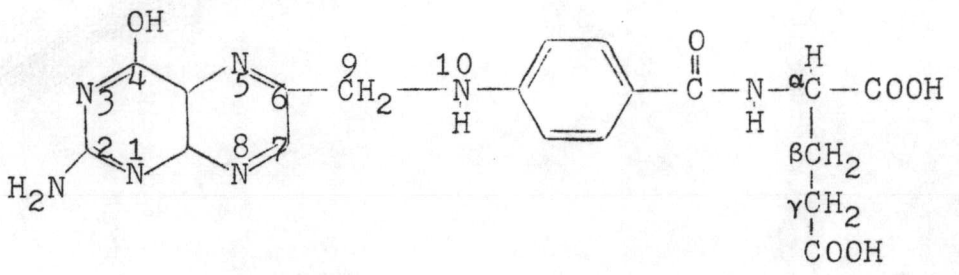
CHAPTER I

INTRODUCTION

Folic acid is one of the water soluble vitamins. It was first known as "Wills factor", because, Wills and her associates found that macrocytic anemia responded to the factor obtained from autolysed yeast or crude liver extracts (Wills et al., 1937). Nowaday it is known that Wills factor is the same substance as folic acid. Folic acid was firstly synthesised by a group of American chemists in 1945 (Angier et al., 1945). The term "folic acid" derived from Latin word "folium" because it was first found in spinach leaves (Mitchell et al., 1941).

Folic acid is essential for man and it is also necessary for the growth of Lactobacillus casei. It was therefore called the "L. casei factor" (Herbert, 1970). Other names of folic acid are folacin, vitamin Bc, vitamin M, vitamin B_{10}, vitamin B_{11} and factor U. The term "folic acid" is not only a generic name but sometimes it was used as a synonym of pteroylglutamic acid. Therefore, when the context does not make the meaning clear, it is preferable to use the term "folate" for the generic meaning (IUPAC-IUB Commission-1966).

The major portions of folic acid molecule are the pteridine moiety linked by a methylene bridge to para-aminobenzoic acid, which itself is joined in peptide-like linkage to glutamic acid (Herbert, 1970). The structural formula of folic acid is :-



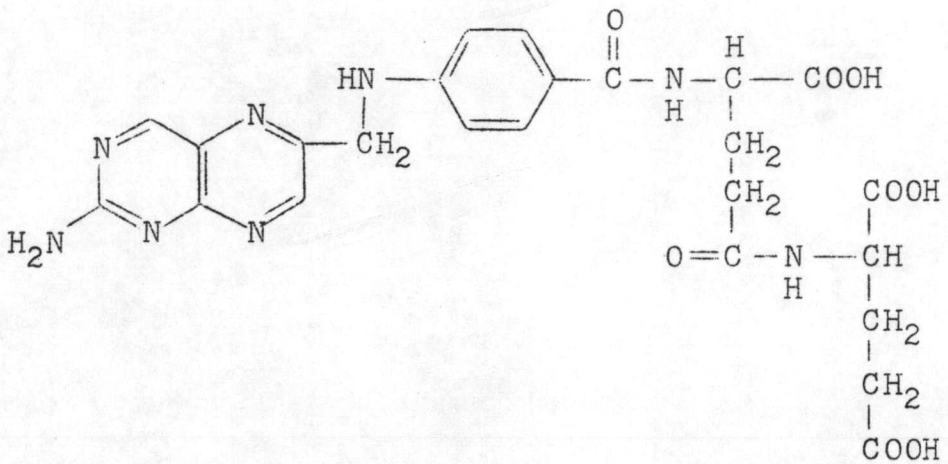
2-Amino-4-hydroxy-6-methylpterin — p-Aminobenzoic acid — Glutamic acid

Pteroic acid

Pteroylglutamic acid

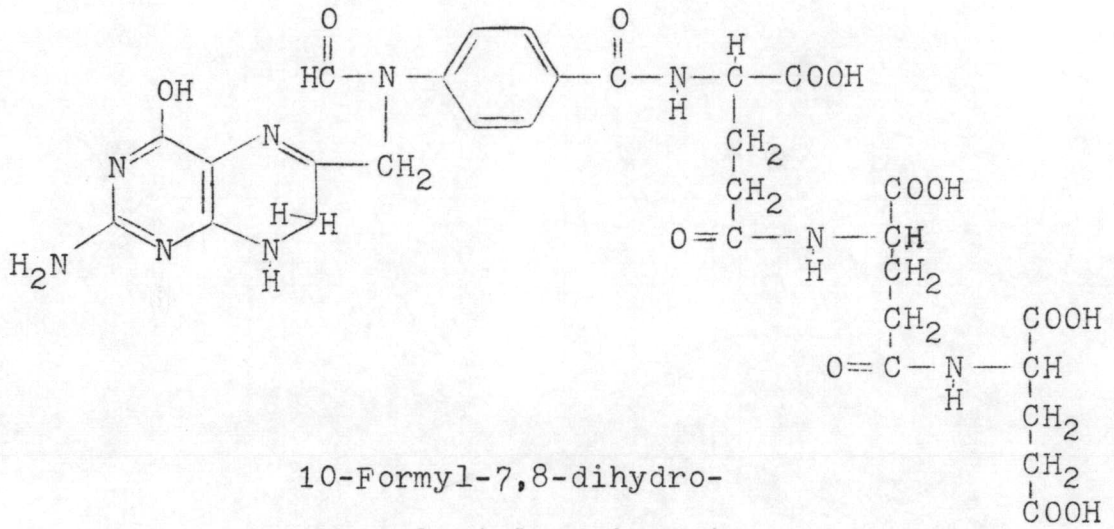
N-{p-([(2-Amino-4-hydroxy-6-pteridinyl)-methyl]amino)benzoyl}-glutamic acid

When pteroic acid conjugates with one molecule of l-glutamic acid, the compound should be called pteroyl-glutamic acid or pteroylmonoglutamic acid. If another glutamic acid residue is linked to the γ-carboxyl group by its amino group, it is called the pteroyldiglutamic acid :-

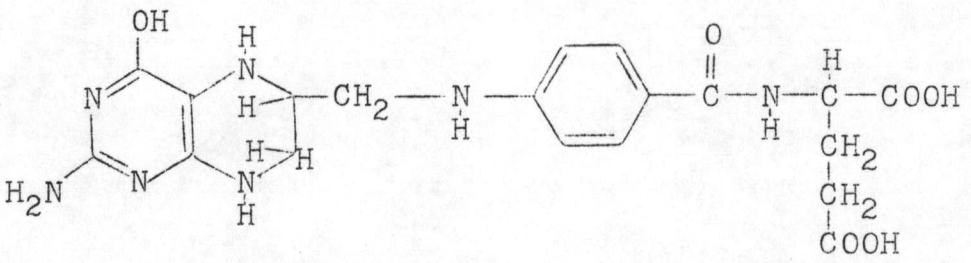


Pteroyldiglutamic acid

Up to seven additional molecules of glutamic acid may be attached to the γ-carboxyl group of pteroylglutamic acid. The conjugate with three glutamic acid molecules is known as pteroyltriglutamic acid, and the compounds with more than three glutamic acid residues are called pteroyl-polyglutamic acid (IUPAC-IUB Commission-1966). Reduced compounds also occur, with the words "dihydro" or "tetra-hydro" precede the word pteroylglutamic acid to indicate the position of the hydrogen atoms as shown in the following formulae :-



10-Formyl-7,8-dihydro-
pteroyltriglutamic acid
(10-HCO-7,8-H_2 PteGlu_3)



5,6,7,8-Tetrahydrofolic acid
(THF; FH_4; THFA)

Folic acid is a yellow crystalline substance and has no melting point. It darkens and chars from about 250° C. The light absorption of folic acid is in the range of 230 - 380 nm. Absorption maxima at pH 13 are : 256, 283 and 368

nm. It is very slightly soluble in cold water and methanol, appreciably less soluble in ethanol and butanol. This substance is insoluble in acetone, chloroform, ether and benzene and relatively soluble in acetic acid, phenol and pyridine. As the disodium salt is more soluble, so that folic acid solution is prepared by dissolving in alkali hydroxides and carbonates. Folic acid is susceptible to oxidative destruction by heating and is destroyed by acidity at pH below 4, but is relative stable at pH above 5, with no destruction in 1 hour at 100° C without oxidising agent (Herbert, 1973[a]). Its molecular weight is 441.41. When the molecule is destroyed it usually splits into pteridine and para-aminobenzoyl glutamate (Herbert, 1973[a]).

Folic acid exists in the polyglutamyl forms in most of the natural food (Hurdle et al., 1968). It is normally hydrolysed to free folic acid by a conjugase enzyme (γ-L-glutamyl carboxypeptidase) which is present in the small intestinal epithelium (Hepner et al., 1968). Free folic acid is actively absorbed from the upper small intestine, and the absorbed folate is reduced to dihydro and then tetra-hydrofolate by folate reductase and then being converted to 5-methyltetrahydrofolate. Folic acid in the blood, serum, liver and other tissues is believed to be in this form (Perry, 1971; Perry and Chanarin, 1968; Herbert, 1968[a]). Absorption of folic acid occurred against a gradient, suggesting an

active transport mechanism (Hepner et al., 1968). The absorbed folate is probably transported in the serum by binding to a protein and it is delivered to bone marrow cells, reticulocytes and other tissue cells (Corcino et al., 1971).

Folic acid itself is coenzymatically inactive. It must be converted into its active coenzyme form, tetrahydrofolic acid. This form of folic acid has an important role in one-carbon transfers in the body. One-carbon units exist in the body at three levels of oxidation, methyl (CH_3-), hydroxymethyl (CH_2OH-), and formate (O=CH-) or in the combination with nitrogen of formimino radicle (NH=CH-) of formiminoglutamic acid (Blakley, 1969; Girdwood, 1973). Metabolic reactions of one-carbon units transfers are important in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis and in purine and pyrimidine synthesis. The interchange and transfer mechanism are catalysed by enzymes, which require folate derivative as coenzymes. In certain steps of reaction, vitamin B_{12} is needed as coenzymes or cofactors. When vitamin B_{12} is deficient, most of the folate is trapped in the methyl-THF form which cannot be used in synthetic reaction, of which the most important for the folate metabolism in the body (Herbert and Zalusky, 1962). The believed actions of folates are shown in a diagram and numbered the steps that might be important in relation to the actions.

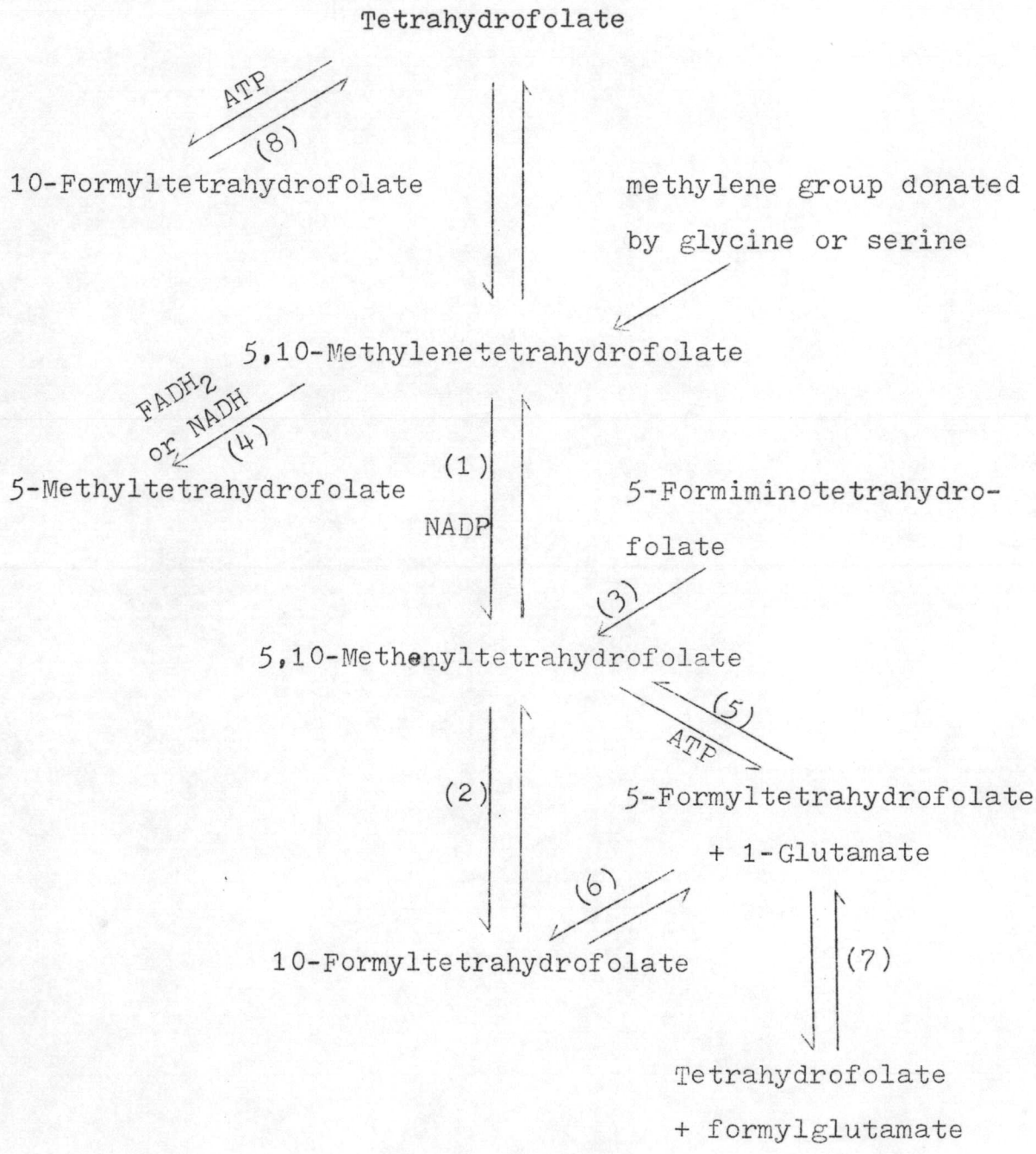


Fig.I Reactions involving 'tetrahydrofolates'

NADP = nicotinamide adenine dinucleotide phosphate;

NADPH = nicotinamide adenine dinucleotide phosphate, reduced;

NADH = nicotinamide adenine dinucleotide, reduced;

ATP = adenosine triphosphate;

$FADH_2$ = flavine adenine dinucleotide, reduced.

The steps numbered in Figure I are as follows :

1. The oxidation of 5,10-methylenetetrahydrofolate is catalysed by methylenetetrahydrofolate dehydrogenase.

2. The hydrolysis of 5,10-methenyltetrahydrofolate to 10-formyltetrahydrofolate is catalysed by 5,10-methenyltetrahydrofolate cyclohydrolase.

3. The conversion of 5-formiminotetrahydrofolate to 5,10-methenyltetrahydrofolate is catalysed by 5-formiminotetrahydrofolate cyclodeaminase.

4. The conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate is catalysed by 5,10-methylenetetrahydrofolate reductase. And in the reaction $FADH_2$, NADH or NADPH has been required.

5. The ATP dependent conversion of 5-formyltetrahydrofolate to 5,10-methenyltetrahydrofolate is catalysed by 5-formyltetrahydrocyclodehydrase.

6. The conversion of 5-formyltetrahydrofolate to 10-formyltetrahydrofolate is catalysed by 5-formyltetra-

hydrofolate : 10-formyltetrahydrofolate mutase.

7. It is possible that the formyl group of 5-formyltetrahydrofolate may be transformylated to 1-glutamate by an enzyme N-formylglutamate : tetrahydrofolate transformylase.

8. In the presence of ATP, tetrahydrofolate may be formylated to give 10-formyltetrahydrofolate. The enzyme has been named 10-formyltetrahydrofolate synthetase.

It is obvious that there must be much interconversion of tetrahydropteroylglutamic acid derivatives in biosynthetic reactions. It was suggested that one-carbon fragments are available from numerous substances, e.g., serine, glycine and histidine. Various enzymes are involved in the processes (Girdwood, 1973).

Megaloblastosis is the end product of deranged DNA synthesis phase of cell replication, usually due to the inadequate availability of vitamin B_{12} or folate. Instead of being in a resting phase, they are in process of attemping to double their DNA in order to devide. This is most striking in bone marrow cells, with the ineffective hematopoiesis resulting in peripheral blood pancytopenia (Herbert, 1970). Megaloblastic hematopoiesis causes by folate deficiency is indistinguishable from that of vitamin B_{12} deficiency, and diarrhea and weight loss are usually the prominent features of the syndrome (B.P.C. 1973).

Blood pancytopenia (anemia, leukopenia, thrombocytopenia) occurred with macroovalocytes and hypersegmented polymorphonuclear leukocytes. The first evidence of hematological damage is increased segmentation. This is followed by macroovalocytosis, with megaloblastic bone marrow and overt anemia rapidly developing. Deficiency of folate in infants may be a cause of mental retardation, which is the result of nerve damage. In adults, folate deficiency may lead to sleeplessness, irritability and forgetfulness (Herbert, 1968[a]).

The causes of folic acid deficiency in man are usually due to one or more of the five basic categories.

1. Inadequate nutrient ingestion is resulted from poor diet or heavily cooked food.

2. Inadequate absorption always appeared in malabsorption in tropical sprue. It is caused by degenerative change in the epithelial tissue e.g. angular stomatitis, achlorhydria with chronic gastritis or gastric mucosa atrophy or jejunitis (Symposium on Sprue, 1968; Klipstein, 1968). Malabsorption of folic acid may occurs in patients with malaria and hookworm infection (Areekul et al., 1974; 1975). The amount of absorbed folate is also limited by the efficiency of the deconjugating mechanism and conjugase inhibitors in some foods, such as yeast (Tamura et al., 1976; Tamura and Stokstad, 1973; Girdwood, 1973).

3. Inadequate utilization of folic acid may be due to vitamin B_{12} deficiency. Recent studies on human bone marrow cultures strongly suggests that the N^5-methyl tetrahydrofolic acid cannot be adequately utilized in the bone marrow of the vitamin B_{12} deficient subjects (Metz et al., 1968). The N^5-methyl form of folate tends to accumulate in the serum of the patients with vitamin B_{12} deficiency and the folate cannot be transported into maturing erythrocytes. The folate activity in the erythrocytes of vitamin B_{12} deficiency patients showed relatively low, as determined by the microbiological assay (Cooper and Lowenstein, 1964). Anticonvulsant drugs used in the treatment of epilepsy, oral contraceptives and folic acid antagonists (methotrexate, pyrimethamine, trimethoprim, triamterene etc.) also induce inadequate utilization of folate (Herbert, 1973; Shojania et al., 1968; 1969; Reynolds, 1967).

4. Increased requirement of folate is usually appeared in pregnant women and hyperthyroidism patients due to the increased metabolic activity. Pregnant women, especially in multiple and twin pregnancies need folate up to 400 µg daily. Folate deficiency in pregnant women is caused by recurrent drainage of residual folate stores, which are taken by the fetus at the expense of the mother. Deficiency of folate in pregnant women has been reported to be common in many areas, especially in developing

countries (Colman et al., 1974; Moscovitch and Cooper, 1973; Cooper et al., 1970; Chanarin et al., 1968; Lowenstein et al., 1966).

5. Increased excretion of folate may play a role in the tissue folate deficiency which eventually appears in vitamin B_{12} deficient patients. There was continued normal excretion of approximately 0.1 mg of folate in bile per 24 hours by a patient with vitamin B_{12} deficiency, suggesting that this may be a major route for depletion of folate stores in the patients with vitamin B_{12} deficiency (Herbert, 1970).

Folic acid deficiency may be detected by measuring serum or red cell folate levels by the microbiological assay. The normal human serum folate level is between 6 and 20 ng/ml. Folate deficiency is possible if the serum level is below 6 ng/ml and certain if below 3 ng/ml (Davidson et al., 1975). The minimal daily requirement (MDR) for folate is in the range of 50 µg for adults (Herbert, 1968[c]), but a daily intake of 200 µg of folate in food is recommended by FAO/WHO (1970) and up to 400 µg for women during pregnancy. For the uncomplicated folate deficiency therapy, a daily oral dose of folic acid of 100 µg is the minimal requirement.

Folic acid that is present in natural food sources has several different forms. Most of them are poly-glutamates. Foodstuffs with high folate content include

yeast, liver, kidney, grains, fruits, nuts and fresh green vegetables. Other good sources are asparagus, spinach, leaf lettuce, broadleaf endive and calabrese broccoli. Low folate content of food is present in white eggs, milk, meats and poultry (Butterworth, 1968; Herbert, 1968[a]). However, large proportions of food folate may be destroyed or leached out from 50 to 95 percent by cooking or canning procedure (Herbert, 1968[b]).

The methods used for assay of folic acid in body fluids and foodstuffs are microbiological techniques. Three organisms (Lactobacillus casei, Streptococcus faecalis, and Pediococcus cerevisiae) are commonly used. L. casei responds to all forms of folic acid with up to three glutamic acid residues which are called the "free folate". Streptococcus faecalis is used for determination of free folic acid and several tetrahydrofolic acid, especially for formyl derivatives. And the Pediococcus cerevisiae requires 5-formyl tetrahydropteroylglutamic acid (folinic acid or citrovorum) as nutritional factor, and it is also called Leuconostoc citrovorum. Most of folate in the foodstuffs are the conjugated form. If to demonstrate the pteroylpolyglutamates, they are treated with a conjugase (obtained from chicken pancreas or hog kidney) and assayed by the L. casei, we obtained the "total folate" (Grossowicz, et al., 1962). The subtraction of the free folate from the

total folate is called the "conjugate folate".

The purpose of this study is to determine both the free and conjugated folic acid content in some Thai foods. The foods studied were fish sauce (Nam-pla), soya-bean sauce, vinegar, milk, fruit juice and fresh fruits. These kinds of food samples were selected for this study because they are consumed very commonly without any processing.

Since there has been very few reports on folate content in Thai foods, this study of the free and conjugated folate content in food may give a further contribution to our knowledge of the availability of folate in some Thai foods.