CHAPTER I

INTRODUCTION

Folic acid, a water soluble vitamin, is essential for normal growth and proliferation of all human cells (Herbert, 1975; Herbert and Das, 1976). It derived its name from the Latin term of leaf (folium) because it was first isolated from spinach leaves (Mitchell, 1941; Mitchell et al., 1944). Several apparently unrelated substances had been isolated in various laboratories before it was realized that they had in common the same parent compound, pteroyl-L-glutamic acid (Osol and Hoover, 1975). Previously, they were called : Factor U (a chick growth factor), vitamin Bc (a chick antianemia factor), vitamin M (a nutritional factor for monkeys), liver and yeast Lactobacillus casei factors (bacterial growth factors) (Chanarin, 1969; Freed, 1966).

The Commission on Biochemical Nomenclature (International Union of Pure and Applied Chemistry - The International Union of Biochemistry Commission, 1966) had designated both "folic acid" and "folate" as generic terms for any member of this family. In 1972 the International Union of Nutritional Sciences Committee on Nomenclature decided to use the term "folacin" as the generic descriptor for folic acid and their related compounds (Osol and Hoover, 1975). The term "pteroylglutamic acid" was proposed by the IUPAC to call the pure

substance hitherto known as folic acid, folacin or vitamin Bc (Herbert, 1973 a).

The structural formula of the parent compound, pteroyl-glutamic acid and the major coenzymically active forms are illustrated in Figure 1 (Herbert and Das, 1976; Mowat et al.,1948; Stokstad, 1967). The major portions of the molecule are the pteridine moiety linked by a methylene bridge to the p-aminobenzoic acid, which is joined in amide linkage to glutamic acid. A series of compounds with several molecules of glutamic acid attached to the first glutamic acid radical in peptide linkage have been synthesized (Munson et al., 1976).

Pteroylglutamic acid (M.W. 441.42) crystallized from cold water, in which it is only slightly soluble, as yellow spear-shaped platelets (Freed, 1966). It dissolves in dilute solutions of alkali carbonates and hydroxides and in the dilute solution of hot sulfuric and hydrochloric acids but is insoluble in alcohol and the usual organic solvents (Mitchell and Williams, 1944). The vitamin is destroyed at below pH 4, but is relatively stable above pH 5 (Herbert, 1973a). Exposure of pure folic acid solutions to the sunlight results in its deterioration; however, it appears to be more stable in natural solutions (Freed, 1966).

Lists of some of the possible one-carbon adducts with THFA. Dashed lines indicate the N-5 or N-10 site of attachment of various one-carbon units for which THFA acts as a carrier.

	R	OXIDATION STATE
N^5 - formyl - THFA	-CHO	formate
N^{10} - formyl - THFA	-CHO	formate
N^5 - formimino - THFA	-CH = NH	formate
$N^{5,10}$ - methenyl - THFA	$\gg$CH	formate
$N^{5,10}$ - methylene - THFA	$>CH_2$	formaldehyde
N^5 - methyl - THFA	$-CH_3$	methanol

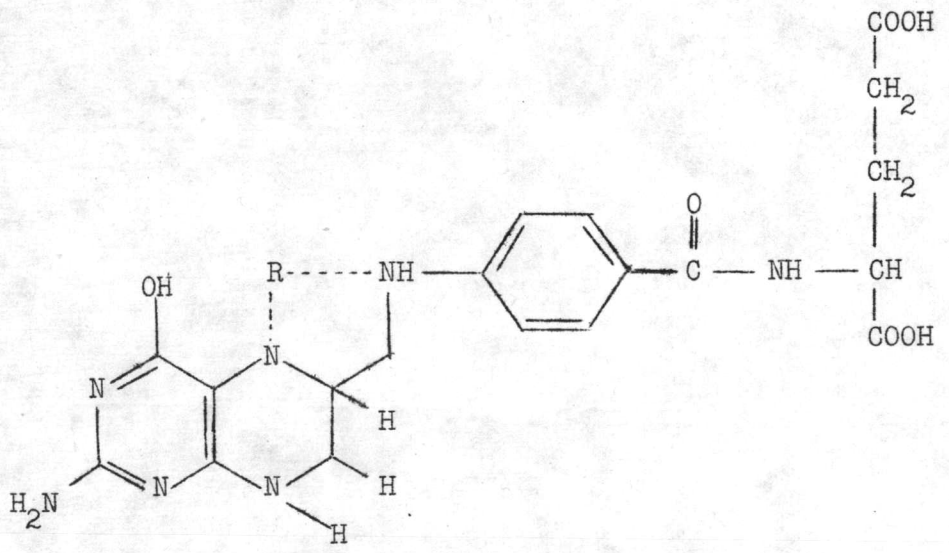

Figure I

Structures and nomenclature of folate derivatives.

Pteroylglutamic acid, the parent compound, is not biochemically active, but becomes so after reduction (in position 5, 6, 7 and 8) and substitution of one - carbon adducts on the N - 5 and/or N - 10 positions. The enzymic reduction of pteroylglutamic acid is catalyzed by dihydrofolate reductase, which is inhibited by various folate antagonists by binding to it, such as methotrexate, aminopterin, pyrimethamine and triamterene (Miller, 1968; Herbert and Das, 1976). Tetrahydrofolic acid (THFA) is an important carrier of one-carbon units which are required for the interconversion of various amino acids and for purine and pyrimidine synthesis (Dyke, 1965). Folic acid plays an essential part in all processes of cell division, particularly in haemopoiesis. Deficiency of folic acid causes a clinical disorder in man known as megaloblastic anemia which is characterized by the increased size and slowed DNA synthesis in all proliferating cells in the body (Herbert and Das, 1976). The primary defect is probably a disturbance of the doubling of DNA in the nucleus during cell division (Diem and Lentner, 1970).

Folates are existed in foods in nature as reduced polyglutamyl derivatives with single carbon moieties in various forms of reduction, substituted on nitrogens 5 and/or 10 (O'Broin, 1975). Compounds with one, two, three and seven glutamic acid groups have been isolated (Osol and Hoover, 1975). The latter three are known as conjugates. Microorganisms can utilize 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. Therefore, the folate in

food can be classified into two main groups according to its availability to Lactobacillus casei i. e. "free" folate, which is available to L. casei without pre-treatment with conjugase and "total" folate, consisting of "free" folate plus those polyglutamates that are only available to L. casei after treatment with conjugase (FAO/WHO Expert group, 1970).

Animals and man can utilize conjugates as a source of pteroylglutamic acid because enzyme conjugases which present in vegetable and mammalian tissues (including human intestine) liberate pteroyldiglutamates and pteroylmonoglutamates from the conjugates, making the folate available for absorption (Herbert, 1973a). About 90 per cent of monoglutamate or only about 50 per cent of hepta-glutamate, is absorbed by the entire length of the human small intestine (Grossowiez et al., 1972). However, there are some evidence that the jejunum is the primary site for active absorption for small quantities of this vitamin (Bernstein et al., 1970) and large amounts probably diffuse passively through the intestinal wall (Herbert, 1973a).

The absorbed folate which was bound to a protein, was transported in the blood to bone marrow cells, reticulocytes, and perhaps other cells. Methyl-folate seems to be the chief form of the vitamin in body tissues. About half of the folic acid stored in the body is in the liver. Some folate is excreted in the bile as well as in the urine (Mitchell et al., 1976).

Foodstuffs with the highest folate content per unit of dry weight were found to be yeast, liver and fresh green vegetables. Milk is a poor source of this vitamin (Lampert, 1975). Cow's milk and human milk have about the same folic acid activity, but goat's milk has appreciably less. Folate is highly susceptible to oxidative destruction, about 50 to 90 per cent of the folate content of foods may be destroyed by protracted cooking or other processing, such as canning (Chanarin, 1969). This is a major reason why folate deficiency is common in man.

Folate deficiency may be divided into primary (dietary) and secondary folic acid deficiency. For the latter, numerous possible causes have been cited, such as the inadequate absorption, inadequate utilization, increased requirement and increased excretion (Herbert, 1973).

The mininum daily requirement of folic acid for the healthy adult was suggested to be 50 µg of PGA (Pteroylglutamic acid) and 20 - 50 µg for infant (Herbert, 1962; Herbert, 1968; Sullivan, 1966; Velez, 1963). In 1970, FAO/WHO Expert Group recommended a daily dietary intake of free folate of 200 µg for adults, 40 µg for infants of 0-6 months, 60 µg for infants of 7-12 months, 100 µg for children, 400 µg during pregnancy and 300 µg during lactation (FAO/WHO Expert Group, 1970; Willoughby and Jewell, 1966).

All cells require folic acid for growth. The greatest need cells are the most active cells such as bone marrow. Lack of folic

acid results in a megaloblastic anemia. Since folic acid is not stored in significant quantities in the liver, the balance is always precarious, particularly in infants (Jolly, 1974). The incidence of folate-deficient megaloblastic anemia in infant usually occurs in the second 6 months of life which usually accompanies by the diarrhea, various infections and malnutrition (Herbert, 1975; Matoth et al., 1964b; Hoffbrand, 1970). The rapidly growing tissues and the intensive hematopoiesis associated with a steadily expanding blood volume cause an increased demand for folic acid in infants.

The quantitative assay of folacin in natural products is mainly by biological or microbiological methods. In the biological assay, the chicks are placed on a folic acid-free diet until they become anemic, after which folic acid supplements and the test material are administered. The degree of recovery is related to the quantity of reference folic acid fed. In microbiological method, the method is based on the fact that pteroylglutamic acid is a required growth factor for certain microorganisms. Using a basal medium complete all respects except folic acid, the growth responses of the organism are compared quantitatively in standard and unknown solutions (Freed, 1966). The organisms mostly used are :-

(1) Lactobacillus casei (Orla - Jensen) Hansen and Lessel which fully utilizes oxidized and reduced folates, regardless of single carbon attachment, with up to three glutamates.

(2) Streptococcus faecalis Andrewes and Horder which utilizes oxidized and reduced folates except methyl derivatives and also responds to pteroic acid.

(3) Pediococcus cerevisiae Balcke which utilizes only nonmethyl tetrahydrofolates (Dong and Oace, 1975).

As milk is the sole source of nutrition for infants in the first few months of life, so folic acid content of milk is very important. Previous reports indicated that milk contained only 1-2 µg of folate activity per litre (Hodson, 1949; Collins et al., 1951) but recent investigations using the improved assay procedure found folic acid up to 100 µg per litre of milk (Naiman and Oski, 1963; 1964; Luhby and Cooperman, 1963; Ghitis, 1966; Dong and Oace, 1975; Areekul et al., 1978; Sunthorntham, 1977; Vongyuthitham, 1977) However, these data are variable and the variations in folate content of milk are influenced by feeding practices and/or other environmental status (Dong and Oace, 1975).

Furthermore, folates in food are easily destroyed by storing, cooking and other processing methods (Mitchell et al., 1976). Hurdle (1968) found no loss of folate content in milk after boiling or pasteurizing but Ghitis (1966) found that 5 minutes' boiling of fresh milk and pasteurized milk resulted in loss of 50-60 per cent and 70-90 per cent of its folate respectively. Areekul et al., (1978) found that the pasteurized milk and sterilized milk lost free folic acid activity 37 per cent and 61 per cent respectively.

The purpose of this study is to determine the free folic acid, conjugated folic acid and total folic acid activity in human milk, fresh cow's milk, and other cow's milk preparations, such as

pasteurized milk, sterilized milk, powdered milk, condensed milk, sweetened condensed milk, butter and cheese. The effect of pasteurization and sterilization on the folic acid activity in fresh cow's milk was also performed.