AN ABSTRACT OF THE THESIS OF

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Title: STUDIES ON DIETARY FACTORS AFFECTING PLASMA AND RED CELL FOLATE

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Lorraine T. Miller

We investigated some dietary factors affecting plasma and red cell folate. From a population of 300 preschool children, we compared the folate intake of 49 children who had a plasma folate level below 5.6 mg per ml and 37 children with a level above 16.7 ng per ml. The total folate intake for the low folate group was 180 μg, ranging from 43 to 374 μg per day; and that for the high folate group was 198 μg, ranging from 77 to 504 μg. No significant correlation was found between dietary folate and plasma folate. There was no difference between the two groups of children in the quantities of folate that they received from the different food groups. All of the children received breads and cereals and milk. Not all of them ate fruits, vegetables and nuts. Twenty-five percent of the children with low plasma folate, however, received less than two-thirds of the Recommended Dietary Allowances (RDA) for folate, whereas 8 percent of those with high plasma folate failed to receive two-thirds of the RDA. Among the children taking vitamin supplements, 6 out of 7 children in the high

plasma folate group and only 1 out of 8 in the low plasma folate group took a supplement containing folate.

In another investigation, the utilization of folate from bread was determined in 9 men by measuring plasma and red cell folate. The three breads studied were white bread enriched with vitamin B_6 plus a supplement of pteroylglutamic acid (PGA) given orally (WB_6+PGA), whole wheat (WW) and white bread plus an oral supplement of vitamin B_6 (W). The total dietary folate when the subjects received WB_6+PGA, WW and W breads was 538, 440 and 303 μg, respectively. Approximately two-thirds of dietary folate came from bread. The subjects received each bread for one week in a three by three Latin Square design. The mean plasma folate was significantly higher ($p < 0.05$) when the subjects were on the WB_6+PGA bread (7.1 ± 1.5 ng per ml) than when they were on the W bread (5.8 ± 1.2 ng per ml). Five out of the 9 responded to WB_6+PGA and WW breads equally well and one utilized WW bread better than WB_6+PGA bread. No clear difference was observed between WW and W breads. From these results, no definite conclusions can be drawn regarding the utilization of folate in breads and cereals which are commonly consumed by preschool children. Red cell folate did not reflect the type of bread consumed or level of dietary folate.

Although an inverse relationship between plasma folate and plasma vitamin B_6 was frequently observed in the preschool children,

and a low plasma folate in patients receiving large amounts of vitamin B_6 was reported by Carson and Carre (Arch. Dis. Child. 44: 387. 1969), a transitory rise in plasma vitamin B_6 in 5 men following 2- and 10-mg loading doses of pyridoxine did not affect the level of plasma or red cell folate. These results do not explain the inverse relationship between plasma vitamin B_6 and folate which was observed in some preschool children.

Studies on Dietary Factors Affecting Plasma and Red Cell Folate

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INTRODUCTION

Inadequate dietary folate may cause low levels of serum folate and consequently, megaloblastic anemia (Cooper and Lowentein, 1964; Leevy et al., 1965; Herbert, 1967). Herbert (1962) reported that serum folate fell below 3 ng per ml in a subject who received 5 μg folate daily for approximately three weeks. Varadi and Elwis (1964) found that the megaloblastic anemia in 52 out of 76 patients was due to dietary folate deficiency. Forshaw, Moorhouse and Harwood (1964) reported that the mean intake of folate in their patients with megaloblastic anemia was approximately 22 μg per day.

Low serum folate levels, however, may not necessarily be associated with megaloblastic anemia or with any other abnormal hematological and biochemical changes (Hoffbrand, Newcombe and Mollin, 1966). A normal healthy person may also have a low plasma folate. Hall et al. (1974) observed a wide variation in plasma folate among 106 subjects. Almost half of their subjects who had low plasma folate levels (1.8-4.9 ng per ml) had no hematological evidence of folate deficiency. The authors suggested that the low plasma folate levels may be a reflection of dietary intake.

Recently the plasma folate levels were measured in 300 Oregon preschool children. It was observed that 49 (16 percent) had low

plasma folate levels. In order to determine the effect of dietary folate on the level of plasma, the dietary intake of folate was compared in the 49 children with low plasma folate (1.5-5.6 ng per ml) and in 37 (13 percent) who had high plasma folate levels (16.7-42.9 ng per ml). Among these 300 preschool children, low levels of plasma folate were observed also in some children with high levels of plasma vitamin B_6. Bread, although not the major source of dietary folate, was commonly consumed by the children.

Thus, the purposes of the research reported in this thesis were to 1) examine the dietary intake of folate by preschool children who have either a low or high plasma folate, 2) measure utilization of folate from bread and 3) observe the relationship between plasma folate and plasma vitamin B_6 in men receiving loading doses of pyridoxine.

REVIEW OF LITERATURE

Chemical Nature of Folic Acid

Folic acid was first recognized as an anti-anemia factor by Wills (1931). The term "folic acid" was introduced later by Mitchell, Snell, and Williams (1941) to describe a factor which was extracted from spinach leaves. This substance was identified as pteroylglutamic acid (PGA). PGA, also the synthetic form of the vitamin, is composed of pteridine linked to para-aminobenzoic acid and L-glutamic acid (Figure 1).

PGA is readily absorbed and effectively utilized by the human body (Jandle and Lear, 1956). However, folic acid in foods is primarily a conjugated form, pteroylpolyglutamate. It is structurally related to PGA but may have up to ten residues of glutamic acid attached to the glutamic acid portion of PGA (Figure 1) in a gamma-peptide linkage (Thorne and Leonard, 1964). Before pteroylpolyglutamate can be utilized by the body, these glutamic acid residues are removed by conjugase to form pteroylmonoglutamate (Figure 2). Pteroylmonoglutamate can then be reduced to the enzymatically active form, tetrahydrofolic acid (THFA), which participates in several metabolic reactions (Stokstad and Koch, 1967). These reactions are summarized in Figure 2.

The function of folic acid as a coenzyme in the metabolic

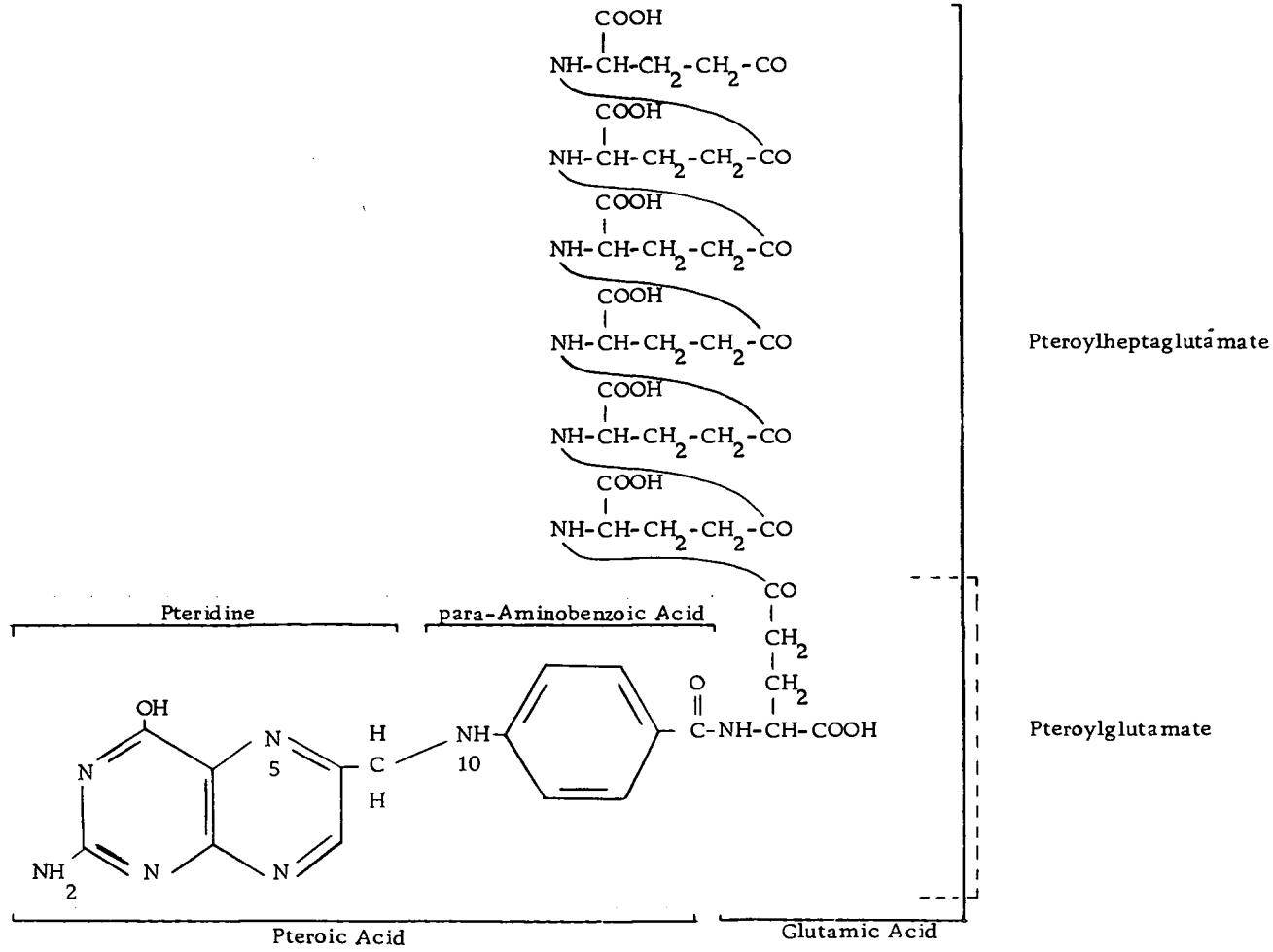

Figure 1. Structure of pteroylglutamate (PGA) and pteroylpolyglutamate.

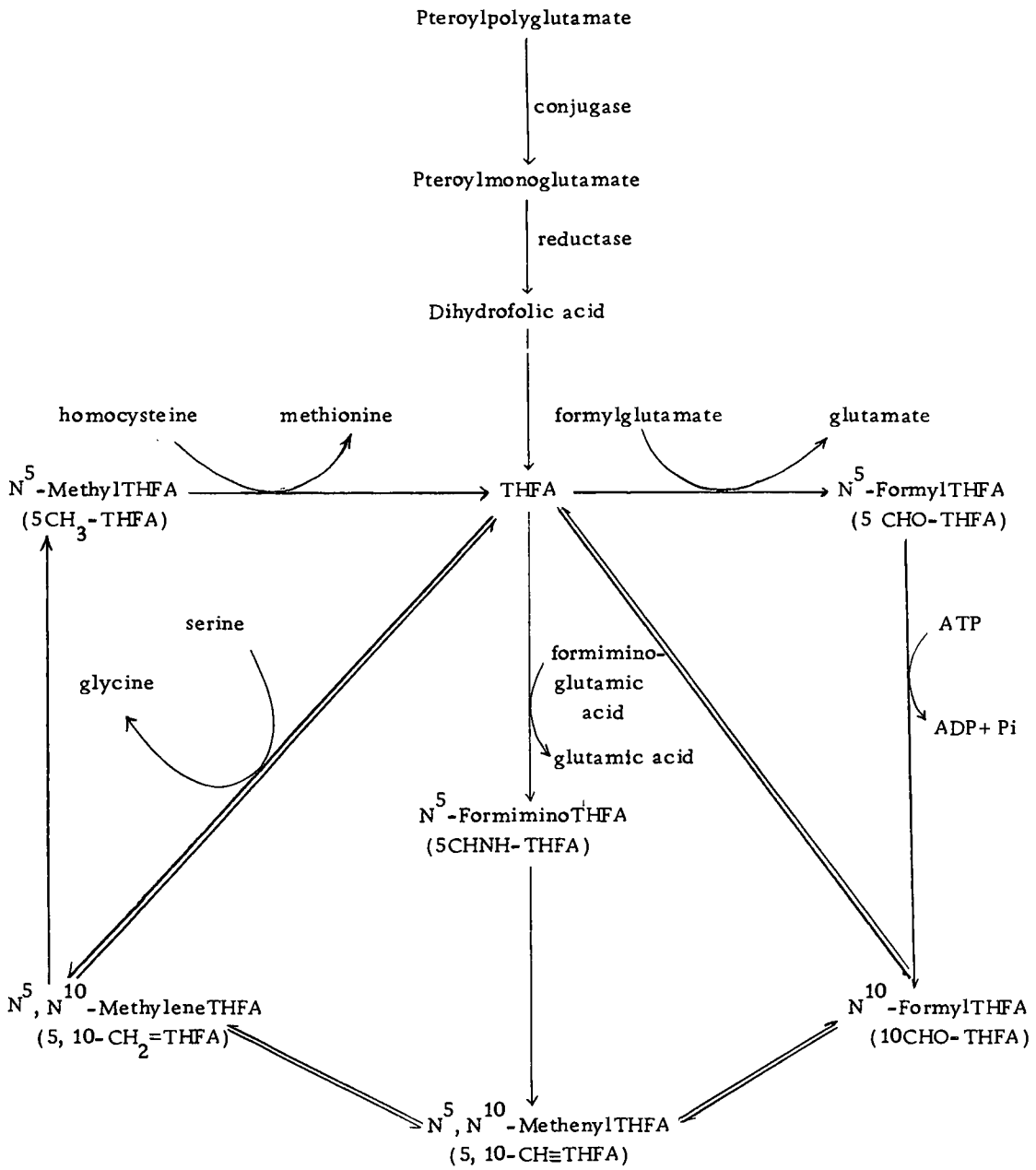

Figure 2. Metabolic reactions of folic acid. Adapted from Stokstad and Koch (1967).

transfer of single carbon units has been reviewed by Vitale (1966), Stokstad and Koch (1967), and Rosenberg and Godwin (1971). These single carbon units (formyl, methenyl, formimino, and methyl groups) may be attached to the N^5 or N^{10} position or form a methylene bridge between N^5 and N^{10} of THFA (see Figure 1). These metabolically active forms of folic acid are listed in Table 1 and also in Figure 2.

These single carbon units of folic acid are involved in purine and pyrimidine (thymine) synthesis. The introduction of the 2-carbon of the purine ring comes from N^{10}-formylTHFA, whereas the 8-carbon is introduced by N^5-N^{10} methenylTHFA. Folic acid is also involved in the biosynthesis of thymidylic acid, a pyrimidine. MethyleneTHFA serves as a single carbon source for methylation of deoxyuridylic acid to form deoxythymidylic acid.

The requirement for folic acid depends on the cell turnover rate. Hence, symptoms of folate deficiency usually appear in rapidly dividing tissues such as red and white blood cells and the gastrointestinal tract. Before a cell can divide, it must precisely double its deoxyribonucleic acid (DNA) content. Thus, a deficiency of folate may result in impaired cellular growth since cell replication depends on DNA synthesis. Morphologically, the nuclear pattern of the red blood cell is altered and the size of the red blood cell increases. The abnormal megaloblast results from prolonged cell divisions. The maturation of granulocytes and megakaryocytes is also altered.

Table 1. Six metabolically active forms of folate.

Form	Carbon Unit
N^5-Formyl THFA	-CHO
N^{10}-Formyl THFA	-CHO
N^5, N^{10}-Methenyl THFA	≡CH
N^5-Formimino THFA	-CH=NH
N^5, N^{10}-Methylene THFA	$=CH_2$
N^5-Methyl THFA	$-CH_3$

Consequently, giant metamyelocytes appear in the bone marrow, and hypersegmented polymorphonuclear leukocytes in the blood (Wintrobe et al., 1970).

In folate deficiency, changes occur in the lining of the intestinal tract, resulting in the impaired absorption. Klipstein, Lipton and Schenk (1973) reported that folate deficiency depresses the concentration of intestinal mucosal folate, and produces certain structural changes of the proximal small intestine.

Biochemical and Hematological Assessment of Folate Status

Herbert (1962) reported the metabolic and hematological changes that occurred in a healthy male physician who received a diet containing 5 μg of folate. After 3 weeks of deprivation the subject's serum folate fell below 3 ng per ml. In response to a test dose of L-histidine, after 14 weeks of folate deficiency the subject excreted large amounts of formiminoglutamic acid (FIGLU), a metabolite of histidine. His level of red cell folate decreased gradually after approximately 120 days of folate deprivation. When the subject was given a supplement of the vitamin, his serum folate level returned to normal. Herbert summarized the sequence of biochemical and hematological events occurring in folate deprivation: reduction in serum folic acid, production of polymorphonuclear leukocytes, excretion of high urinary FIGLU after a histidine loading test, reduction in red blood cell folate,

production of macroovalocytosis, and finally development of megaloblastic marrow anemia.

Herbert (1966) later determined the serum folate in 4,500 individuals (assayed with Lactobacillus casei). He suggested the following criteria for using serum folate values to judge folate status.

	Folate values (ng per ml)
Low	< 3
Suggestive of deficiency	3- 4.9
Borderline low	5- 6.9
Normal	7-15.9
Borderline high	16-24.9
High	>25

Herbert proposed that serum folate values suggestive of deficiency are between 3-4.9 ng per ml. Levels below 3 ng per ml indicate folate deficiency.

Low serum folate values do not necessarily mean, however, that the tissues are depleted of the vitamin. Hall et al. (1974), who studied the plasma folate levels of 106 healthy persons, found much variation in plasma folate among their subjects. Approximately 30 percent of their subjects had subnormal plasma folate (3-4.9 ng per ml), and 12 percent had levels below 3 ng per ml. All of these subjects had normal hematological values.

Age may also play a significant factor in the level of plasma

folate. As people become older their concentration of plasma folate decreases (Daniel and Bennett, 1975). Few studies have been done on red cell folate. Red cell folate levels between 160-600 ng per ml are considered normal (O'Neal, Johnson and Schaefer, 1970; Beck, 1973).

Values for normal serum folate in adults differ from laboratory to laboratory (Table 2). Information on normal values of serum folate in children is limited. The normal range of plasma folate from one group of presumably healthy subjects may not apply to another population of healthy subjects examined by the same laboratory. Hall et al. (1974) measured the plasma folate levels among healthy groups of black migrant workers, urban blacks, white laboratory personnel, and white nursing students. Approximately 31 percent of the 29 white and 30 percent of the 77 black subjects had plasma folate values between 3.0-4.9 ng per ml. Seventeen percent of blacks and none of whites had plasma folate levels below 3 ng per ml. None of these subjects had symptoms of folate deficiency. Low levels of serum folate were found also in the studies (Table 2) of Spray (1964, 1969); Chanarin, Kyle and Stacey (1972); and Daniel and Bennett (1975). Hence serum folate values may be misleading if compared to an inappropriate reference group. To set up normal values, Hall and his co-workers suggested that it is necessary to include all segments of a population.

In addition to serum, red cell folate has been used to determine

Table 2. The plasma and red cell folate concentration in normal subjects from different laboratories.

Reference	Age Range (yrs)	Subject (no.)	Serum Folate (ng per ml)		Red Cell Folate (ng per ml)	
			Range	Mean	Range	Mean
Spray, 1964		94	2.1-28.0	7.8		
Herbert, 1966		2,161	7.0-15.9			
Hoffbrand et al., 1966	19-42	40	6.0-18.6	9.7	166-640	316
Spray, 1969	16-63	81	2.1-13.0	4.9	80-470	192
Chanarin et al., 1972		69	4.0-28.0	9.8	152-568	299
Liu, 1974	21-56	35	7.2-21.3	10.6	161-710	416
Hall et al., 1974		106	1.8-17.0	5.6		272
Daniel and Bennett, 1975	12-17	451	2.0-10.0			

the folate status in human subjects. When either PGA or conjugated folate was given orally, the rise in plasma folate was detected as early as 15 minutes, reaching its peak between 30 to 60 minutes. Plasma folate declined rapidly afterwards (Perry and Chanarin, 1968; Rosenberg, Streiff and Godwin, 1969; Grossowicz and Izak, 1972). A longer time was required to produce a change in the folate concentration of red cells than in plasma. Red cell folate rose slowly, reaching a plateau, when an oral dose of the vitamin was given daily for two months.

Since low serum folate may indicate a temporary reduction in the intake of the vitamin or an increase in the requirement, serum folate values are of limited value for indicating folate stores. Red cell folate is probably a better indicator of folate status than the plasma folate (Chanarin et al., 1968). Low intake of the vitamin was followed by a rapid fall of serum folic acid within a few weeks, while more time was required for a reduction of red cell folate.

High serum and low red cell folate levels may be associated with vitamin B_{12} deficiency (Cooper and Lowenstein, 1964; Saraya, Choudhry and Ghai, 1973). Armstrong et al. (1974) reported that vegetarians have higher mean serum folate values than non-vegetarians. Vitamin B_{12} deficiency, in part, may be responsible for the elevation of serum folate in strict vegetarians (vegans). In ova-lacto or lacto vegetarians supernormal levels of serum folate more likely

reflect the high consumption of vegetables that are rich in the vitamin.

Folate and Vitamin B_6

The interconversion of the amino acids, serine and glycine, requires both reduced folic acid (THFA) and vitamin B_6.

$$\text{Serine} + \text{THFA} \underset{\text{serine transhydroxymethylase}}{\overset{\text{vitamin } B_6}{\rightleftharpoons}} N^5,N^{10}\text{-Methylene THFA} + \text{Glycine}$$

The beta-carbon of serine serves as a source of a single carbon unit for THFA. THFA, the active form of folic acid, reacts with serine to form N^5, N^{10}-methyleneTHFA and glycine. The enzyme, serine transhydroxymethylase, which catalyzes this reaction is vitamin B_6-dependent (Stokstad and Koch, 1967). Since this enzyme requires vitamin B_6, it is possible that a defect in this enzyme will increase the requirement for vitamin B_6 to pharmacologic levels. In the absence of sufficient vitamin B_6, folic acid metabolism may be impaired (Beck, 1973).

Patients with homocystinuria, however, who were given supplementary vitamin B_6, exhibited low serum folate values (Carson and Carre, 1969). No explanation was offered for this observation except that a large amount of vitamin B_6 may influence the metabolism of folate. Miller (1977) observed that preschool children with high levels

of serum vitamin B_6 frequently had low levels of serum folate.

Assay Methods for Foods and Blood

A radioisotopic method for measuring folic acid in plasma and red cells has been developed recently (Waxman, Schreiber and Herbert, 1971). However, microbiological assays are still commonly used to measure folic acid. L. casei is the organism most widely used to measure folate in plasma, red cells and foods.

L. casei responds to the N^5-methylTHFA, the major form of folate in plasma and red cells (Usdin, 1959; Herbert, Larabee and Buchanan, 1962), and utilizes mon-, di- and tri-glutamyl folic acid. This organism responds to folic acid with four to seven glutamates to a very limited extent. Thus food folate, which is in polyglutamic form, can not be estimated unless foods are treated with conjugase before assay.

A superior stabilizing agent for folic acid is ascorbic acid (O'Broin et al., 1975), which prevents the oxidation of THFA (Stokes et al., 1975). When ascorbic acid is used in the assay procedure, there is a ten-fold increase in the folate value of some foods. The values for food folate in most of the published tables were determined without the addition of ascorbic acid to the assay medium, and should, therefore, not be used for estimating food folate. Unfortunately, the values in the most extensive reference on food folate (Toepfer et al.,

1951) were obtained by procedure that did not utilize ascorbic acid in the assay medium. However, the information in this reference is valuable for suggesting foods rich or poor in the vitamin.

There are limited data on the folate content of foods with ascorbic acid protection are by Hurdle, Barton and Searles (1968), Streiff (1971), Butterfield and Calloway (1972), Hoppner et al. (1972), Dong and Oace (1973), Tamura and Stokstad (1973), and Dong and Oace (1975). These authors have reported both free and total folate.

Dietary Forms of Folate

Many food products contain both free (monoglutamate) and total (polyglutamate) folate. Hexa- and hepta-glutamate probably are the most abundant forms in foods. Pteroylheptaglutamate is the primary form of folate in yeast (Pfiffner, Calkins and O'Dell, 1945), and pteroylpentaglutamate in liver (Houlihan and Scotch, 1972). The predominant form in wheat, vegetables, meat, and milk products is pteroylpolyglutamate. It has been inferred that similar conjugates may also predominate in other plant and animal tissues, as evidenced by the increase in folic acid activity after conjugase treatment.

The distribution of folic acid-active compounds in individual foods has been studied by using column chromatography (Noronha and Silverman, 1962; Santini, Brewster, and Butterworth, 1964). Formyl derivatives constitute the most abundant form of folate in vegetables;

approximately 90 percent of folate in vegetables are either 5- or 10-formyl derivatives. Methylfolate compounds are found in yeast, liver, beans, and black-eyed peas. The major portion of liver folate is made up polyglutamates of 5-methylTHFA. Small amounts of di- and tri-glutamates are also present.

Composite samples of typical American diets have been analyzed. The free folate content was 50 μg per day and the total folate content after conjugase treatment was about 180 μg per day (Butterworth, Santini and Frommeyer, 1963). Thus, the content of folate in food can not be determined unless it is treated with conjugase before assay.

Dietary Sources of Folate

Folates are widely distributed in foods. Liver and yeast are the richest sources of folate, 100-gram portions (fresh weight) containing 0.5 mg (Hurdle et al., 1968) and 3 mg (Butterfield and Galloway, 1972), respectively. Significant amounts of folate are also present in dried beans and nuts as well as in fresh green leafy vegetable, e.g. spinach, broccoli, and lettuce. Fresh fruit, milk, poultry and egg white are relatively poor in folate (Toepfer et al., 1951; Santini et al., 1962).

Orange juice and whole wheat products are also good sources of food folate: orange juice for its high content of monoglutamate

(Streiff, 1971), and whole wheat products for the quantities that are consumed. Thus, the amount of folate contributed by these two products to the diet can not be neglected. Foods made with whole wheat are richer in folate than those made with white flour. Folate content in whole wheat bread is 54 μg per 100 g, while white bread contains 35.5 μg per 100 g. Among the wheat fractions, the germ is higher in folate than the bran (Butterfield and Calloway, 1972).

Folate is heat labile and light sensitive. Up to 90 percent of the folate content of foods may be lost during processing and cooking, especially boiling (Herbert, 1963; Hurdle et al., 1968). Free folate is more sensitive to destruction by heat than is total folate (Perloff and Butrum, 1977).

Recommended Dietary Allowances

The normal intake of folate is not well established because data on the folic acid content of foods are limited. In addition, some confusion exists with respect to the minimum requirements for this vitamin. The requirement for folate depends on metabolic and cell turnover rates. It is accepted that the minimum requirement for folate as PGA is approximately 50 μg (Herbert, 1968). A daily allowance of 400 μg of folate has been recommended for adolescents, men and women. The Recommended Dietary Allowances (RDA) for infants under one year of age is 50 μg; those for children 1 to 3, 4 to 6, and

7 to 10 years old are 100, 200, and 300 μg, respectively. The RDA for pregnant and lactating women is 800 and 600 μg, respectively (Food and Nutrition Board of the National Research Council, 1974).

Availability of Dietary Folate in Man

Food folate is not identical with crystalline PGA or with crystalline pteroylheptaglutamate. Folate is probably best absorbed in its monoglutamate form. Jandl and Lear (1956), who measured the urinary excretion of folate as an index of folate absorption, found that 95 percent of a 1.5-mg dose of PGA was absorbed. Synthetic pteroylheptaglutamate is absorbed nearly as well as synthetic pteroylmonoglutamate (Swendseid, Bird and Brown, 1947). Perry and Chanarin (1968) gave five subjects oral doses of PGA and yeast pteroylheptaglutamate that were equivalent to 20 μg of PGA per kg body weight. The yeast pteroylheptaglutamate was utilized and absorbed about one-third as well as that of PGA, as judged by comparing the serum folate levels, red cell folate, and urinary excretion of folate following the oral administration of the vitamin.

Some foods contain inhibitors which may limit the availability of dietary polyglutamic folate. According to Swendseid et al. (1947) heptaglutamates from crude preparations of yeast were not utilized completely, which may be due to the presence of a conjugase inhibitor in the yeast extracts. Whether or not such inhibitors also exist in

other folate-containing foods is not clear. Luther et al. (1965) suggested that some foods may contain substances, such as proteins or cellulose, which may interfere with absorption through binding of folate.

The absorption of dietary folate may vary from time to time, even in the same person, because of changes in the concentration of intestinal conjugase and intestinal pH (Tamura and Stokstad, 1973).

Orange juice, which contains large amounts of monoglutamate, is recommended as an excellent source of dietary folate. A controversy exists, however, whether or not the folate in orange juice is available to the human. Tamura and Stokstad (1973) studied the absorption of folate by measuring the urinary concentration of folate in subjects who were saturated with the vitamin. According to these authors, only 31 percent of the folate in orange juice is available. Tamura and Stokstad suggested that certain factor(s) in orange juice may inhibit the absorption of monoglutamate. On the contrary, Nelson, Streiff and Cerda (1974), by using intra luminal perfusion of the human small intestine, demonstrated no difference between the absorption of folate in orange juice and that of PGA in solution.

The availability of folate in bananas, lima beans, liver and brewers' yeast are relatively high, from 50 to 96 percent. The availability of the vitamin in romaine, lettuce, egg yolk, cabbage, defatted soybeans is relatively poor, from 25 to 47 percent. The

availability of folate in wheat germ, which is high in the vitamin, is only 30 percent (Tamura and Stokstad, 1973).

Several investigations on fortifying certain staple foods with folate have been conducted recently. Colman et al. (1975a) compared the utilization of folate in cereal and bread fortified with different levels of PGA. They observed increases in serum folate values which were about half of those observed in the ones receiving PGA solution. Fortified bread, however, produced an increment about one-third that of PGA solution. Colman et al. (1975b) and Margo et al. (1975) also showed that staple foods fortified with folate increased folate stores and prevented deficiency of the vitamin in pregnant women.

It is not clear whether the fiber in bread can also form insoluble complexes with folate in the intestinal tract, and consequently reduce the availability. Russell, Ismail-Beigi and Reinhold (1976) conducted sequential folate absorption tests using tritiated pteroylmonoglutamate by gradually increasing the dietary fiber content. No interference with folate absorption was found. In addition, they also demonstrated no formation of insoluble complexes in vitro between bread fiber and folic acid.

MATERIALS AND METHODS

Study I. The Effect of Dietary Folate on Folate Status in Oregon Preschool Children

It has been suggested that plasma folate reflects the intake of the vitamin (Herbert, 1962; Forshaw et al., 1964). The objective of this study was to investigate the effect of dietary folate in preschool children who have a low or high level of plasma folate.

Subjects

This study is a part of the investigation on nutritional health, food intake, and socio-environmental profiles in Oregon preschool children. Participating in this study were 300 healthy preschool children who were from 11 of Oregon's 36 counties. The subjects attended the Well Child and Multiphasic Screening Clinics set up by the individual counties in collaboration with the State Health Division and the Cooperative Extension Service. This investigation was submitted to the Oregon State University Human Subject Committee by Ms. Eva Benson. It was approved by this committee on March 23, 1973.

Among these 300 children, 49 children (16 percent) were observed to have low plasma folate values, ranging from 1.5 to 5.6 ng per ml, and 37 children (12 percent) were observed to have high

plasma folate levels, ranging from 16.7 to 42.9 ng per ml. Children from these two groups were chosen for studying the effect of dietary folate on the level of this vitamin in plasma. The following table gives the age range and number of girls and boys in this sub-study.

	Age Range months	Boys no.	Girls no.	Vitamin Supplement no.
Low plasma folate group (1.5-5.6 ng per ml)	30-103	27	22	8
High plasma folate group (16.7-42.9 ng per ml)	12- 87	21	16	7

Blood Drawing

Venous blood was drawn by a medical technician from non-fasting subjects and was transferred to vacutainer tubes containing heparin. The blood was used for the determination of erythrocyte transaminases, hemoglobin, hematocrit, plasma vitamin B_6, vitamin A and folic acid. Reported here are the values of plasma folate,[1] hemoglobin and hematocrit as well as the folate intake of these 86 children.

[1] Assayed by Ms. Eva Benson and Ms. Elena Brineman using L. casei as assay organism. The method will be described in this section under "Assay Methods."

Dietary Analysis

Twenty-four hour recall or three-day dietary records, which were obtained by a nutritionist employed by the Foods and Nutrition Department, were used to evaluate the children's total folate intake. The children's intake of dietary folate was calculated from data which were obtained from food treated with added ascorbate (Herbert, 1963; Hurdle et al., 1968; Streiff, 1971; Hoppner et al., 1972; Butterfield and Calloway, 1972; Hoppner, Lampi and Perrin, 1973; Dong and Oace, 1973). Most of these food tables gave the folate value for raw foods. Thus, whenever the folate value from a cooked food could not be obtained, the value for the raw food was used for the calculation. In order to determine the major sources of dietary folate, the foods were divided into food groups.

Study II. The Utilization of Folate from Bread

The nutritional quality of foods with respect to folate is frequently judged from food composition data, assuming that all of the folate is completely available to the body. Recent research by Tamura and Stokstad (1973), however, shows that the folate in foods is not always completely available. Thus, the aim of this study was to determine the utilization of folate from three breads: whole wheat, white plus an oral supplement of vitamin B_6 and white enriched with

vitamin B_6 plus an oral supplement of PGA. The preschool children, who were discussed in another section of this thesis, obtained approximately 15 percent of their dietary folate from bread and cereal products. This study was done in conjunction with Anne Perera (1977) and Dianne Peffers (1977) who studied the bioavailability of vitamin B_6 in wheat bread.

Subjects

Nine apparently healthy men, ranging in age from 21 to 33 years, served as subjects. They were students at Oregon State University and maintained their normal class schedule and physical activity throughout the study. They were free from any known metabolic illness and were taking no drugs, medications or vitamin supplements. The vital statistics of the subjects are listed in Table 3. This investigation has been approved by the Human Subjects Committee at Oregon State University on December 5, 1975.

Procedure

This study was conducted over a span of four weeks, starting on April 27, 1976 and ending on May 23, 1976. The subjects received all of their meals in the metabolic unit of the Foods and Nutrition Department at Oregon State University.

In order for the subjects to adapt to the large amount of bread

Table 3. The vital statistics of the subjects and their bread assignments.

Subject no.	Age (yrs)	Height (cm)	Weight (kg)	Bread Assignments Period I	Period II	Period III
1	32	180	89	W[a]	WB_6+PGA[b]	WW[c]
2	29	170	96	WB_6+PGA	W	WW
3	21	178	61	WB_6+PGA	WW	W
4	27	183	66	WW	W	WB_6+PGA
5	32	168	52	WB_6+PGA	WW	W
6	25	180	75	W	WW	WB_6+PGA
7	23	178	66	W	WB_6+PGA	WW
8	23	185	86	WW	WB_6+PGA	W
9	27	168	64	WW	W	WB_6+PGA

[a] White bread - subject received white bread plus an oral supplement of 0.84 mg pyridoxine·HCL which was divided evenly among the three meals.

[b] White bread enriched with vitamin B_6+PGA - subject received white bread containing 0.20 mg of pyridoxine (PIN) per 100 g plus an oral supplement of 200 µg of PGA which was divided evenly among the three meals.

[c] Whole wheat bread.

(570-600 g), the first week of the study was an adjustment period. During this time the amount of bread consumed by the subjects was gradually increased; the details of this diet are shown in Table 4. At the end of the adjustment period, the study was divided into three seven-day periods: periods I, II and III. Two three by three Latin Square designs were used to assign breads to the subjects.

Bread as the major source of protein, vitamin B_6, and folate in the meals was emphasized in this study. The three types of bread for this study were whole wheat (WW) bread, white bread enriched with vitamin B_6 plus a PGA supplement given orally (WB_6+PGA) and white bread with an oral vitamin B_6 supplement but no PGA (W). The subjects received each bread for a week. Approximately two-thirds of dietary folate came from bread when they received the WW bread or WB_6+PGA bread. The order in which the subjects received these breads is presented in Table 3. A supplement of PGA was given along with the WB_6+PGA bread.

Diet

The subjects received a constant diet which met the RDA for all nutrients (Food and Nutrition Board of National Research Council, 1974). The subjects ate sufficient quantities of margarine, sugar, hard candies, and lemonade to maintain their weight. Table 5 gives the daily diet during the experimental periods.

Table 4. Daily diet during adjustment period (April 27-May 2).

	April 27, 28		April 29, 30		May 1, 2	
	Food	Weight (g)	Food	Weight (g)	Food	Weight (g)
Breakfast	Orange juice	170	Orange juice	170	Orange juice	170
	Milk, 2%	240	Milk, 2%	240	Milk, 2%	120
	Cream of wheat	12[a]	Cream of wheat	12	Cream of wheat	12
Lunch	Milk, 2%	240	Milk, 2%	120	Milk, 2%	120
	Cheese	60	Cheese	30	Cheese	30
	Peaches, canned	100	Peaches, canned	100	Peaches, canned	100
Dinner	Milk, 2%	240				
	Hamburger	60[b]	Hamburger	90		
	Rice casseroles[c]	121	Rice casseroles	121	Rice casseroles	121
	Pears, canned	100	Pears, canned	100	Pears, canned	100
Bread (Whole wheat) for the day		175		275		550

[a] Dry weight.

[b] Uncooked weight.

[c] Ingredients included 25 g rice, 2 g dehydrated onion, 34 g tomato juice, 25 g celery, and 10 g olive.

Table 5. The daily diet[a] during experimental periods I, II and III (May 3 to May 23).

Food Items	Weight (g)
Orange juice	170
Milk, 2 percent	240
Cream of wheat, dry	12
Peaches, canned	100
Pears, canned	100
Rice casseroles[b]	121
White bread[c] or	600
Whole wheat bread	570
Miscellaneous[d]	

[a] This diet supplied 66 g protein, 11.08 g fiber, 877 mg calcium, 1827 mg phosphorus, 19.3 mg iron, 4327 IU vitamin A, 2.06 mg thiamin, 1.34 mg riboflavin, 18.80 mg niacin, 92.7 mg vitamin C, and 1.64 mg vitamin B_6 in whole wheat bread. Whereas white bread contains the same amount of protein, vitamin A and C; 818 mg calcium, 903 mg phosphorus, 20.6 mg iron, 1.85 mg thiamin, 1.81 mg riboflavin, 16.65 mg niacin, 1.61 mg vitamin B_6. The content of nutrients was calculated from Handbook no. 8 (Watt and Merril, 1963). Vitamin B_6 content assayed in foods by Ms. Anne Perera using Saccaromyces carlsbergensis as the assay organism.

[b] Ingredients are given in Table 4.

[c] White bread enriched with pyridoxine (PIN) or white bread with an oral supplement PIN.

[d] Variable amount of margarine, jelly, hard candy and lemonade (Wyler Foods, Borden, Inc., Northbrook, Ill.) were consumed by individual subjects. They were also allowed to have coffee, tea, one bottle 7-UP (Bottled under the authority of 7-UP Services Inc., St. Louis, Missouri) and 6 tsp Tang (General Foods Corporation, White Plains, New York) per day.

Due to a probable contamination of the L. casei culture, falsely high folate values were obtained for the breads from the first few assays before the study started. For this reason 200 μg of PGA rather than 100 μg were given orally along with the WB_6+PGA bread. In order to keep everything standardized, it was decided to continue giving each subject 200 μg of PGA when they were receiving the WB_6+PGA bread. A food composite for one day, excluding bread, was assayed at the end of each experimental period. The three breads were assayed separately, each bread being assayed before and during the study periods.

Table 6 gives the daily folate intake of the subjects when each type of bread was fed. The total daily folate consumed during the week with WW, WB_6+PGA and W breads was 440, 538 and 303 μg, respectively. Since subject 5 was unable to eat this large amount of bread, his amount of bread was reduced by two-thirds and the amount folate consumed was decreased correspondingly.

Schedule of Blood Drawing

On days 1, 3 and 5 of each experimental period, blood samples were drawn by a medical technologist into heparinized vacutainer tubes before breakfast. Since the blood was obtained from fasting subjects, that drawn on days 3 and 5 of one period and day 1 of the subsequent period correspond to 2, 4 and 7 days of receiving one type

Table 6. Daily total folate intake for the subjects[a] when they received each type of bread.

Item	Folate Content			
	Bread	PGA	Food[b]	Total
	---------- μg ----------			
WW Bread[c] (570 g)	331		109	440
WB_6 +PGA Bread[d] (600 g)	229	200	109	538
W Bread[e] (600 g)	194		109	303

[a] Except subject 5 had daily total folate intake 329 μg on the WW bread diet, 401 μg on the WB_6+PGA bread, and 238 μg on the W bread.

[b] Basic diet given in Table 5.

[c] Whole wheat bread.

[d] Enriched with 0.20 mg of pyridoxine (PIN) per 100 g.

[e] Containing no additional vitamin B_6; subjects received an 0.84-mg oral supplement of PIN.

of bread. The first blood sample that was obtained from each subject during the adjustment period served as a baseline value for that subject. These blood samples were analyzed by a microbiological method utilizing L. casei as the assay organism for the determination of plasma and red cell folate. Procedure for folate determination will be described subsequently.

Study III. Influence of Vitamin B_6 on Plasma and Red Cell Folate

This study attempted to find out if a transitory rise in plasma vitamin B_6 following either a 2-mg or 10-mg loading dose of pyridoxine would affect plasma and red cell folate. Five apparently healthy men served as subjects in this study conducted by Janet Wozenski (1977) who kindly supplied the blood samples. She was studying vitamin B_6 metabolism in men receiving loading doses of pyridoxine in different amounts (0. 5 to 10. 0 mg), and equivalent amounts of pyridoxine, pyridoxal and pyridoxamine. Her data will be presented elswwhere. The subjects' age ranged from 24 to 32 years. Their mean height was 173±4 cm and their mean weight was 74. 2 ±7. 4 kg. All subjects were of normal weight for their height.

On the day before the loading dose, these subjects received a controlled diet of known composition which was adequate in all nutrients known to be required by men. On the following day, blood samples were drawn at 0, 1/2, 1 and 3 hours after the 2-mg loading

dose of pyridoxine was taken. No food or liquid except water was allowed during these three hours. The pyridoxine dose was given in 200 ml of water. A week later the same procedure was performed except 10-mg of pyridoxine was taken. The group plasma folate values at each time interval as well as red cell folate following the 2-mg pyridoxine loading dose were compared to the values following 10-mg of pyridoxine. The Student's t test was used to determine if there was a significant difference at the 0.05 level between the levels of vitamin B_6 at 0 and 1/2 hours.

Assay Methods

The folic acid content of the food, plasma and red cells was determined with L. casei as the assay organism. The method for measuring the total and free folate in food was adapted from the procedure recommended by Hurdle et al. (1968). The procedure for plasma and red cell folate was based on the method of Scott, Ghanta and Herbert (1974). In both procedures, ascorbic acid was incorporated in the buffer solution which was used for extracting the folic acid from the foods and in the assay medium.

Foods

I. Folate Extraction. The food was homogenized in a food blender. From this, 1.5 g of food was blended in 75 ml of 0.1 M

potassium phosphate buffer at pH 6.0 containing 0.15 percent ascorbic acid. This mixture was homogenized in a blender for three minutes at room temperature, autoclaved for 10 minutes at 10 psi, cooled, and centrifuged. After centrifugation, the supernatant was divided into two parts: one was stored at -10° C until assay, while the other was treated with conjugase as given below. Both samples were later assayed with L. casei.

II. Conjugase Treatment. "Difco" desiccated chicken pancreas (Difco Laboratories, Detroit, Michigan) was suspended in water, 3 mg per ml. The suspension was centrifuged and the precipitate was discarded. Seven ml of food homogenate were incubated with 1 ml of conjugase preparation for 16 hours at 37° C. A layer of benzene was placed on top of the samples to prevent microbial growth.

III. Food Assay. The double strength commercial folate-free medium (Difco Laboratories, Detroit, Michigan) containing 0.1 percent of ascorbic acid was used. Aliquots of untreated and conjugase-treated supernatant, standards, and blanks were diluted with redistilled water. After autoclaving, the tubes were inoculated with one drop of L. casei suspension and incubated for 18 hours at 37° C. The standard curve for the food assay was 0.1, 0.3, 0.5, 1.0, 1.5, 2.0, 3.0, and 5.0 ng per ml. Five levels (1, 2, 3, 4, 5 ml) of untreated and conjugase-treated supernatant samples were assayed. Each level was assayed in duplicate.

Blood

I. Preparation of Plasma and Red Cell Folate. For red cell folate, exactly one-half ml of whole blood was removed from the vacutainer tube, diluted 1:10 into redistilled water containing 1 percent ascorbic acid, and mixed gently. For plasma folate, the plasma was separated from the red cells by centrifugation at 3,000 rpm for 15 minutes at 0° C. Both plasma and whole blood were stored at -10° C until assay.

II. Plasma and Red Cell Folate Assay. A quarter-strength assay medium was added in appropriate amounts of redistilled water containing 0.1 percent ascorbic acid. This medium was dispensed with a syringe pipette in 10-ml amounts into test tubes. Tubes were plugged with cotton and autoclaved at 15 psi for 15 minutes before using.

Two levels of sample, 50 µl and 100 µl, were assayed. Each level was analyzed in triplicate. To correct for the color produced by the presence of the hemolysate, one tube of each concentration of blood was not inoculated and was used as a blank. The reading of this uninoculated tube containing hemolysate was subtracted from the test reading. This value gives the whole blood folate. A hematocrit was obtained at the time the blood was drawn for calculating red cell folate. The red cell folate is calculated from the whole blood folate

as follows:

$$\text{Red Cell Folate} = \frac{\text{Whole Blood Folate - Plasma Folate (1-Hematocrit)}}{\text{Hematocrit}}$$

The turbidity was read at 660 nm in an Evelyn Colorimeter for food, plasma and red cell folate assays. The folate concentrations from food, plasma and red cells were calculated from standard curves and expressed as ng per gram or ng per ml.

Blood Drawing

Blood drawn for Study I was described under Study I. For Study II and III blood was drawn from the antecubital vein into heparinized vacutainer tubes by a registered medical technician. Blood hematocrit and hemoglobin content were determined by standard methods.

Statistical Treatment

The statistical tests used in this study are F test, and Student's t test. Statistically significant correlations of tested parameters have been determined at the 0.05 level.

RESULTS AND DISCUSSION

Study I. The Effect of Dietary Folate on Folate Status in Preschool Children

Besides the free folate already present in foods, experimental evidence shows that the conjugase present in the human intestinal mucosa releases additional free folate from polyglutamyl folate, the predominate form of this vitamin in foods (Rosenberg, Streiff, and Godwin, 1969). Since total folate, which is obtained after treatment of the food with conjugase before assay, more accurately reflects the folate available to the human, total folate values from food tables were used to estimate the amounts of the vitamin that were consumed by the children.

Table 7 gives the calculated total folate intake for each of 49 children in the low plasma folate group (1.5-5.6 ng per ml) and Table 8 for the 37 children in high plasma folate group (16.7-42.9 ng per ml). The mean total folate intake per day for the low plasma folate group was 180 μg and the range was from 43 to 374 μg, whereas that for the high plasma folate group was 198 μg and the range was from 77 to 504 μg. Correlation coefficients between the dietary folate and plasma folate were estimated. No correlation ($r=0.002$) was found between dietary folate and plasma folate for all of the 86 children. Furthermore, dietary folate did not correlate with plasma for

Table 7. Vital statistics of the children who have low plasma folate values (1.5-5.6 ng per ml), their hematological values and the mean individual dietary folate intake.

Age Groups	Age	Subject	Sex	Height	Weight	Hemoglobin[a]	Hematocrit[a]	Plasma Folate	Total Dietary Folate
(mo.)	(mo.)	(no.)		(cm)	(kg)	(g/100 ml)	(%)	(ng/ml)	(μg)
12-47	30	54	F	99.1	16.3	--[b]	--	3.3	208
RDA for total	36	363	F	--	13.7	12.7	37	4.6	92
dietary folate	40	185	M	99.1	16.8	11.4	38	2.4	105
- 100 μg	42	155	M	96.5	14.5	13.5	38	5.5	143
	42	240	M	99.1	15.9	12.9	38	4.4[c]	275
	44	209	M	96.5	15.9	12.3	36	5.2[c]	71
	47	56	F	104.1	17.2	12.8	38	2.7	150
	47	211	M	99.1	15.9	13.4	40	4.5	164
48-83	48	172	M	104.1	16.3	12.6	34	4.2	374
RDA for total	48	202	M	109.2	15.9	11.8	36	4.9	89[d]
dietary folate	53	147	M	99.1	19.5	11.2	34	4.3	149
-200 μg	54	46	M	76.5	13.6	11.5	37	3.5	112[d]
	54	200	M	104.1	18.1	12.6	38	2.9	174
	56	164	M	111.8	19.9	12.7	38	5.5	197
	57	197	M	111.8	19.9	13.5	39	3.8	248
	58	356	F	102.0	16.4	13.9	41	4.0	270
	59	337	M	109.0	21.0	13.7	39	4.0	277
	61	150	F	109.2	17.2	12.8	38	2.0	79[d]
	61	182	M	116.8	20.8	11.7	37	5.0[c]	136
	63	153	F	109.2	20.8	12.5	39	5.5[c]	84[d]
	64	174	M	91.4	24.5	13.2	39	2.7	312
	64	298	F	114.0	18.4	14.7	41	5.3	273

Table 7. (Continued)

Age Groups	Age	Subject	Sex	Height	Weight	Hemoglobin[a]	Hematocrit[a]	Plasma Folate	Total Dietary Folate
(mo.)	(mo.)	(no.)		(cm)	(kg)	(g/100 ml)	(%)	(ng/ml)	(μg)
48-83	64	304	M	102.0	18.2	12.9	36	3.0	173
	66	160	F	111.8	24.9	12.5	36	4.4	139
	66	194	F	119.4	23.6	13.2	40	4.4	153
	66	203	M	114.3	18.1	12.9	40	5.5	64[d]
	55	213	F	109.2	18.6	13.3	40	2.7[c]	185
	66	291	M	119.0	21.0	12.6	35	4.2	247
	68	144	M	119.4	22.6	11.8	37	5.6	116[d]
	68	214	F	114.3	20.4	14.0	40	3.4	123[d]
	68	215	M	116.8	26.7	14.3	42	3.3	43[d]
	69	201	F	119.4	21.3	12.5	37	4.5	158
	72	210	M	91.4	17.7	13.3	39	4.9	185
	74	122	M	121.9	24.9	12.7	37	4.8[c]	223
	74	159	F	109.2	18.1	12.5	35	1.5	234
	75	116	M	129.5	33.6	14.0	40	5.2	121[d]
	75	198	M	124.5	23.6	14.2	43	3.1	200
	75	300	F	119.0	21.0	14.7	41	4.6	228
	75	327	M	125.0	23.6	12.4	36	3.6	250
	76	135	F	124.5	25.4	--	36	4.8	178
	79	106	M	119.4	20.4	13.1	39	5.4	117[d]
	80	170	M	127.0	26.7	12.4	35	3.0[c]	253

Table 7. (Continued)

Age Groups	Age	Subject	Sex	Height	Weight	Hemoglobin[a]	Hematocrit[a]	Plasma Folate	Total Dietary Folate
(mo.)	(mo.)	(no.)		(cm)	(kg)	(g/100 ml)	(%)	(ng/ml)	(μg)
84-120	84	322	F	119.0	23.2	12.6	37	5.2	344
RDA for total	89	173	F	109.2	18.6	11.6	32	5.0	143[d]
dietary folate	93	143	F	111.8	20.8	14.2	44	5.2	186[d]
- 300 μg	93	320	F	122.0	21.8	14.4	41	4.9	213
	103	171	F	137.2	32.6	12.9	34	3.5[c]	215
----------	--	237	F	--	--	13.1	38	5.2	105
	--	263	F	111.8	18.6	12.2	--	5.2	234

[a] The acceptable value of hemoglobin for age 1 to 5 and 6 to 10 is > 11.0 and > 11.5 g per 100 ml, respectively, whereas the hematocrit is > 34 and > 36 percent, respectively.

[b] Data unavailable.

[c] Took a vitamin supplement.

[d] Failed to receive two-thirds of the RDA for folate.

Table 8. Vital statistics of the children who have high plasma folate values (16.7-42.9 ng per ml), their hematological values and the mean individual dietary folate intake.

Age Groups	Age	Subject	Sex	Height	Weight	Hemoglobin[a]	Hematocrit[a]	Plasma Folate	Total Dietary Folate
(mo.)	(mo.)	(no.)		(cm)	(kg)	(g/100 ml)	(%)	(ng/ml)	(μg)
12-47	12	49	M	78.7	10.0	13.0	36	22.1	219
RDA for total	17	55	M	88.9	12.7	12.9	38	21.0	85
dietary folate	25	231	M	86.4	13.1	12.3	33	34.6[c]	136
- 100 μg	32	256	F	94.0	11.8	12.6	37	42.9	144
	32	258	F	91.4	11.3	11.9	35	26.1	147
	33	216	M	--	--[b]	12.1	37	17.2[c]	504
	33	239	M	94.0	14.9	12.7	37	18.4	130
	33	278	M	91.4	13.6	13.6	44	31.5	301
	34	161	M	96.5	15.9	11.3	34	17.6[c]	149
	35	233	F	96.5	14.9	12.0	36	24.2	144
	29	255	F	81.3	10.4	12.9	38	37.8	77
	40	259	F	99.1	14.9	13.2	39	17.9	263
	40	350	M	94.0	12.7	--	39	21.4	157
	41	276	M	91.4	11.8	12.9	39	17.9	123
	41	352	F	99.0	16.5	12.9	38	32.7	242
	43	265	F	94.0	12.2	13.9	--	22.5[c]	198
	44	178	M	101.6	16.8	12.4	38	19.4	163
	46	186	M	--	--	12.8	40	18.1	302
	47	353	F	107.0	18.4	13.0	39	22.4	87

Table 8. (Continued)

Age Groups	Age	Subject	Sex	Height	Weight	Hemoglobin[a]	Hematocrit[a]	Plasma Folate	Total Dietary Folate
(mo.)	(mo.)	(no.)		(cm)	(kg)	(g/100 ml)	(%)	(ng/ml)	(μg)
48-83	50	261	M	104.1	18.1	12.6	--	17.8	188
RDA for total	50	279	F	111.8	19.5	13.5	42	28.2	156
dietary folate	53	53	M	99.1	16.3	12.0	35	21.5[c]	167
- 200 μg	54	284	M	109.2	19.5	13.0	38	18.0	260[d]
	57	217	M	111.6	12.7	12.3	36	17.8	96
	57	269	F	106.7	17.2	12.6	37	18.0	146
	60	232	F	104.1	18.1	12.3	37	28.0	138
	60	266	F	109.2	18.6	12.4	--	18.8	285
	63	175	M	116.8	21.3	14.3	44	27.8[c]	172
	63	287	F	94.0	13.1	13.3	39	16.8	254
	65	354	M	91.0	15.5	15.8	43	19.0	344
	67	283	M	116.8	22.6	12.8	39	21.0	223
	70	268	M	109.2	16.8	12.3	38	19.5	308
	74	275	F	114.3	19.0	14.0	42	18.6	168
	74	330	M	122.0	22.2	13.1	37	17.4	208
	75	257	F	119.4	22.2	14.2	41	38.0	162
	75	324	M	114.0	18.2	13.4	--	23.8[c]	223
84-120	87	357	F	127.0	21.8	14.2	40	16.7	195[d]
RDA for total dietary folate - 300 μg									

[a] The acceptable values of hemoglobin and hematocrit for age 1 to 5 and 6 to 10 are given in Table 9.

[b] Data unavailable.

[c] Took a vitamin supplement.

[d] Failed to receive two-thirds of the RDA for folate.

the children in either the low (r=-0. 122) or high (r=-0. 282) plasma folate groups. This suggests that neither the high nor the low level of plasma folate was affected by the dietary intake of the vitamin. However, about 25 percent of the children in the low plasma folate group while only 8 percent in the high folate group received less than two-thirds of the RDA for folate. This supports the suggestion that an inadequate diet may cause low serum folate (Herbert, 1962; Forshaw et al., 1964).

When the dietary record was broken down into food groups (Table 9), it was observed that approximately 40 percent of daily total folate intake came from dairy foods. Most of the children drank each day from 1 to 3 cups of milk, which contains approximately 0. 15 μg of total folate per gram of milk. Milk contains a firm binder for folic acid (Metz et al., 1968; Ford, Salter and Scott, 1969; Ingemann Hansen, Holm and Lyngbye, 1977). Metz et al. (1968) demonstrated the preferential uptake of PGA by milk compared to serum. Milk removed PGA bound to serum but not vice versa in vitro. These authors also observed that in the lactating women who had a folate deficiency severe enough to produce megaloblastic anemia, orally administered PGA appeared to be taken up by breast milk in preference even to the hematopoietic system. It seems possible that the folate binder may influence the nutritional availability of milk folate. Bioavailability of folate in milk has not been studied in man.

Table 9. The mean and range of dietary total folate intake from different food groups by children who have a low plasma folate (1.5-5.6 ng per ml) or a high plasma folate (16.7-42.9 ng per ml).

Food Groups	Low Plasma Folate Group			High Plasma Folate Group		
	Mean (μg)	Percent	Range (μg)	Mean (μg)	Percent	Range (μg)
Dairy foods[a]	84 ±51	47	2 - 223	82 ±37	41	18 - 177
Meat	6 ± 4	3	0 - 18	7 ± 7	4	0 - 40
Vegetables	31 ±26	17	0 - 160	39 ±32	20	0 - 165
Fruits	24 ±35	14	0 - 155	33 ±41	17	0 - 184
Bread and Cereal Foods	28 ±22	16	1 - 147	30 ±20	15	6 - 75
Nut products	7 ±10	4	0 - 51	7 ±12	4	0 - 42
Total Folate Intake	178 ±75		43 - 374	198 ±85		77 - 504

[a] Includes eggs.

Moderate amounts of dietary folate (14 to 20 percent) came from vegetables, fruits, bread and cereal foods. Meat provided the least amount (approximately 3 percent). Bread and cereals as well as milk were the only foods commonly consumed by all of the 86 children (Table 9).

None of the children were vegetarians. Armstrong et al. (1974) reported that vegetarians have higher serum folate than non-vegetarians, although inadequate vitamin B_{12}, in part, may also be responsible for high levels of serum folate. The children in the high plasma folate group may have eaten more fruits and vegetables than those in the low plasma folate group. The children with low folate values had a slightly higher intake of dairy foods (47 percent) than the one with high folate values (41 percent). The high plasma folate group had a slightly higher intake of vegetables (20 percent) and fruits (17 percent) than the low plasma folate group (17 and 14 percent, respectively). The Student's t test was used to determine if there was a significant difference between two groups of children in their intake of different food groups. No significant differences, however, were found among these food groups.

There is little information on the folate requirement of children from one to ten years old. Since the Food and Nutrition Board, National Research Council (1974), recommends a different level of folate for each age group, the adequacy of children's total dietary

folate intake must be discussed in terms of age groups.

The estimated intake of dietary folate in almost half of the 86 children was below the RDA (Food and Nutrition Board of National Research Council, 1974). Dietary folate intake below the RDA was observed in the low plasma folate group as well as in the high plasma folate group. The preschool children by age groups who failed to receive two-thirds of the RDA for folate were as follows:

Age groups (yrs)	Low folate group	High folate group
1- 3	0[2]/8[3]	0/19
4- 6	10/34	2/17
7-10	2/5	1/1

More children than these results indicate may have received less than two-thirds of the RDA for folate. Since the vitamin is heat-sensitive (Perloff and Butrum, 1977), calculating food folate in cooked foods from raw foods may overestimate the folate content of the diet.

Fifteen children (17 percent) in this study took a vitamin supplement (Tables 7 and 8). Among these, seven took a supplement containing folic acid. It is interesting to note that 6 out of these 7 children who took the folate-containing supplement had a high plasma folate value, whereas 1 out of 8 children who had low plasma folate

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[2]Number of children failed to receive two-thirds of the RDA for folate.

[3]Total number of children in the age group.

values took a folate-containing supplement. Thus, these 6 children with high folate values may have had a high plasma level of the vitamin because of the folate-containing supplement.

It has been felt that dietary recall may not be a valid method to estimate folate intake. Not only is there a lack of standardized and extensive food composition tables for folate, but also dietary histories of some individuals may be either atypical for that day or incompletely recorded. Thus, values for daily total folate intake may be inaccurate.

One- to three-day recall may not be sufficient for estimating the food intake. Hegsted (1975) examined the problem of accuracy in dietary surveys. He pointed out that many individuals who have an apparently inadequate intake of the nutrient may not necessarily be at risk of deficiency because of the poor quality of the dietary data that had been taken. It may be simply that the individual's dietary was atypical for that day, since intake of nutrients varies from day to day and from week to week. The shorter the dietary recall, the greater the variability will be. A simple summation of the number of diets found in surveys to be below standard may be an overestimate of the individuals at risk of deficiency. Therefore, he suggested that dietary surveys must be designed in a way so that day-to-day variation in intake can be estimated and appropriate statistical procedures developed to estimate the variation in usual intake. In addition, the RDA usually is estimated to exceed the requirements of most

individuals (Food and Nutrition Board of National Research Council, 1974).

Hall et al. (1974) who studied variation in plasma folate levels among groups of healthy persons found that groups considered to be healthy differ in plasma folate values. A low plasma folate does not necessarily mean that the person is deficient in folate unless actual clinical symptoms are present. Individual variation is probably an important reason for the wide range in plasma folate among the children. As can be seen from Tables 7 and 8, plasma folate ranged from 1.5 to 42.9 ng per ml in spite of a relatively small variation in folate intake.

Vitale et al. (1966) and Toskes et al. (1974) proposed that low serum folate may be accompanied by iron deficiency. However, Burns and Spray (1969) and Omer et al. (1970) disagree with this. All of the 86 children in this study had normal hemoglobin levels (Tables 7 and 8). Five of them had low hematocrit values. Although 4 out of these 5 children with low hematocrit had a low plasma folate, their hematocrits were within the low limit of normal range, which is 30 to 35 percent (O'Neal et al., 1970; Sauberlich et al., 1974).

The children's body weight and height were compared with standard references (National Center for Health Statistics Growth Charts, 1976). Most children's body weight and height were within

normal growth range. Nine of the children with low plasma folate values and 7 of those with high plasma folate were either below or above the 5th or 95th percentile for height and weight (Tables 7 and 8).

Evaluated by the data presented in Tables 7 and 8, these children in the present study are considered normal and healthy. Thus, Herbert's (1966) proposed criteria for diagnosis of folate deficiency may not apply to children. In addition, the normal range of plasma folate levels for adults are different from laboratory to laboratory (Table 2). Hall et al. (1974) proposed that there will not be a satisfactory range for normal values of plasma folate until these values are obtained from all segments of the population. The mean value for plasma folate for the 300 Oregon preschool children was 10.69 ± 6.36 ng per ml (Miller, 1977). Red cell folate was not measured in these children. Since so little information is available from studies in children for the normal range of plasma folate, it is probably necessary to set up our own normal values for these children.

Other nutrients which were not included in this study may affect plasma folate. One of these nutrients may be vitamin B_6. An inverse relationship between plasma folate and vitamin B_6 was observed in many of the children in the present study. In view of the fact that Carson and Carre (1969) had observed that high vitamin B_6 intake (from supplement) resulted in low plasma folate, it was decided to determine the effect of vitamin B_6 on plasma folate in another study.

Study II. The Utilization of Folate from Bread

The subjects' hemoglobin and hematocrit values are given in Table 10. Both values were relatively consistent in each subject throughout the entire study. The values for all of the subjects, except those for subject 3, were within the acceptable ranges for hemoglobin and hematocrit in the adult male, which have been defined as levels above 14 g per 100 ml and 44 percent, respectively (O'Neal et al., 1970). Subject 8 had hematocrit values slightly lower than 44 percent. His hemoglobin levels, however, were normal. Although subject 3 was diagnosed as anemic during period I of the diet study, his values were not in the deficiency range (<37 percent and <12 g per 100 ml, respectively).

During the three periods of the diet study, the levels of plasma folate varied within each individual (Table 11). According to the guidelines for assessing plasma folate which were proposed by Herbert (1966), none of the subjects had levels of plasma folate that were consistently within the normal range (7-15.9 ng per ml). Most of the subjects had plasma folate values that were either at the lower end of the normal range or borderline low (5.0-6.9 ng per ml). Subject 3, whose plasma folate levels fell either into the borderline low or in suggestive deficiency stage (3.0-4.9 ng per ml) during the diet study will be discussed later. Subject 9 had plasma folate levels that

Table 10. The hematocrit and hemoglobin values for individual subjects during different periods of the diet study.

Period \ Subjects	Hematocrit (percent)									Hemoglobin (g per 100 ml)							
	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
Basal value	50	44	43	46	47	48	45	47	45	17.6	17.6	15.4	15.7	16.8	17.2	17.1	15.8	17.2
Adjustment Period																		
Day 4	51	45	39	42	46	47	45	43	48									
Day 7	49	45	38	43	45	43	43	41	47	17.2	16.3	13.1	15.6	16.0	17.5	16.0	15.0	18.2
Period I																		
Day 2	48	43	40	44	47	48	45	41	45									
Day 4	48	44	39	45	44	48	44	42	45									
Day 7	49	43	40	45	47	48	44	43	44	17.2	15.4	13.8	14.5	16.1	17.1	15.3	15.3	17.0
Period II																		
Day 2	48	45	40	43	45	48	44	43	44									
Day 4	49	43	41	43	46	49	44	42	44									
Day 7	48	45	41	45	48	48	43	42	44	16.7	16.6	13.9	15.4	16.9	17.6	16.5	15.3	17.1
Period III																		
Day 2	45	45	41	46	46	47	45	43	45									
Day 4	47	43	41	48	47	47	45	44	45									
Day 7	47	45	42	44	45	47	43	42	46	15.6	16.2	13.5	14.9	15.1	15.9	15.4	14.5	17.8

Table 11. The effect of different breads on the level of plasma folate in ng per ml.

Period \ Subjects	1	2	3	4	5	6	7	8	9
Basal value	6.7	7.5	3.8	6.0	11.4	6.0	4.1	14.2	3.4
Adjustment Period									
Day 4	8.7	8.1	5.0	9.5	5.7	7.6	5.6	19.3	2.7
Day 7	7.0	6.7	4.5	8.1	4.7	6.3	5.0	13.6	2.5
WB_6 + PGA Bread Period									
Day 2	8.1	7.8	3.9	8.6	6.3	8.8	6.6	8.9	4.0
Day 4	6.8	8.7	5.2	8.4	6.0	8.4	6.5	10.0	4.1
Day 7	6.9	7.5	6.8	7.9	5.7	6.7	7.5	9.1	5.0
WW Bread Period									
Day 2	6.6	6.7	5.5	8.5	6.4	5.8	6.8	13.4	2.1
Day 4	7.3	6.0	5.2	7.1	7.4	5.8	6.9	9.8	2.6
Day 7	6.7	6.1	6.0	8.7	7.2	6.6	6.8	8.8	3.4
W Bread Period									
Day 2	7.2	7.1	5.0	6.7	6.2	7.5	4.9	9.4	3.3
Day 4	7.2	7.6	6.0	5.7	7.0	7.8	4.1	6.7	2.9
Day 7	5.9	6.6	5.9	5.8	6.5	4.6	5.4	5.4	3.3

fell in the deficiency range (<3 ng per ml). Even though these subjects had folate levels that fell into either the low or deficiency stage, according to these guidelines by Herbert, they had normal hematocrit and hemoglobin values, except possibly subject 3. In addition, all of the subjects had levels of red cell folate (Table 12) which were within the acceptable range, defined as levels above 160 ng per ml (O'Neal et al., 1970; Beck, 1973). These subnormal plasma folate values were obtained despite the fact that these subjects received a diet containing two-thirds or more of the RDA for folate (Food and Nutrition Board of National Research Council, 1974).

The effect of each type of bread on the level of plasma folate in the individual subjects is presented in Table 11. As can be noticed immediately, the level of plasma folate varied widely among the subjects and within each subject. The range (for all values) was 2.1 to 19.3 ng per ml. Red cell folate (Table 12) also varied among the subjects, ranging from 381.8 to 769.1 ng per ml (for all values). These variations in plasma and red cell folate have been observed in other studies (Hoffbrand et al., 1966; Spray, 1969; Chanarin et al., 1972; Hall et al., 1974).

The individual plasma and red cell folate values on day 7 (Tables 11 and 12, respectively) and the mean of days 4 and 7 (Tables 13 and 14, respectively) for each bread period were arbitrarily selected for statistical analysis. Means from days 4 and 7 were chosen

Table 12. The effect of different breads on the level of red cell folate in ng per ml.

Subjects / Period	1	2	3	4	5	6	7	8	9
Basal value	381.8	588.8	543.0	484.4	423.4	463.0	512.5	639.4	527.9
Adjustment Period									
Day 4	412.2	619.1	465.6	501.2	399.7	498.0	510.4	683.9	499.0
Day 7	486.9	565.7	517.2	492.6	418.7	532.6	526.8	644.7	474.8
WB_6 +PGA Bread Period									
Day 2	410.9	605.1	528.1	597.9	450.4	455.1	499.6	756.2	545.3
Day 4	428.7	575.2	478.0	533.5	500.8	486.3	527.1	731.7	560.7
Day 7	469.1	583.4	604.9	516.5	474.8	497.1	513.9	745.6	593.1
WW Bread Period									
Day 2	473.4	593.8	541.3	481.3	393.2	435.6	552.6	678.0	503.7
Day 4	465.7	626.7	560.4	528.5	448.4	460.6	534.2	730.1	501.9
Day 7	543.5	612.3	529.8	579.4	495.7	470.4	574.3	704.5	502.5
W Bread Period									
Day 2	449.4	598.6	553.5	519.0	472.8	495.0	530.0	702.8	511.4
Day 4	459.7	642.2	569.6	575.4	441.3	467.8	567.5	769.1	540.1
Day 7	408.0	602.0	618.0	512.9	506.9	410.2	524.2	738.0	545.5

Table 13. The effect of different breads on the mean plasma folate values in ng per ml of each subject on days 4 and 7.

Breads / Subjects	WB_6+PGA (538 µg daily)[a]	WW (440 µg daily)[b]	W (303 µg daily)[c]
1	6.9	7.0	6.6
2	8.1	6.1	7.1
3	6.0	5.6	6.0
4	8.2	7.9	5.8
5	5.9	7.3	6.8
6	7.6	6.2	6.2
7	7.0	6.9	4.8
8	9.6	9.3	6.1
9	4.6	3.0	3.1
Mean ± SD[d]	7.1 ± 1.5[e]	6.6 ± 1.7	5.8 ± 1.2[e]

[a, b, c] Total daily folate intake when the subjects received the WB_6+PGA, WW and W breads, respectively.

[d] Standard deviation.

[e] Statistically different at 0.05 level.

Table 14. The effect of different breads on the mean red cell folate values in ng per ml of each subject on days 4 and 7.

Breads / Subjects	WB_6+PGA (538 µg daily)[a]	WW (440 µg daily)[b]	W (303 µg daily)[c]
1	448. 9	504. 6	433. 9
2	579. 3	619. 5	622. 1
3	541. 5	545. 1	593. 8
4	525. 0	554. 0	544. 2
5	487. 8	472. 1	474. 1
6	491. 7	465. 5	439. 0
7	520. 5	554. 3	545. 9
8	738. 7	717. 3	753. 6
9	576. 9	502. 2	542. 8
Mean ± SD[d]	545. 6 ± 83. 7	548. 3 ± 79. 4	549. 9 ± 100. 2

[a, b, c] Total daily folate intake when the subjects received the WB_6+PGA, WW and W breads, respectively.

[d] Standard deviation.

for measuring utilization of folate from the three breads because of the day-to-day variation of these values in each subject. The value from day 2 was not included because it may represent an adjustment to the bread.

Since folic acid as PGA in WB_6+PGA bread was 200 μg higher than that of W bread, noticeable changes in plasma folate levels were observed following the ingestion of these two breads. The mean plasma folate values on days 4 and 7 as well as plasma folate value at day 7 for the WB_6+PGA bread period were significantly higher ($p < 0.05$) than those for the W bread period. The mean plasma folate value for all subjects during the WB_6+PGA bread period was 7.1 ± 1.5 ng per ml, whereas during the W bread period it was 5.8 ± 1.2 ng per ml (Table 13). Tamura and Stokstad (1973) stated that 200 μg of PGA would effectively change plasma folate levels.

Plasma folate levels in 7 subjects increased with WB_6+PGA bread, indicating plasma folate responded to these extra 200 μg of PGA very well. Subject 3 utilized both types of bread equally well, and subject 5 showed a decrease in plasma folate when receiving 200 μg of additional PGA (Table 13 and Figure 3). Thus, the changes in plasma folate levels in 7 subjects showed that plasma folate does reflect the dietary folate intake, since the difference between total folate content of WB_6+PGA and W bread was 200 μg.

The mean plasma folate values following WB_6+PGA bread

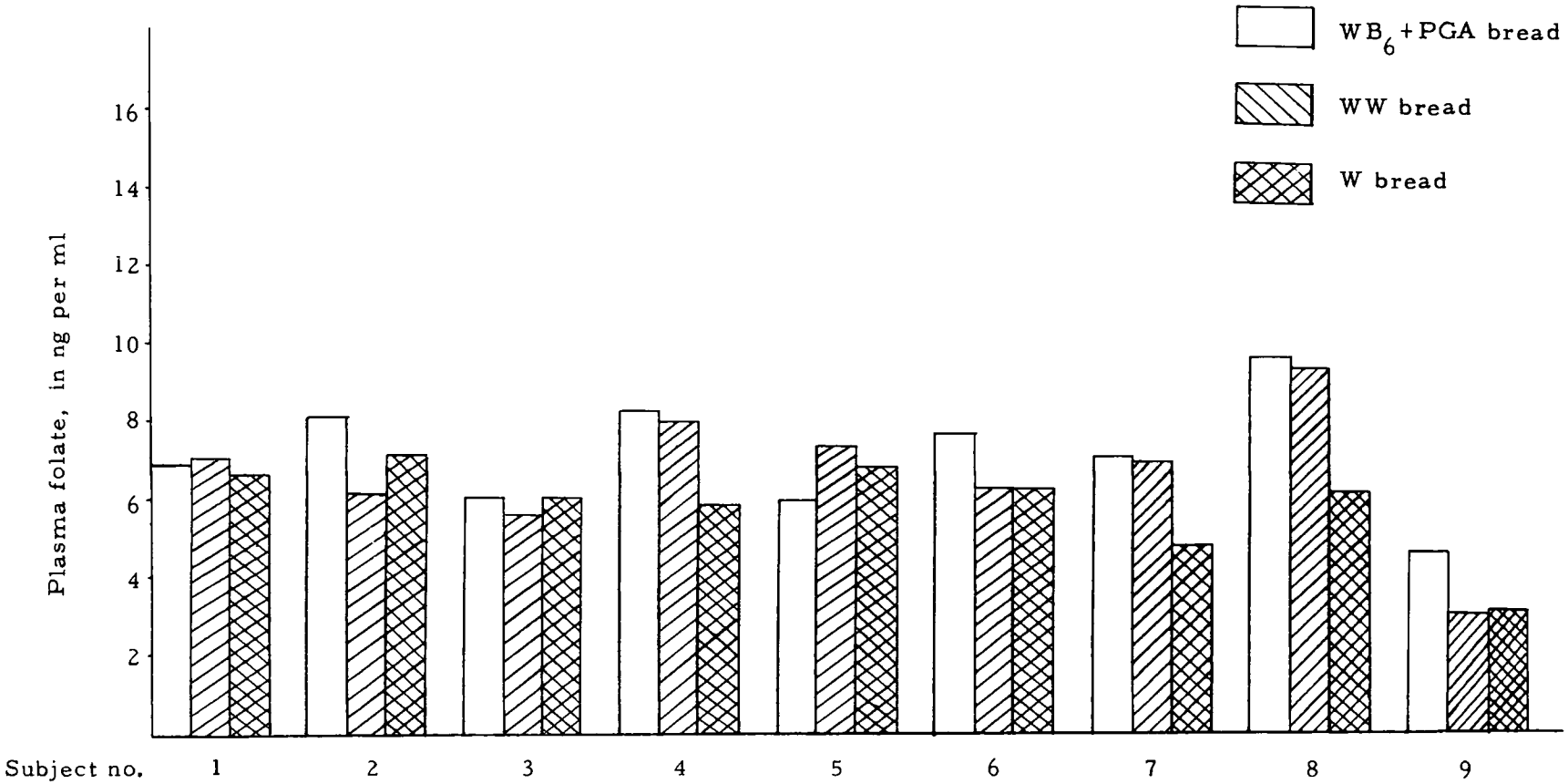

Figure 3. The effect of different breads on plasma folate. Each value represents the mean of days 4 and 7 of each experimental period. The total daily folate intake when the subjects received WB_6+PGA, WW and W breads was 538, 440 and 303 µg, respectively.

(7. 1±1. 5 ng per ml) ingestion was not significantly different from that of WW bread (6. 6±1. 7 ng per ml). Subjects 1, 3, 4, 7 and 8 utilized the folate in WB_6+PGA and WW breads equally well. Plasma folate values of subjects 2, 6 and 9 decreased following the WW bread period indicating that the plasma folate did not respond to the 100 μg difference between the two breads. The plasma folate level of subject 5, on the other hand, was higher on the WW bread diet than on WB_6+PGA (Table 13 and Figure 3). In view of these results and of the fact that the WW bread supplied 100 μg less folate than the WB_6+PGA bread, these results indicate that plasma folate may respond better to the folate in WW bread than in WB_6+PGA bread. This would apply to at least 6 of the 9 subjects.

There was no significant difference in plasma foltate value between the WW bread (6. 6±1. 7 ng per ml) and the W bread (5. 8±1. 2 ng per ml) diet, although folate in WW bread was 100 μg higher than that in W bread. Again there was much variation among the subjects in their response to the WW and W breads. The level of plasma folate in subjects 4, 5, 7 and 8 were higher when they received the WW bread than when they consumed the W bread. Subjects 1, 3, 6 and 9 responded equally to the two breads, whereas subject 2 had higher plasma folate when consuming the W bread than when he received WW bread (Table 13 and Figure 3). No clear differences were observed between the WW and W bread which was 100 μg lower in folate than

the WW bread, because this level of folate does not effectively raise plasma folate (Tamura and Stokstad, 1973). Since no clear cut differences were observed in utilization of WW and W breads, no definite conclusion can be drawn from the present study regarding the utilization of folate in breads and cereals which are commonly consumed by preschool children. Periods longer than one week may be necessary to show differences in utilization.

No difference was observed in red cell folate due to the type of bread consumed (Table 14 and Figure 4). The level of red cell folate did not reflect the differences in response that were observed in plasma folate. In fact, Herbert (1962), Perry and Chanarin (1968) observed that a prolonged period is required to change the level of red cell folate. Apparently, in the present study seven days for each bread was not long enough to detect changes in the red cell folate. Results from this study indicated that plasma folate is a better indicator of folate utilization in a shorter period of time than in red cell folate.

It was interesting to examine the plasma folate levels in subjects 5 and 8. Their plasma folate values dropped dramatically during the adjustment period (Table 11). Their 24-hour recall and food frequency data revealed that both subjects had a diet high in fruits and vegetables before starting the study.

Because of a mild anemia subject 3 started taking 5 gr of iron

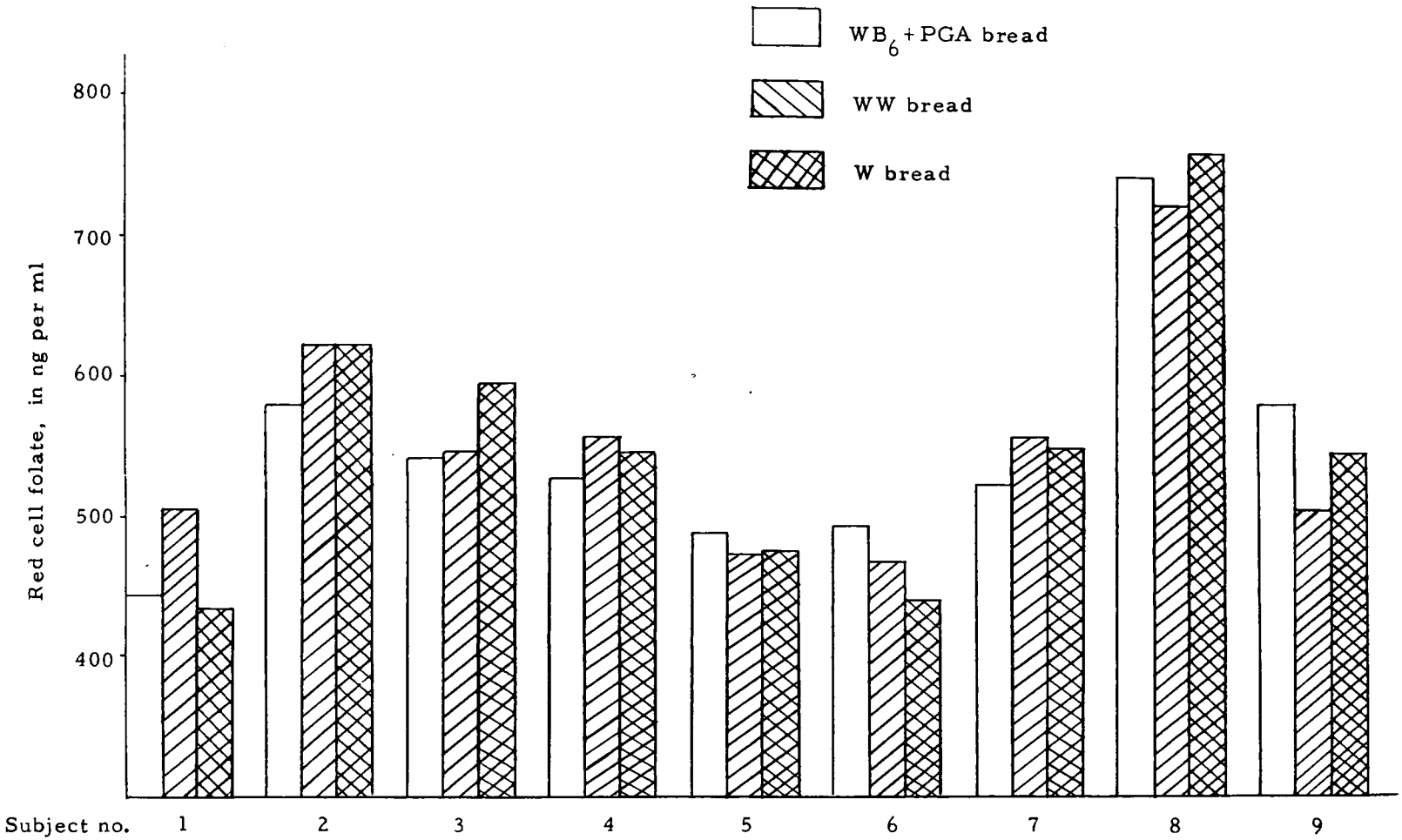

Figure 4. The effect of different breads on the level of red cell folate. Each value represents the mean of days 4 and 7 of each experimental period. The total daily folate intake when the subjects received WB_6 + PGA, WW and W breads was 538, 440 and 303 ug, respectively.

sulfate on day 4 of experimental period I. As a result, his hematocrit and hemoglobin level gradually increased (Table 10) during the study, but did not approach normal levels.

Iron deficiency may produce a secondary deficiency of folic acid (Velez et al., 1966). Vitale et al. (1966) and Toskes et al. (1974) demonstrated that iron deficiency in rats results in decreased serum folate levels. On the other hand, Omer et al. (1970) who studied plasma folate in iron deficiency, found similar levels of serum folate in patients deficient in iron and in the controls. In all these investigations, an increase in serum folate was observed after iron therapy.

In the present study, subject 3 had low plasma folate values of 3.8 and 3.9 ng per ml (Table 11), respectively, before starting the study and before starting the iron sulfate therapy. These low plasma folate values probably resulted from the secondary folate deficiency in anemia. After he started the iron therapy on day 4 of experimental period I, his plasma folate rose and stayed between 5.0-6.8 ng per ml throughout the remainder of the study. The period he started iron sulfate therapy was also the period during which he was receiving WB_6+PGA bread. Therefore, the combination of iron sulfate and synthetic PGA in diet produced a rise in plasma folate levels. His red cell folate values remained steady throughout this study (Table 12). Hershko et al. (1975) indicated that red cell folate is unaffected by coexistent iron deficiency.

Study III. Influence of Vitamin B_6 on Plasma and Red Cell Folate

An inverse relationship was observed between plasma vitamin B_6 and plasma folate in the preschool children (Miller, 1977). Plasma vitamin B_6 rises rapidly following an oral dose of vitamin B_6 and reaches a peak within half an hour (Wozenski, 1977). The purpose of this study was to determine if the transitory rise in plasma vitamin B_6 following either a 2- or a 10-mg loading dose of pyridoxine would affect plasma and red cell folate.

The plasma and red cell folate values for each subject at 0, 1/2, 1 and 3 hours after the loading doses of 2- or 10-mg pyridoxine are presented in Table 15. The hematocrit values for three subjects were within the acceptable range, which is above 44 percent (O'Neal et al., 1970). Those for subjects 4 and 5 were slightly lower, 43 percent, which is in "lower limit" (37-43 percent) of normal value.

The individual variability in plasma and red cell folate values were observed in this study as well as in the previous studies. The plasma folate levels of the subjects at 0 hour ranged from 3.9 to 10.3 ng per ml, whereas red cell folate levels ranged from 321.5 to 457.7 ng per ml. According to Herbert (1966), only subjects 2 and 3 had plasma folate values that were consistently above the normal values and the plasma folate values of subjects 1, 4 and 5 were "low" (Table 15). However, results from this thesis and several other

Table 15. The effect of 2- or 10-mg loading dose of pyridoxine (PIN) on the levels of plasma and red cell folate at 0, 1/2, 1 and 3 hours.

Subjects	Oral PIN	Hematocrit[a] (percent)	Plasma Folate (ng/ml)				Red Cell Folate (ng/ml)			
			0	1/2	1	3	0	1/2	1	3
1	2 mg	53	5.2	4.5	5.2	4.4	414.8	378.7	375.2	443.3
2		45	10.3	8.0	8.1	9.5	358.5	475.8	447.9	463.9
3		47	8.4	9.2	8.2	9.0	433.1	488.6	506.7	455.4
4		43	4.3	8.2	6.0	5.8	364.1	360.1	378.8	363.9
5		43	4.5	3.7	3.9	5.2	431.9	405.1	406.5	417.5
MEAN			6.5	6.7	6.3	6.8	400.5	421.7	423.0	428.8
SD			2.7	2.5	1.9	2.3	36.6	57.7	55.1	40.3
1	10 mg	53	3.9	3.9	5.3	5.7	380.5	363.5	379.8	395.0
2		44	7.1	7.0	7.0	6.9	338.2	390.6	346.3	404.4
3		47	7.3	5.9	7.6	8.5	457.7	509.9	360.2	373.4
4		43	4.8	4.3	4.1	3.5	367.6	371.7	417.4	367.0
5		43	4.8	3.6	4.3	3.9	321.5	327.8	321.7	350.6
MEAN			5.6	4.9	5.7	5.7	373.1	392.7	365.1	378.1
SD			1.5	1.5	1.6	2.1	52.7	69.4	36.1	21.7

[a] Done once only.

studies (Hoffbrand et al., 1966; Hall et al., 1974; Hershko et al., 1975) have indicated the low plasma folate levels, which may be due to individual variation, do not necessarily mean that the person is deficient in folate. In addition, since all of these subjects had normal red cell folate values (> 160 ng per ml, O'Neal et al., 1970; Beck, 1973; Sanberlich et al., 1974) and normal hemoglobin and hematocrit, their folate status was considered satisfactory.

The means of the responses of the 5 subjects to the 2- or 10-mg loading dose of pyridoxine are shown in Figure 5. There was greater rise in plasma vitamin B_6 (assayed with the Saccharomyces uvarum) following the 10-mg pyridoxine loading dose than after the 2-mg one. The level of plasma vitamin B_6 in all of the subjects reached its maximum peak at 1/2 hour following the 2-mg pyridoxine loading dose. Following 10-mg pyridoxine loading dose, subjects 1, 2 and 5 reach the maximum peak at 1/2 hour and subjects 3 and 4 at 1 hour instead.

In contrast to the changes in plasma vitamin B_6, no obvious changes were observed in plasma folate. The plasma folate levels of all the subjects were lower following the 10-mg dose than those following 2-mg pyridoxine loading dose (Table 15), except those of subject 5 which were constant following the two pyridoxine loading doses. The group means of plasma folate at 0, 1/2, 1, 3 hours after 10-mg loading dose were also slightly lower than that after the 2-mg

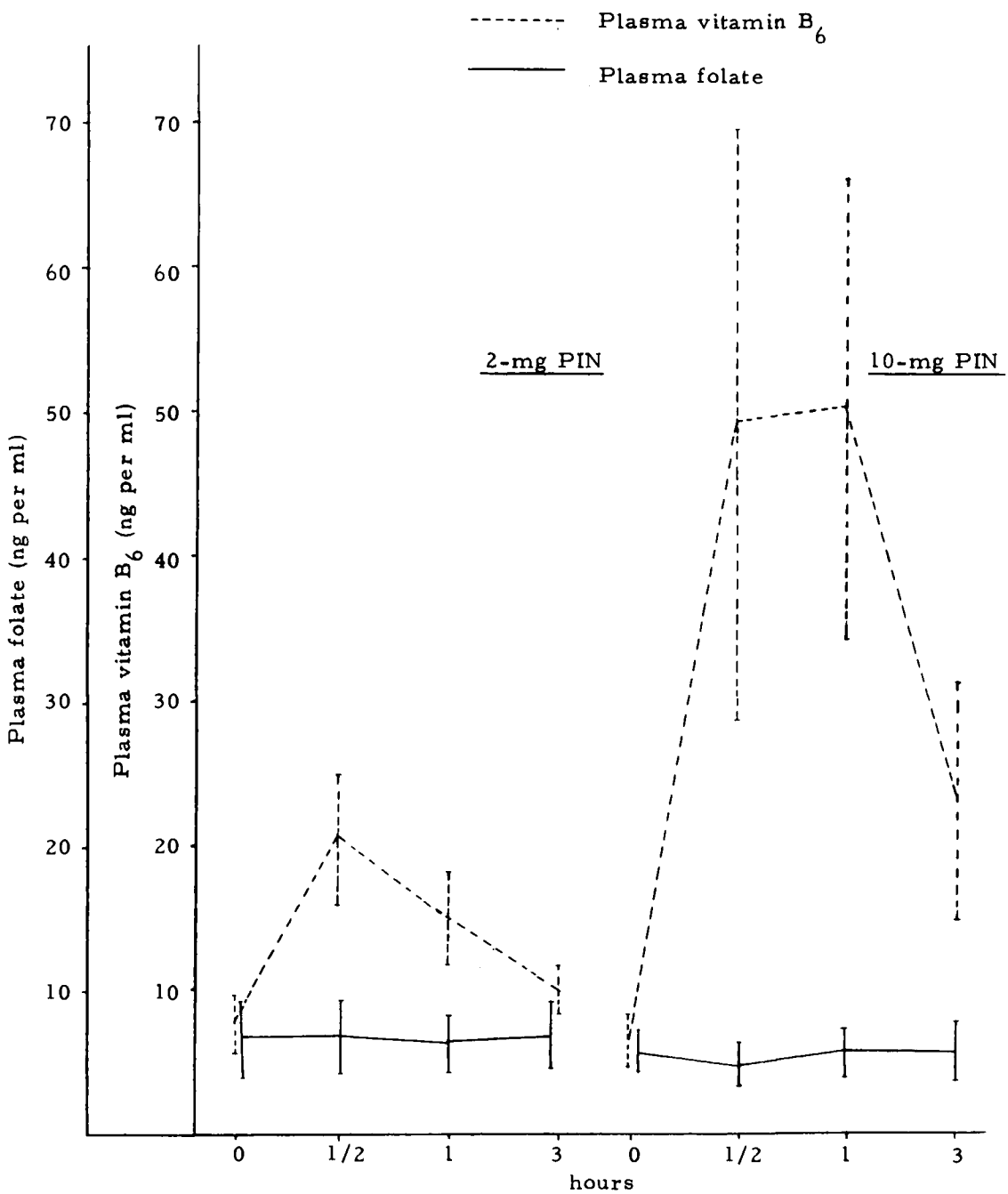

Figure 5. The effect of a transitory rise of plasma vitamin B_6 (group mean) on plasma folate (group mean) following 2- or 10-mg loading dose of pyridoxine (PIN).

loading dose (Figure 5).

In order to see if the transitory rise in plasma vitamin B_6 would affect plasma and red cell folate, the percent decreases in plasma and red cell folate values between 0 and 1/2 hour after the two pyridoxine loading doses were calculated for each individual. The Student's *t* test was used to determine if there were significant changes in plasma as well as red cell folate levels following the two loading doses of pyridoxine.

The percent decreases in plasma and red cell folate values following the 10-mg oral pyridoxine dose were not significantly lower than those after the 2-mg pyridoxine loading dose. In other words, transitory rise in plasma vitamin B_6 following pyridoxine loading did not affect the plasma and red cell folate levels. The results of this study do not explain the inverse relationship between plasma folate and plasma vitamin B_6 that was observed in some of the preschool children studied by Miller (1977). Carson and Carre (1969) reported a significant drop of serum folate in their patients with homocystinuria who were treated with large amounts of vitamin B_6 over a period of time. A daily supplement of vitamin B_6 over a long period of time, instead of one day as in the present study, may be necessary for the changes in plasma and red cell folate to occur.

SUMMARY AND CONCLUSIONS

Low serum folate is frequently attributed to an inadequate dietary intake of the vitamin (Herbert, 1962; Forshaw et al., 1964). Thus, the purpose of the research presented in this thesis was to investigate some dietary factors affecting plasma and red cell folate.

Among 300 preschool children recently studied by Miller (1977), 49 (16 percent) children had plasma folate values less than 5.6 ng per ml. The folate intake of these children were compared to those 37 (12 percent) with a high plasma folate (16.7-42.9 ng per ml).

Breads and cereals as well as milk were sources of folate consumed by all of the children. Fruits, vegetables and nuts were also sources for most of the children, but not all. The total folate intake for the low folate group was 180 μg, ranging from 43 to 374 μg per day; and that for high folate group was 198 μg, ranging from 77 to 504 μg per day. There was no correlation between dietary folate and plasma folate. There was no difference between the two groups of children in the quantities of folate they received from the different food groups. However, 25 percent of the children with low plasma folate and 8 percent of those with high plasma folate received less than two-thirds of the RDA for folate (Food and Nutrition Board of the National Research Council, 1974). Among the children in the high folate group, 6 out of 7 were taking a vitamin supplement which

included folate, and among those in the low folate group, 1 out of 8 was receiving a folate-containing supplement.

In another study, the utilization of folate from bread was determined in 9 men by measuring plasma and red cell folate. These men received two-thirds of their folate from WB_6+PGA, WW and W breads. The breads plus their constant diet supplied 538, 440 and 303 μg of folate daily, respectively. The subjects received each bread for one week in a three by three Latin Square design. The plasma folate was significantly higher ($p < 0.05$) when subjects received WB_6+PGA bread (7.1±1.5 ng per ml) than when they consumed W bread (5.8±1.2 ng per ml) which was 200 μg lower in folate. Five out of the 9 responded to WB_6+PGA and WW breads equally well and one utilized WW bread better than WB_6+PGA bread. No clear cut difference was observed between WW and W breads. In view of these results, no definite conclusions can be drawn from this study regarding the utilization of folate in breads and cereals which are commonly consumed by preschool children. Periods longer than one week may be necessary to show clear differences in utilization. No difference was observed in red cell folate due to the type of bread consumed.

Carson and Carre (1969) observed low plasma folate in patients receiving large amounts of vitamin B_6. However, a transitory rise in plasma vitamin B_6 in 5 men following 2- and 10-mg loading doses did not affect the level of plasma or red cell folate. These results

do not explain the inverse relationship between plasma vitamin B_6 and folate which were observed in some preschool children.

BIBLIOGRAPHY

Anonymous. 1974. The availability of food folate in man. Nutrition Review 32(6):167.

Armstrong, B. K., R. E. David, D. J. Nicol, A. J. V. Merwyk and C. J. Larwood. 1974. Hematological, vitamin B_{12}, and folate studies on Seventh-Day Adventist vegetarians. Am. J. Clin. Nutr. 27:712.

Beck, W. S. 1973. Hematology. Harvard Pathophysiology series volume 1, The MIT Press, Cambridge, Massachusetts, and London, England. p. 114.

Burns, D. G. and G. H. Spray. 1969. Normal folic acid metabolism in iron-deficient rats. Brit. J. Nutr. 23:665.

Butterfield, S. and D. H. Calloway. 1972. Folacin in wheat and selected folacin in wheat and selected foods. J. Am. Dietet. Assoc. 60:310.

Butterworth, C. E., R. Santini and W. B. Frommeyer. 1963. The pteroylglutamate components of American diets as determined by chromatographic fractionation. J. Clin. Invest. 42:1929.

Carson, N. A. J. and I. J. Carre. 1969. Treatment of homocystinuria with pyridoxine. Arch. Dis. Child. 44:387.

Chanarin, I., B. Rothman, J. Perry and D. Stratfull. 1968. Normal dietary folate, iron and protein intake with particular reference to pregnancy. Birt. Med. J. 2:394.

Chanarin, I., R. Kyle and J. Stacey. 1972. Experience with microbiological assay for folate using a chloramphenicol-resistant L. casei strain. J. Clin. Path. 25:1050.

Colman, N., R. Green and J. Metz. 1975a. Prevention of folate deficiency by food fortification. II. Absorption of folic acid from fortified staple foods. Am. J. Clin. Nutr. 28:459.

Colman, N., J. V. Larsen, M. Barker, E. A. Barker, R. Green and J. Metz. 1975b. Prevention of folate deficiency by food fortification. III. Effect in pregnant subjects of varying amounts of added folic acid. Am. J. Clin. Nutr. 28:465.

Cooper, B. A., and L. Lowenstein. 1964. Relative folate deficiency of erythrocytes in pernicious anemia and its correction with cyanocobalamin. Blood 24:502.

Daniel, W. A., Jr., and D. L. Bennett. 1975. Dietary intakes and plasma concentrations of folate in healthy adolescents. Am. J. Clin. Nutr. 28:363.

Dong, F. M., and S. M. Oace. 1973. Folate distribution in fruit juices. Am. J. Dietet. Assoc. 62:162.

Dong, F. M., and S. M. Oace. 1975. Folate concentration and pattern in bovine milk. J. Agric. Food Chem. 23(3):534.

Food and Nutrition Board, National Research Council. Recommended dietary allowances. 1974. Eighth Revised Edition, Washington, D. C., National Academy of Sciences. p. 71.

Ford, J. E., D. N. Salter and K. J. Scott. 1969. The folate-binding protein in milk. J. Dairy Res. 36:435.

Forshaw, J., E. H. Moorhouse and L. Harwood. 1964. Megaloblastic anemia due to dietary deficiency. Lancet 1(2):1004.

Grossowicz, M. R., and G. Izak. 1972. Absorption of pteroylglutamate and dietary folates in man. Am. J. Clin. Nutr. 25: 1135.

Hall, C. C., S. A. Bardwell, E. S. Allen and M. E. Rappazzo. 1974. Variation in plasma folate levels among groups of healthy persons. Am. J. Clin. Nutr. 28:854.

Hegsted, D. M. 1975. Dietary standards. J. Am. Dietet. Assoc. 66:13.

Herbert, V. 1962. Experimental nutritional folate deficiency in man. Trans. Assoc. Am. Physic. 75:307.

Herbert, V., A. R. Larabee and J. M. Buchanan. 1962. Studies on the identification of a folate compound of human serum. J. Clin. Invest. 41:1134.

Herbert, V. 1963. A palatable diet for producing experimental folate deficiency in man. Am. J. Clin. Nutr. 12:17.

Herbert, V. 1966. Aseptic addition method for Lactobacillus casei assay of folate activity in human serum. J. Clin. Path. 19: 12.

Herbert, V. 1967. Biochemical and hematologic lesions in folic acid deficiency. Am. J. Clin. Nutr. 20:562.

Herbert, V. 1968. Nutritional requirement for vitamin B_{12} and folic acid. Am. J. Clin. Nutr. 21:743.

Hershko, C., N. Grozzowicz, M. Rachmilewitz, S. Kesten and G. Izak. 1975. Serum and erythrocyte folates in combined iron and folate deficiency. Am. J. Clin. Nutr. 28:1217.

Hoffbrand, A. V., B. F. A. Newcombe and D. L. Mollin. 1966. Method of assay of red cell folate activity and the value of the assay as a test for folate deficiency. J. Clin. Path. 19:17.

Hoppner, K., B. Lampi and D. E. Perrin. 1972. The free and total folate activity in foods available on the Canadian market. J. Inst. Can. Technol. Aliment. 5(2):60.

Hoppner, K., B. Lampi and D. E. Perrin. 1973. Folacin activity of frozen convenience foods. Am. J. Dietet. Assoc. 63:536.

Houlihan, C. M., and J. M. Scott. 1972. The identification of pteroylpentaglutamate as the major folate derivative in rat liver and the demonstration of its biosynthesis from exogenous |3H| pteroylglutamate. Biochem. Biophys. Res. Comm. 48: 1675.

Hurdle, A. D. F., D. Barton and I. H. Searles. 1968. A method for measuring folate in food and its application to hospital diet. Am. J. Clin. Nutr. 21:1202.

Ingemann Hansen, S., J. Holm and J. Lyngbye. 1977. Mechanism of folate binding in milk. Clinica Chemica Acta. 75:321.

Jandl, J. H., and A. A. Lear. 1956. The metabolism of folic acid in cirrhosis. Ann. Internal Med. 45:1027.

Klipstein, F. A., S. D. Lipton and E. A. Schenk. 1973. Folate deficiency of the intestinal mucosa. Am. J. Clin. Nutr. 26: 728.

Leevy, C. M., L. Cardi, C. Frank, R. Geelene and H. Baker. 1965. Incidence and significance of hypovitaminemia in a randomly selected municipal hospital population. Am. J. Clin. Nutr. 17:259.

Liu, Y. K. 1974. Microbiologic assay of erythrocytic folate content by the aseptic addition method. Am. J. Clin. Path. 62(2):688.

Luther, L., R. Santini, C. Brewster, E. Perez-Santaigo and C. E. Butterworth. 1965. Folate binding by insoluble components of American and Puerto Rican diets. Alabama J. Med. Sci. 2: 389.

Margo, H., M. Barker, F. Fernandes-Costa, N. Colman, R. Green and J. Metz. 1975. Prevention of folate deficiency by food fortification. VII. The use of bread as a vehicle for folate supplementation. Am. J. Clin. Nutr. 28:761.

Metz, J., R. Zalusky, and V. Herbert. 1968. Folic acid binding by serum and milk. Am. J. Clin. Nutr. 21:289.

Miller, L. T. 1977. Personal communication.

Mitchell, H. K., E. E. Snell and R. J. Williams. 1941. The concentration of "folic acid." J. Am. Chem. Soc. 63:2284.

National Center for Health Statistics: NCHS Growth Charts. 1976. Monthly Vital Statistics Report 25(3) supp (HRA):76.

Nelson, E. W., R. R. Streiff and J. J. Cerda. 1975. Comparative bioavailability of folate and vitamin C from a synthetic and a natural source. Am. J. Clin. Nutr. 28:1014.

Noronha, J. M., and M. Silverman. 1962. Distribution of folic acid derivatives in natural material. I. Chicken liver folates. J. Biol. Chem. 237:3299

O'Broin, J. D., I. J. Temperley, J. P. Brown and J. M. Scott. 1975. Nutritional stability of various naturally occurring monoglutamate derivatives of folic acid. Am. J. Clin. Nutr. 28:438.

Omer, A., N. D. C. Finlayson, D. J. C. Shearman, R. R. Samson and R. H. Girdwood. 1970. Plasma and erythorcyte folate in iron deficiency and folate deficiency. Blood 35:821.

O'Neal, R. M., O. C. Johnson and A. E. Schaefer. 1970. Guidelines for classification and interpretation of group blood and urine data collected as part of the national nutrition survey. Pediat. Res. 4:103.

Peffers, D. 1977. Bioavailability of Vitamin B_6 from wheat breads in humans. Master thesis.

Perera, A. 1977. Vitamin B_6 enrichment of wheat flour: stability and bioavailability. Ph. D. thesis.

Perloff, B. P., and R. V. Butrum. 1977. Folacin in selected foods. J. Am. Dietet. Assoc. 70:161.

Perry, J., and I. Chanarin. 1968. Absorption and utilization of polyglutamyl forms of folate in man. Brit. Med. J. 4:546.

Pfiffner, J. J., D. G. Calkins and B. L. O'Dell. 1945. Isolation of an antianemia factor (vitamin B_c conjugate) in crystalline form from yeast. Sicence 102:228.

Rosenberg, I. H., R. R. Streiff and A. Godwin. 1969. Absorption of polyglutamic folate: participation of deconjugase enzymes of the intestinal mucosa. New Engl. J. Med. 280:985.

Rosenberg, I. H., and H. A. Godwin. 1971. The digestion and absorption of dietary folate. Gastroenterology 60(3):445.

Russell, R. M., F. Ismail-Beigi, and J. G. Reinhold. 1976. Folate content of Iranian breads and the effect of their fiber content on the intestinal absorption of folic acid. Am. J. Clin. Nutr. 29:799.

Santini, R., F. M. Berger, G. Berdasco, T. W. Sheeny, J. Aviles and L. Davila. 1962. Folic acid activity in Puerto Rican foods. J. Am. Dietet. Assoc. 41:562.

Santini, R., C. Brewster and C. E. Butterworth. 1964. The distribution of folic acid active compounds in individual foods. Am. J. Clin. Nutr. 14:205.

Saraya, A. K., V. P. Choudhry and O. P. Ghai. 1973. Interrelationships of vitamin B_{12}, folic acid, and iron in anemia of infancy and childhood: effect of vitamin B_{12} and iron therapy on folate metabolism. Am. J. Clin. Nutr. 26:640.

Sauberlich, H. E., R. P. Dowdy and J. H. Skala. 1974. Laboratory tests for the assessment of nutritional status. CSC Press, Inc. 18901 Cranwood Parkway, Cleveland, Ohio. p. 49-60, 114-124.

Scott, J. M., V. Ghanta and V. Herbert. 1974. Trouble-free microbiologic serum and red cell folate assays. Am. J. Med. Tech. 40:125.

Spray, G. H. 1964. Microbiolotical assay of folic acid activity in human serum. J. Clin. Path. 17:660.

Spray, G. H. 1969. Estimation of red cell folate activity. J. Clin. Path. 22:212.

Stokes, P. L., V. Melikian, R. L. Leeming, E. Portman-Graham, J. A. Blair and W. T. Cooke. 1975. Folate metabolism in scurvy. Am. J. Clin. Nutr. 28:126.

Stokstad, E. L. R. and J. Koch. 1967. Folic acid metabolism. Physiol. Rev. 47:83.

Streiff, R. R., and I. H. Rosenberg. 1967. Absorption of polyglutamic folic acid. J. Clin. Invest. 46:1121.

Streiff, R. R. 1971. Folate levels in citrus and other juices. Am. J. Clin. Nutr. 24:1390.

Swendseid, M. E., O. D. Bird and R. A. Brown. 1947. Metabolic function of pteroylglutamic acid and its hexaglutamyl conjugate. II. Urinary excretion studies on normal persons. Effect of a conjugase inhibitor. J. Lab. Clin. Med. 32:23.

Tamura, T., and E. L. R. Stokstad. 1973. The availability of food folate in man. Brit. J. Haematology 25:513.

Thorne, C. B., and C. G. Leonard. 1958. Isolation of D and L glutamyl polypeptides from culture filtrates of Bacillus subtilis. J. Biol. Chem. 233:1109.

Toepfer, E. W., E. G. Zook, M. L. Arr and L. R. Richardson. 1951. Folic acid content of foods. Microbiological assay by standardized methods and compilation of data from the literature, Agriculture Handbook No. 29 Washington, D. C., U. S. Department of Agriculture.

Toskes, P. P., G. W. Smith, T. A. Bensinger, R. A. Giannella, and M. E. Conrad. 1974. Folic acid abnormalities in iron deficiency: the mechanism of decreased serum folate levels in rats. Am. J. Clin. Nutr. 27:355.

Usdin, E. 1959. Blood folic acid studies. VI. chromatographic resolution of folic acid-active substances obtained from blood. J. Biol. Chem. 234:2373.

Varadi, S. and A. Elwis. 1964. Megaloblastic anemia due to dietary deficiency. Lancet 1:1162.

Velez, H., A. Restrepo, J. J. Vitale and E. E. Hellerstein. 1966. Folic acid deficiency secondary to iron deficiency in man. Am. J. Clin. Nutr. 19:27.

Vitale, J. J. 1966. Present knowledge of folacin. Nutr. Rev. 24: 289.

Vitale, J. J., A. Restrepo, H. Velez, J. B. Riker and E. E. Hellerstein. 1966. Secondary folate deficiency induced in the rat by dietary iron deficiency. J. Nutr. 88:315.

Watt, B. K. and A. L. Merrill. 1963. Composition of foods. U. S. Department of Agriculture Handbook, no. 8. Washington, D. C.

Waxman, S., C. Schreiber and V. Herbert. 1971. Radioisotopic assay for measurements of serum folate levels. Blood 38:219.

Wills, L. 1931. Treatment of "pernicious anemia of pregnancy" and "tropical anemia" with special reference to yeast extract as a curative agent. Brit. Med. J. 1:1059.

Wintrobe, M. M., G. W. Thorn, R. D. Adams, I. L. Benett, Jr., E. Braunwald, K. J. Isselbacher and R. G. Petersdorf. 1970. Harrison's Principles of Internal Medicine. Sixth edition. A Blackiston Publication McGraw-Hill Book Co. p. 1595.

Wozenski, J. 1977. The metabolism of Vitamin B_6 in humans and guinea pigs. Ph. D. thesis.