AN INVESTIGATION INTO THE FOLATE STATUS OF HUMAN SUBJECTS IN SELECTED VILLAGES AROUND ZARIA (NORTHERN NIGERIA)

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BY

RAZIA SULTANA HUQ (MISS)
(B.Sc. Hons) A.B.U.,

Department of Biochemistry
Ahmadu Bello University
Zaria, Nigeria

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THESIS APPROVAL

This thesis by Razia S. Huq meets the regulations governing the degree of Master of Science of Ahmadu Bello University and is approved for its contribution to scientific knowledge and literary presentation.

Internal Examiners	External Examiner
Dr. J.A. Abalaka, (Supervisor), Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria.	Professor O.L. Oke, Department of Biochemistry, University of Ife, Ile-Ife, Nigeria.
Ahmad Abalaka	
Professor M.M. Rahman Ag. Head of Department, Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria.	
	Date: 24/2/81

DEDICATION

DEDICATED TO MY BELOVED PARENTS

Dr. & Mrs. HUQ.

TABLE OF CONTENTS

Contents | Page No.

TABLE OF CONTENTS (Conted.)

Contents | Page No.

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R. S. Huq (Miss)

DECLARATION

I declare that this work is wholly the author's and has not been part of any presentation for any other qualification.

Date: ....24/1/81....................

Signed by:- Razia Sultana
Miss R. S. Huq (Author)

J.A. Abalaka
Dr. J.A. Abalaka (Supervisor)

M.M. Rahman
Professor M.M. Rahman
Ag. Head of Department.

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

The .normal ranges for serum and red cell folate levels in a Fulani community around Zaria have been determined to be 1.28-5.96 and 124. - 359 ng/ml respectively. The red blood cell folate (RCF) levels of Fulani children and adult males were significantly lower than those of the control subjects.

Among the raw foods consumed by the Fulanis from the selected population, locust bean cake (Daddawa) was found to have the highest concentration of folates (0.95 Ug folate/g dry wt. of food) .

The seasonal variation in folate levels of raw food items was not significant. However, a clear picture has not emerged concerning the cooked foods because of the unco-operative attitude of human subjects.

Attempts have been made to study the effect of cooking on food folate levels. Most of the folates in food were found to be d s-troyed to various degrees depending on the length and temperature of the heat treatment.

From the results, an individual from the population under study ingests 80-1 20 Ug of folates in a day. This level of folates clearly falls short of the recommended level by authorities in this area.

Chapter 1

INTRODUCTION AND LITERATURE REVIEW

BACKGROUND:

The matter of greatest interest to man is how to obtain energy in the form of food from the environment and to use it to perform other activities of his interest. However, it is necessary to consider the quality as well as the quantity of food consumed. Besides carbohydrates and fat as main energy sources, food must contain protein, vitamins and mineral salts in adequate quantities. This has inspired scientists all over the world to analyze food both qualitatively and quantitatively, although more attention is paid to protein and carbohydrate while comparatively less has been done on vitamins and minerals.

It has been found that the diets of most people in developed countries is adequate qualitatively and quantitatively while malnutrition and undernutrition are common in developing and underdeveloped countries, especially those with denser populations, which also seem to have a high incidence of chronic infection. Due to these reasons, methods for the assessment of nutritional status which have mostly been established in western countries may not apply equally to developing countries. Nigeria also faces undernutrition and malnutrition to some extent and the main problem seems to be one of dietary deficiency of protein as well as a deficiency in the B group of vitamins (Oke, 1972).

Folic acid, which is a B vitamin possessing probably a greater number of derivatives than any other vitamin (Rodriguez, 1978), has been the subject of several investigations since yeast extracts were found to improve tropical macrocytic anaemia (Hurdle, 1968). Megaloblastic anaemia is commonly associated with kwashiorkor (Ebbs, 1976) and children suffering from this have been shown to respond to an oral dose of 5 - 10mg of folic acid per day (Redden, 1976).

Among the richest sources of folic acid are yeast, liver, lettuce and spinach (Hurdle, 1968). However, there is a lack of tables of folate contents for foods due to the late development of sensitive assay methods. Folacin intake has been underestimated in tables compiled before the significance of protecting folic acid during assay by using ascorbic acid was realized (Rodriguez, 1978).

Factors affecting human folic acid (folacin) requirement include availability and absorption. Rodriguez (1978) has reported that availability depends on the type of food consumed, ability of individuals to absorb the folacins from different foods and method of preparation of food. Folacin from liver, spinach and peas is more easily absorbed than that from pumpkin and tomato, while orange juice has been found to inhibit the availability of pteroyl-heptaglutamate added to food.

The difficulties faced when estimating availability of folates in foods have been described (Nutrition Reviews, 1974) as follows:-

A high intake of folic acid may cause only a small rise in excretion of folate in the urine. Levels of red cell folate are not affected during short-term experiments with food-stuffs while changes in serum folate are difficult to quantitate when slight, and depend on the previous folate status of the individual. Ethical reasons prevent administration of isotopically labelled food to healthy individuals. Polyglutamates constitute a major portion of food folates and although their availability is a matter of question, most workers agree that folates possessing more than three glutamic acid residues are poorly absorbed and their inclusion in estimates of folate intake gives falsely high values. This is supported by the findings of Baugh _et al._ (Rodriguez, 1978) which revealed that the absorption rate of polyglutamates is inversely proportional to the γ-glutamyl side chain.

Allowing for the biological activity and absorbability of folic acid and its derivatives, the recommended daily allowances (RDA) of 50 μg for infants, 400 μg for adolescents, 600 μg for lactating mothers and 800 μg for pregnant women have been set by the U.S. Food and Nutrition Board of the National Research Council (Redden, 1976).

Normal stores of folic acid range from 5-10mg (Redden, 1976). The person most likely to get folic acid deficiency is a pregnant or lactating mother as the foetus or infant draws on the mother's folate stores. Feeding experiments indicate that, unlike other constituents of food, the dietary levels of water soluble vitamins influence their excretion in milk (Thomson and Hytten, 1966). Therefore, a folate deficient mother is expected to have a foetus or infant who is also folate deficient and therefore a high standard of nutrition for her, as among the general population, must be unquestionably maintained. Nutrition status surveys conducted in California and Canada (Rodriguez, 1978) showed that approximately 50 % of the adolescents had low serum folacin values and 10 to 13 % were in the high risk group. This may be due to increased requirements during growth or the unbalanced food usually consumed by adolescents.

A study of the seasonal distribution of folic acid deficiency in pregnant women has been undertaken by some workers (Chanarin et al. 1968; Fleming, 1970), who believe this distribution to be related to variation in availability of dietary folic acid and lack of fresh vegetables at different times of the year.

Fleming (1968) measured the serum folic acid level in a chosen population of Southern Nigerians over a period of one year and noted a drop in the level from February to mid July (mid-dry to mid-wet season). He suspected that the vegetables available during this season were either folate deficient or were rendered so by prolonged cooking. Little meat and fruits are consumed due to prohibiting costs and to lack of their availability. Because of this, the serum folate level of Southern Nigerians (1.6 - 28 ng/ml; mean = 9.18 ng/ml), was found to be lower than that of Europeans (5-31.6 ng/ml; mean = 11.2 ng/ml).

The work of Fleming raised some interesting questions such as:-

(1) Is there folic acid deficiency among the Northern Nigerians?

(2) Is there a marked seasonal variation in the incidence of folate deficiency ?

(3) Is the deficiency caused by a dietary lack of the vitamin?

(4) What is the folate content of some common raw food materials, traditional dishes and meal composites ?

(5) How are the folate levels affected by heat, storage and dietary composition of meals ?

For the purpose of answering the above questions, the Fulani community in three villages (Giwa, Maigana and Hunkuyi) around Zaria in the Northern part of Nigeria, was selected. As an extention of this investigation some foods were selected from Zaria market in order to evaluate their folate content.

Blood samples were collected from adults and children of both sexes in order to study the incidence of folate deficiency in the chosen population, and to assess the influence of age and sex on the folate levels. Serum folic acid (SFA) levels were measured to evaluate the current folate status of the individuals while red blood cell folate levels (RCF) offered an indication of a long standing folate deficiency. Camparisons were made with the serum and red blood cell folate levels (RCF) of fifty healthy blood donors (controls) at the Ahmadu Bello University Teaching Hospital (ABUTH), Zaria.

Raw food samples were purchased and assayed over a period of one year to study seasonal and storage effects. Samples of cooked food collected from the subjects provided knowledge about the folate content of the foods in the form actually eaten. This was important because cooking is known to be a factor that probably destroys folates.

FOLIC ACID: The folates form a complex group of water-soluble compounds within the vitamin B complex (Malin, 1975) and may include more than one hundred and forty closely related pteroic acid compounds of which about thirty have been identified (Baugh *et al.*, 1974). The term, folic acid, which specifically refers to the non-reduced pteroyl monoglutamic acid, generally describes a whole range of folates (Malin, 1975). Folates are important for the physiological and biochemical metabolism of many organisms, specially the higher animals and man (Malin, 1975).

DISCOVERY OF FOLIC ACID: Wills (1931) described the tropical macrocytic anaemia observed in female patients, especially pregnant ones, in Bombay. The anaemia was characterised by macrocytic red blood cells. Symptoms of pernicious anaemia, such as nervous disorders, were absent. Marmite, an extract of autolyzed yeast, expected to be ineffective against pernicious anaemia cured this disease (Wills, 1931; Wills and Naish, 1933). Wills concluded that the deficiency of an extrinsic factor, present in plant and animal matter, was responsible for this anaemia.

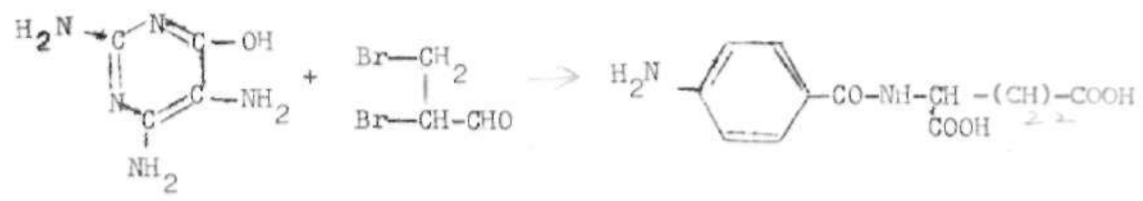
Mitchell, Snell and Williams (1941) discovered that the compound they had extracted and concentrated lacked sulphur, contained nitrogen and had an approximate molecular weight of 500; it was an active growth factor for rats and Streptococcus lactis. They named the compound 'folic acid' from folium, the Latin word for leaf, because, apart from liver and kidney, it was found in all green leafy plants examined.

Hall (1947) observed synergism between synthetic pteroylglutamic acid and a folic acid concentrate prepared from spinach and between pteroylglutamic acid and a concentrate from poppy straw, but not a similar concentrate from liver. He concluded that "folic acid" of plant sources was different from "folic acid" of animal origin.

SYNTHESIS: It was demonstrated, by degradation and synthesis, that the structure of a liver Lactobacillus casei factor was N(4-(2-amino-4-hydroxy-6-pteridymethyl-amino)-benzoyl) glutamic acid (Malin, 1975). The name pteroylglutamic acid was suggested for this compound by the same workers who synthesized it by four methods (Robinson, 1951):

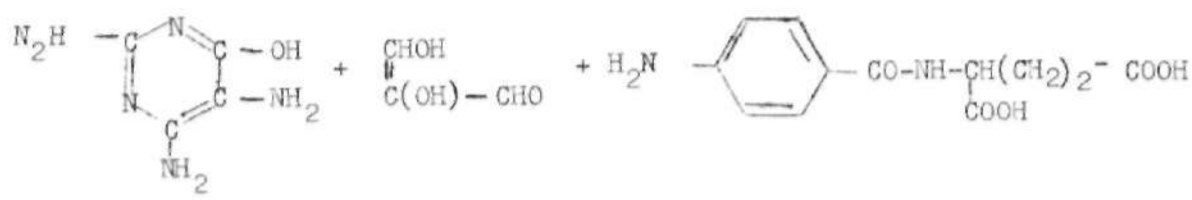
(1) Triamino-6-hydroxypyrimidine (2:4:5-), p-amino benzoyl-L-glutamic acid and 2:3-dibromo-propionaldehyde were reacted

together in the presence of an acetate buffer, to give first, the dihydro-compound which was oxidized during the reaction to give the aromatic compound, pteroyl glutamic acid:



2). Dibromo-propionaldehyde (2:3-) was reacted with pyridine and then condensed to give 15 % of the active product.

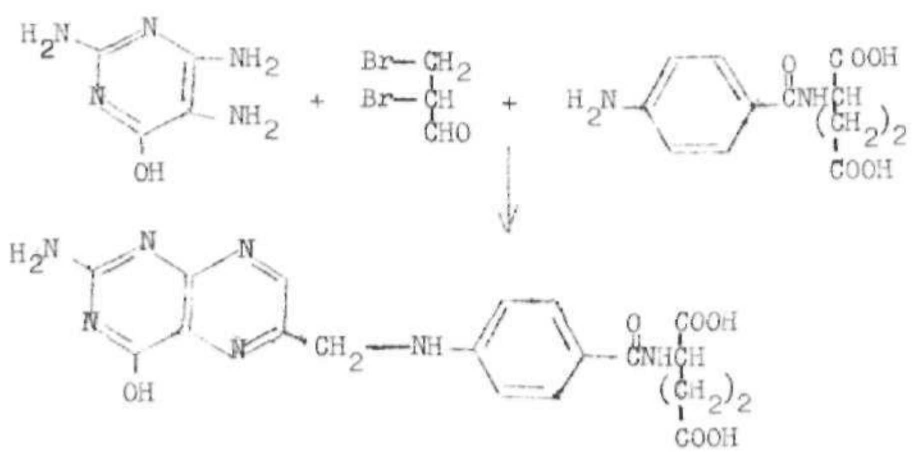
3). Reductone (2:3-dihydroxyacrylaldehyde) was treated with p-amino benzoylglutamic acid and the product was esterified and condensed to give pteroylglutamic acid.



4). Reduction of 2-amino-4-hydroxy-6-pteridyl-methyl pyridinium iodide yielded 2-amino-4-hydroxy-6-methyl-pteridine which was either brominated or chlorinated and the product condensed with the diethyl ester of p-aminobenzoyl-glutamic acid.

American Cyanamid Company filed a patent to cover the synthesis of pteroylglutamic acid from the corresponding diaminopteridine by heating in an aqueous solvent under alkaline conditions (Robinson, 1951).

A 15 % yield of pteroylglutamic acid, which was identical to the chick anti-anaemia factor and the monkey factor, 'vitamin M', (Malin, 1975) was obtained by reacting 2: 4: 5·-triamino-6-hydroxypyrimidine, 2: 3-dibromo propionaldehyde and p-aminobenzoyl-1(+)-glutamic acid in aquoeus sodium acetate buffer:



It was reported that two workers, Karrer and Schwyzer synthesized pteroylglutamic acid by condensing 2:4:5 -triamino-6-hydroxy-pyrimidine with glyceraldehyde or dihydroxyacetone or by reacting it with glyceraldehyde di-p-toluenesulphate and para aminobenzoyl glutamic acid in presence of sodium iodide. It was also reported that the following methods were patented by Roche Products Ltd. for the preparation of pteroyl-glutamic acid. (Robinson, 1951):

(1) 2-amino-4-hydroxy-6-hydroxymethyl pteridine was reacted with thionyl chloride and the product was treated with para aminobenzoyl glutamic acid.

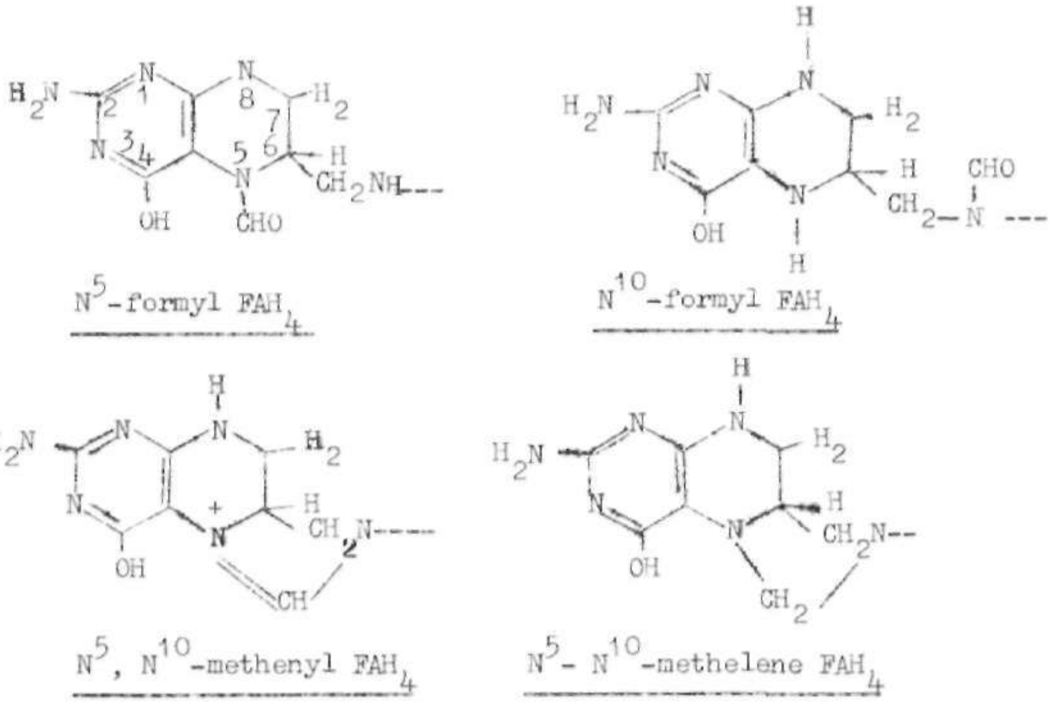
(2) A mixture of 2-amino-4; 6-hydroxymethylpteridine and para nitrobenzoylglutamic acid was hydrogenated.

Fedec and Cossins (1977) found that aeration of carrot storage tissue discs in water was accompanied by folate synthesis. Aeration of the discs in 0.1 mM gibberellic acid resulted in higher production of methyl tetrahydrofolate and also increased the specific activities of 5; 10-methylene tetrahydrofolate reductase, serine hydroxy methyl transferase and 5-methyl tetrahydrofolate homocysteine transmethylase, all involved in the metabolism of folic acid. The same process was also found to enhance the incorporation of formate (^{14}C) into serine, glycine and methionine.

OCCURRENCE OF FOLATES IN NATURAL PRODUCTS: Folic acid has been reported to be present in varied concentrations in many food substances, especially liver, kidney, yeast and green leaves. It is also present in many micro-organisms, of which the highest yields were obtained from Bacillus subtilis, Bacillus vulgatus, Serratia marcescens and a Gram-negative bacillus from chick embryo (Robinson, 1951).

According to Malin (1975), many compounds possessing folic acid activity have been reported since 1931. These are either combinations of folic acid with varying numbers of glutamic acid residues or reduced and non-reduced analogues of folic acid possessing formyl, methyl, and other radicals attached to the pteridine ring.

The functional forms of folic acid are derivatives of 5:6:7:8-tetrahydrofolic acid and are shown below:



N^5-formyl FAH_4 N^{10}-formyl FAH_4 N^5, N^{10}-methenyl FAH_4 N^5- N^{10}-methelene FAH_4

The predominant forms of folate have been shown to be folic acid, the N^5 and N^{10} formyl tetrahydrofolates (N^{10} formyl FAH_4) and N^5-methyl FAH_4 in yeast, N^5 formyl FAH_4 in liver, N^5 -formyl pteroyl triglutamic acid, N^{10}-formyl pteroyl-glutamic acid and N^{10}-formyl pteroic acid in green leaves of soy plants, N^5-methyl FAH_4 in serum, and polyglutamates and a derivative of N^5-methyl FAH_4 in the red blood cells.

Zamierowski (1976) studying the high molecular weight forms of folates by employing experimental injection of (^{3}H)-folic acid found most of the radioactivity bound to proteins in liver cytoplasm and mitochondria, kidney microsomes, and intestine. The bound folates were largely polyglutamates while most of the monoglutamates were free. Cytochrome II contained the largest amount of bound endogenous folate polyglutamates of which the predominant one was 5-methyl-FAH_4-polyglutamate. The _in vivo_ experiment produced more bound folate than the _in vitro_.

PROPERTIES OF FOLIC ACID: Mitchell and Williams (1944) reported that the folic acid from spinach leaves had an absorption spectrum resembling that of xanthopterine and that it therefore contained a xanthopterine-like moeity. He also claimed that it was free from sugars or polyhydroxy compounds.

The crystalline vitamin Bc (the original name of folic acid) isolated from liver by Stokstad (1943) was shown to be insoluble in common organic solvents but soluble in methanol, phenol and glacial acetic acid. It showed a melting point above 360°C and had a solubility of 10 µg/ml in water at pH3 and at 2°C; at 100°C, its solubility in water was shown to be 0.5mg/ml while its extinction coefficients in 0.1N sodium hydroxide were shown to be

$E_{1\,cm}^{1\,\%}$ at 255nm, 565; at 282nm, 350; and at 365nm, 195.

Folic acid is partially inactivated by sulphite (Daniel and Kline, 1947) and lead and mercury salts (O'Dell and Hogan, 1943) and is destroyed by low pH (Robinson, 1951) and autoclaving, especially at low pH. In pure solution, it is inactivated by light to give para aminobenzoyl glutamic acid and 2-amino-4-hydroxy-6-formyl-pteridine which eventually forms 2-amino-4-hydroxypteridine.

FOLATE CONJUGASE: The more complex folates, known as the polyglutamates cannot be utilized by organisms and need to be broken down to simpler folates by an enzyme, conjugase, which is known to be of two types (Tsukahara and Yamada, 1971). The carboxypeptidase occurs widely in nature while the γ-glutamic acid carboxypetidase was isolated from chick pancreas.

Leichter, Landymore and Krumdieck (1979) determined the conjugase in fresh asparagus, broccoli, Brussels sprouts, cabbage, cauliflower, lettuce and spinach. The conjugase activity varied from 0.3 ± 0.21 in lettuce to 106.2 ± 6.6 μ mole/hr/100 mg of fresh weight in spinach. The workers suggested that the only way of obtaining reliable free folate values in fresh vegetables is by inactivating the naturally present conjugase in the sample to prevent the hydrolysis of the polyglutamates in the vegetable.

TRANSPORT AND METABOLISM: Active transport is exhibited by both the natural (L) and the unnatural (D) isomers of the monoglutamate forms of folic acid, but the rate limiting step in their utilization is their storage. The polyglutamate forms of folic acid are limited by inefficient transport although they are better retained by tissues (Shane and Stokstad, 1976).

Normal people excrete 2 to 4 µg of pteroylglutamic acid per day in the urine. An average of 28.5 % of the daily oral dose is excreted by this route (Steinkamp et al., 1946).

It has been suggested that folic acid is metabolized to p-acetamidobenzoylglutamic acid by a cleavage of the molecule at C^9-N^{10} positions. This product has been found in the urine following administration of tritiated folic acid to rats (Keating, 1978). The above author has also suggested that the major product of 5-methyl-5: 6-dihydrofolic acid is p-aminobenzoylglutamic acid.

Folic acid has an enterohepatic circulation and is stored mainly in the liver and kidneys (Himanen, 1977). The amount stored in a given organism after a parenteral dose is greater than an equal dose administered perorally. Himanen (1977) also stated that the decrease of administered tritiated folic acid, in serum, occurs in three phases:

(1) Distribution phase: upto thirty minutes there is a sharp decrease.

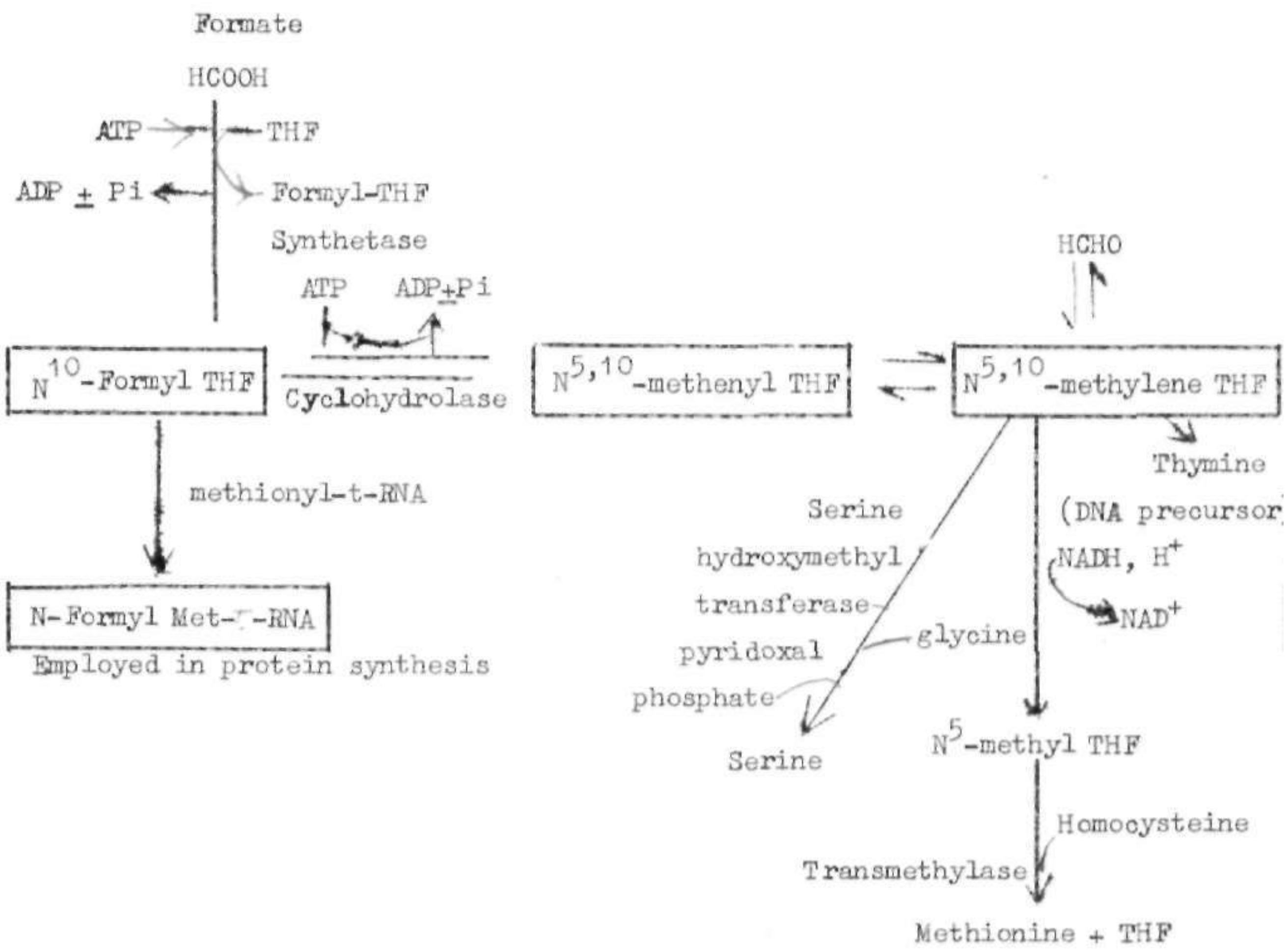
(2) Elimination phase: This takes place for about twelve hours.

(3) Slow phase: The peak retention of folic acid occurs after twenty-four hours.

The hydrolysis of N^{10}-formyl tetrahydrofolate (N^{10}- formyl THF) is catalyzed by N^{10}-formyl THF dehydrogenase (E.C.1.5.1.6) [Scrutton and Beis, 1979] with a Km of 23.8 μM. THF inhibits this reaction non-competetively with a Km of 21.5 μM.

FUNCTIONS: Tetrahydrofolate acts mainly as a carrier for a one-carbon unit at the oxidation level of formate, which is used in the biosynthesis of pyrimidines, purines, serine and glycine.

Some of the reactions in which tetrahydrofolate and its carbon (C_1) derivatives participate are given below (Conn and Stumpf, 1976):



Folic acid is necessary for the haemopoietic system and for reticulocytosis (Robinson, 1951). It has been suggested (Robinson, 1951) that folic acid may participate in the choline oxidase system because folate deficient monkeys were found to be deficient in choline oxidase. Rodney, Swendseid and Swanson (1947) observed a similar influence on tyrosine oxidation in rats.

Citrovorum factor, a cofactor of folic acid, is an antidote for the toxicity of methotrexate (Folkers et al., 1979).

AVAILABILITY AND REQUIREMENT: The joint FAO/WHO Expert Group (Malin, 1975) made two conclusions on the availability and requirements of folate:

(1) Until further knowledge concerning folate absorption is available, only the 'free' or unconjugated forms of folate in food should be considered and the conjugated folates with more than three glutamic acid residues should be ignored.

(2) A healthy individual absorbs only half of the folates available in his diet. However, 80 % absorption of a tritiated dose of synthetic pteroylglutamic acid has been demonstrated (Malin, 1975).

The absorption of polyglutamates is a controversial subject. Experiments have shown 60 - 70 percent absorption of synthetic polyglutamates, whereas natural polyglutamates have not shown the same haematological response as equimolar amounts of monoglutamates.

It has been suggested that the availability of folate depends on the type of food being consumed (Malin, 1975). Some foods may contain inhibitors or at times, some drugs being taken at the time may inflict an inhibitory effect. Certain conditions, such as the tropical sprue (Malin, 1975) or removal of jejunum (Baker et al., 1969), prevent absorption of polyglutamates since the jejunum is the major site of absorption (Hegsted, 1974). Hegsted (1974) also stated that the optimum pH for folate conjugase activity is 4.5, while that of the jejunum is higher than this pH value. In pernicious anaemia, the pH of gastric contents does not go below neutrality. These two factors may explain why conjugase cannot act efficiently in healthy as well as anaemic subjects.

Perry and Chanarin (Hegsted, 1974) suggested that the polyglutamates may be split in the instestinal cell wall by conjugase present in the gut secretions. The conjugase is derived from desquamated epithelial cells. The same authors also suggested that conjugase cannot be active in the intestinal lumen since the mean peak serum and urinary concentrations of mono and polyglutamates in anaemic and healthy individuals were found to be comparable. Hagsted (1974) also confirmed that the removal of glutamic acid side chains from folate facilitates their absorption.

The knowledge of absorption of folates from foodstuffs is important if accurate estimates of folate requirements or the adequacy of a particular diet as a folate source is to be known. However, it is beset by many difficulties. Sometimes a folate-rich food product may show only a small rise in urinary excretion. Slight rises in serum folate are hard to estimate. Similarly, it is against ethics to give isotopically labelled food to human beings.

No unanimous or direct information is available as to the amount of folate required in the diet to maintain a normal folate level in an individual as the requirement seems to be influenced by many factors (Malin, 1975; Rodriguez, 1978) such as age, state of health, for example, tropical versus non-tropical sprue, pernicious anaemia, iron status etc., drug use and abuse, and dietary intake. Nevertheless, an estimate can be made considering the amount of folate required to be given to a patient with pernicious or other types of anaemia to maintain a normal blood level. The Expert Group of FAO/WHO (Malin, 1975) recommended as daily doses, 40 µg for children of 0-6 months, 60 µg for children of 7-12 months, 100 µg for 1-12 years of age, 200 µg for those of 13 years and above and 300-400 µg for pregnant or lactating women.

DEFICIENCY: Folate deficiency is more rampant in premature infants, pregnant women, geriatrics and in patients with tropical sprue (Malin, 1975).

Many causes may be responsible for the deficiency (Hallaway, personal communication). These include the following:

(1) Dietary: A low intake due to poverty, famine, alcoholism, destruction while cooking, institutional cooking, high cost of food at certain times of the year, etc., may cause deficiency.

(2) Malabsorption: This may be caused by tropical or non-tropical sprue, intestinal bypass, inhibitors in certain foods or pH that is not optimal for conjugase activity.

(3) Increased requirements: These requirements may be due to growth in children and due to pregnancy and lactation in women.

(4) Pathological: Increased DNA synthesis due to rapid, cell division under the following conditions may cause deficiency of folates:-

(a) In haemolytic anaemia due to malaria, sickle cell anaemia or haemolysis due to other causes, red blood cells are made rapidly and therefore DNA synthesis is greater. As acquired resistance of foreign body (malaria) is lost during pregnancy to allow the mother to "tolerate" the foetus, a deficiency of folates is more serious and the demand of folates is high to make up for the haemolysis and need of the foetus for folates.

(b) Cancers and tumours also increase demand by rapid division of cells.

In man folate deficiency causes a megaloblastic anaemia which is almost indistinguishable from that due to a deficiency of vitamin B_{12} (Malin, 1975). However, FAO/WHO experts (Malin, 1975) state that folate deficiency is the more common cause of this type of anaemia. Sleeplessness, irritability, forgetfulness (Herbert, 1962), mental retardation (Sanada *et al.*, 1975; Thornton and Thornton, 1972), polyneuropathy and cerebral atrophy (Botez *et al.* 1979) are some of the other conditions resulting from folate deficiency.

Chapter 2.

MATERIALS AND METHODS: The procedure adopted followed the method of Fleming, Comley and Stenhouse (1971) and that of Hurdle, Barton and Searles (1968).

REAGENTS:

Water: Distilled deionized water was used in preparing all reagents, at every stage of the assay, and for the final rinsing of glassware.

Media: Bacto microassay culture agar (Difco Laboratories), Bacto B_{12} inoculum broth (Difco Laboratories), and folic acid (PGA) assay medium (Baltimore Biological Laboratory) were kindly supplied by Professor Fleming, Ahmadu Bello University Teaching Hospital, Zaria and were used according to the manufacturer's instructions.

Test Organism: *Lactobacillus casei* was also obtained from Professor Fleming, stored at 4°C and maintained by subculturing once in three months.

Standard solution: Pteroylglutamic acid (10mg) [British Drug House Ltd.], was dissolved in 100 ml of 0.1M phosphate buffer pH7.0. The stock standard was portioned into 1 ml fractions in disposable plastic vials and stored at - 20°C. When required for assay, a vial was thawed on the day of the assay, used once, and discarded.

Control Serum and Haemolysate: Plasma from discarded unhaemolyzed stored blood from the blood transfusion service of Ahmadu Bello University Teaching Hospital, Zaria, was used. Ascorbic acid (0.5g)

[Nutrition Biochemicals Corporation] was added to 100 ml of plasma, which was divided into fractions of approximately 1ml and stored at -20°C. A fraction was thawed on the day of the assay, used once, and discarded.

Five ml of whole blood from a normal person was diluted with 95ml of ascorbic acid solution (1g/100ml) to ensure complete rupture of the red blood cells (RBC). The haemolysate was divided into 1 ml portions, stored, and used in the same manner as the control serum.

Apparatus and Glassware: Seventy-five by 12mm/tubes (75 x 12) were held in a stainless steel rack capable of holding 144 tubes at a time and the rack was covered by a close-fitting lid. The rack was filled with tubes the night before the assay and autoclaved at 15lb/square inch (psi) and 121°C for 15 minutes. The pipettes were soaked overnight in Pyroneg (Diversay, obtained from A.B.U. Teaching Hospital) solution after use, rinsed with tap water for 6 hours, rinsed finally in deionized water and then autoclaved for 15 minutes at 15 psi and 121°C. All glassware was soaked overnight in Pyroneg solution, rinsed, at least three times in tap water, and finally in deionized water before being dried and autoclaved.

Serum Storage Bottles: The tip of a Pasteur pipette was broken off and the pipette was weighed. The tip was pressed into ascorbic acid, and the amount adjusted until the pipette contained 5mg of ascorbic acid. The top of the solid column of ascorbic acid was marked and the pipette used to distribute ascorbic acid to bottles with push-in caps. The pipette delivered approximately 5mg ascorbic acid to each bottle.

Specimen: Raw food samples were purchased randomly from the markets in three villages around Zaria; namely, Giwa, Maigana and Hunkuyi and once every six weeks from Giwa market to study the seasonal variation in folate levels of the food items.

Samples of cooked food were obtained during the mid-dry (December) season and beginning of wet (April) season from people in the three concerned villages. As an extension to this project, raw food samples were purchased from the main Zaria market for the purpose of comparison. Blood was collected by venipuncture (5ml per person) from 74 individuals from the villages under study and from 50 healthy adult donors from the blood transfusion service, A.B.U. Teaching Hospital, Zaria.

The blood was divided into two fractions of 2.5ml. The first fraction was allowed to clot and 1ml of the serum separated from it was stored in a storage bottle containing 5mg of ascorbic acid. The second fraction was stored in a specimen bottle containing Ethylenediamine tetraacetic acid (EDTA) as an anticoagulant. Freshly prepared ascorbic acid solution (1.9ml)[1g/100ml] was added to 0.1ml of blood from the EDTA-treated fraction in order to haemolyze it. The serum and the haemolysates were stored at -20°C until they were assayed. The remaining EDTA-treated specimen was used for haematocrit (packed cell volume, PCV) reading at the Haematology Laboratory, A.B.U Teaching Hospital. The haemolysates were centrifuged

A known volume of stock PGA standard solution was diluted 1 in 500, 000 with sterile ascorbic acid solution. Multiple standards were set up in the tubes containing 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.6, 2.0ml of the dilute standard solution and the volume was made up to 2ml with ascorbic acid solution.

The specimens assayed were pipetted into two sets of triplicate tubes of 0.04ml and 0.02ml respectively. The control serum and haemolysate were included in each assay. Sterile ascorbic acid solution (2ml) was added to all tubes by means of a microlitre dispenser (Pipettman). The assay medium was dispensed in 2ml portions into all standard and assay tubes after the addition of ascorbic acid solution. One drop of inoculum was added to the first triplicate blank standard and to the first of each triplicate assay tubes. The tight-fitting lid was placed onto the rack, and the mixture in the tubes was incubated at 37°C for 48 hours.

INOCULUM: On the day before the assay, a subculture of *L. casei* was inoculated into Bacto B_{12} inoculum broth from Bacto microassay culture agar stock and incubated overnight at 37°C. The overnight culture was then inoculated into the broth on the morning of the assay. Four 30ml screw-capped bottles containing 10ml assay medium and 10ml ascorbic acid solution were autoclaved at the same time as the bulk of the assay medium and ascorbic acid solution. The culture was centrifuged after 6 hours of incubation, and the supernatant broth was discarded. The organisms were resuspended and washed three times in sterile 20 ml of single strength assay medium; the final resuspension was used as the inoculum in the assay.

READINGS: All cells were shaken to disperse the *L. casei* cells before reading turbidity on an Electro Engineering Laboratories (EEL) nephelometer using a red filter. The average reading of the triplicate uninoculated standard tubes was subtracted from the average of the triplicate standard readings, and the appropriate blanks were subtracted from the mean of the duplicate assay readings.

CALCULATION: A curve was constructed relating turbidity to folic acid content of the standard tubes. From this standard curve the folate content of the assay tubes was read. If the first serum tubes (0.04 ml) contained "X" ng and the second tubes (0.02ml) contained "y" ng, the serum folic acid (SFA) was 25 x ng/ml and 50y ng/ml respectively. If the haemolysate tubes contained "a" ng, and the second tubes contained "b" ng, then the whole blood folate (WBF) activity was 500 a ng/ml and 1,000 b ng/ml. Red cell folate activity was calculated from the equation:

$$RCF = \frac{WBF - SFA\ (1 - (PCV/100)}{(PCV/100)}\ ng/ml$$

Where RCF = Red blood cell folate (ng/ml)

WBF = Whole blood folate (ng/ml)

SFA = Serum folic acid (ng/ml)

and PCV = Packed cell volume (%).

CHAPTER 3
RESULTS

FIG. 1 SERUM FOLATE LEVELS IN 50 HEALTHY BLOOD DONORS FROM A.B.U. TEACHING HOSPITAL, ZARIA.

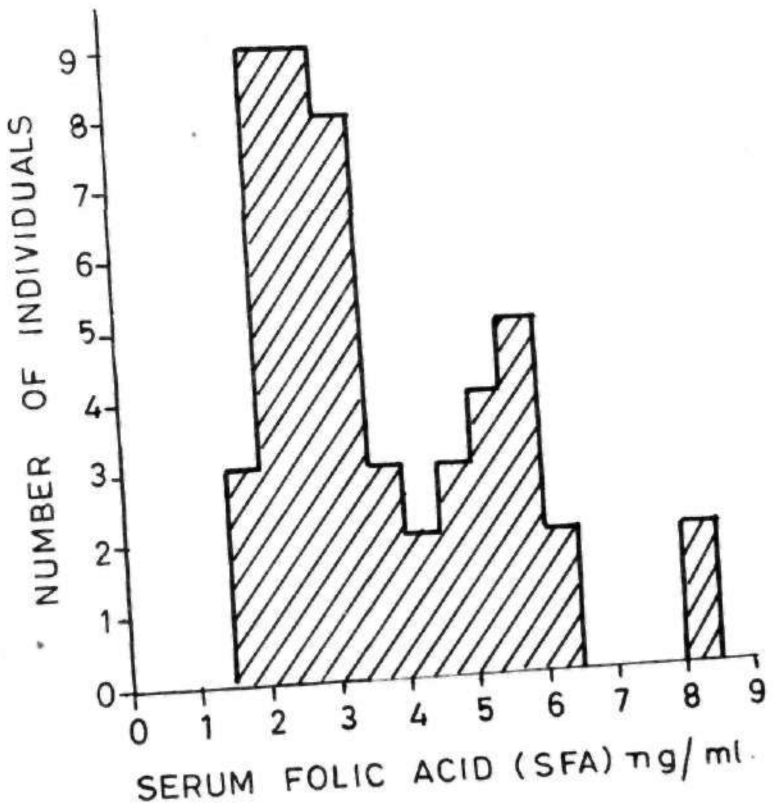
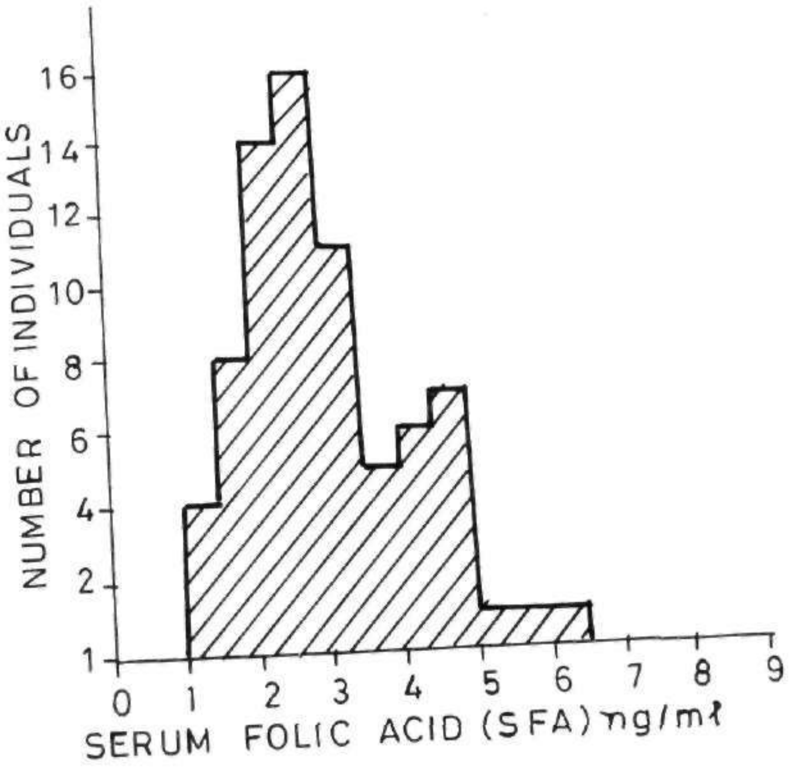


FIG 2 SERUM FOLATE LEVELS IN A SELECTED FULANI POPULATION* AROUND ZARIA



* These folate levels are irrespective of sex and age.

FIG.3 SERUM FOLATE LEVELS IN A SELECTED FULANI POPULATION AROUND ZARIA

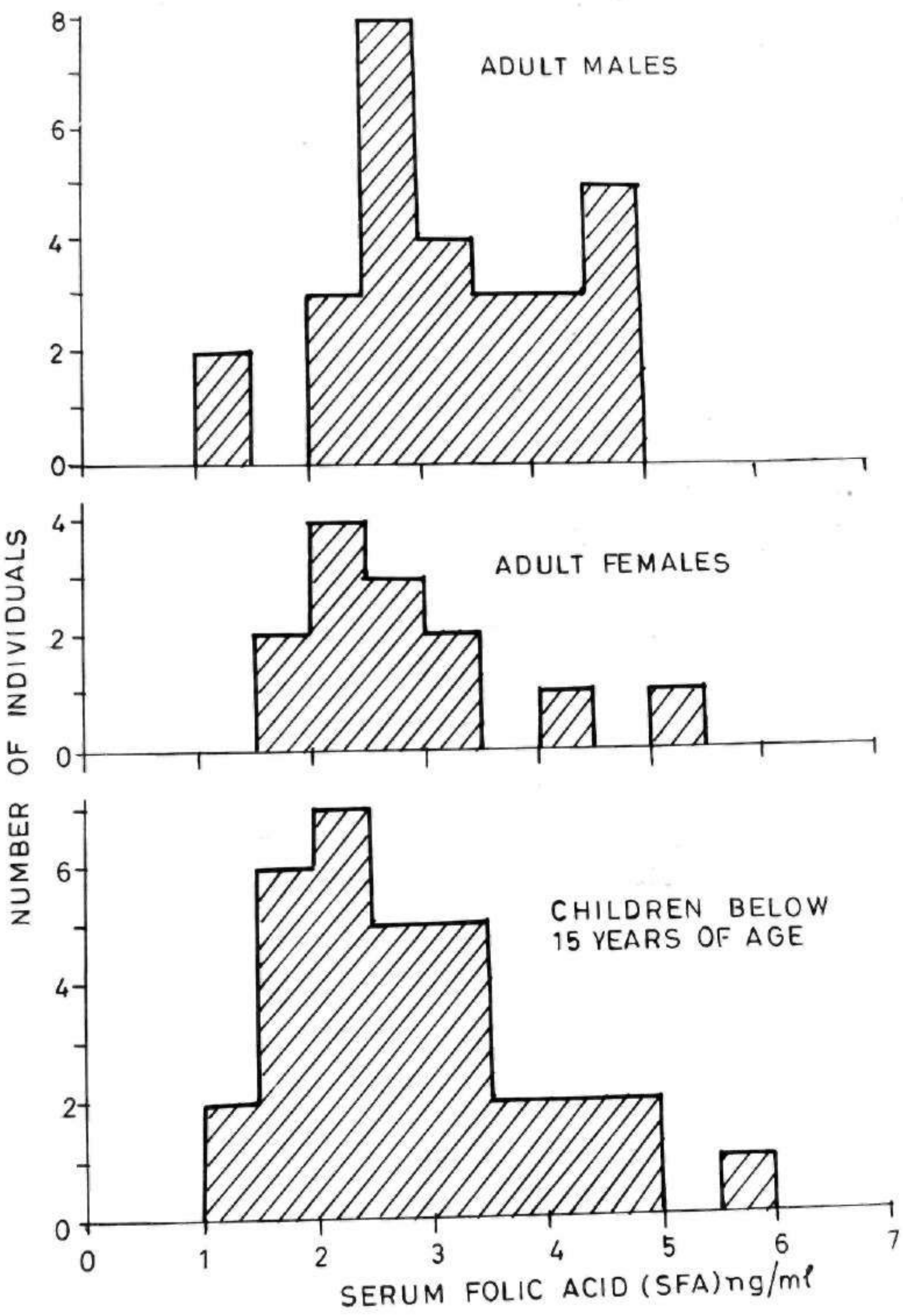


FIG 4 RED BLOOD CELL FOLATE LEVELS IN 50 HEALTHY BLOOD DONORS FROM A.B.U. TEACHING HOSPITAL ZARIA

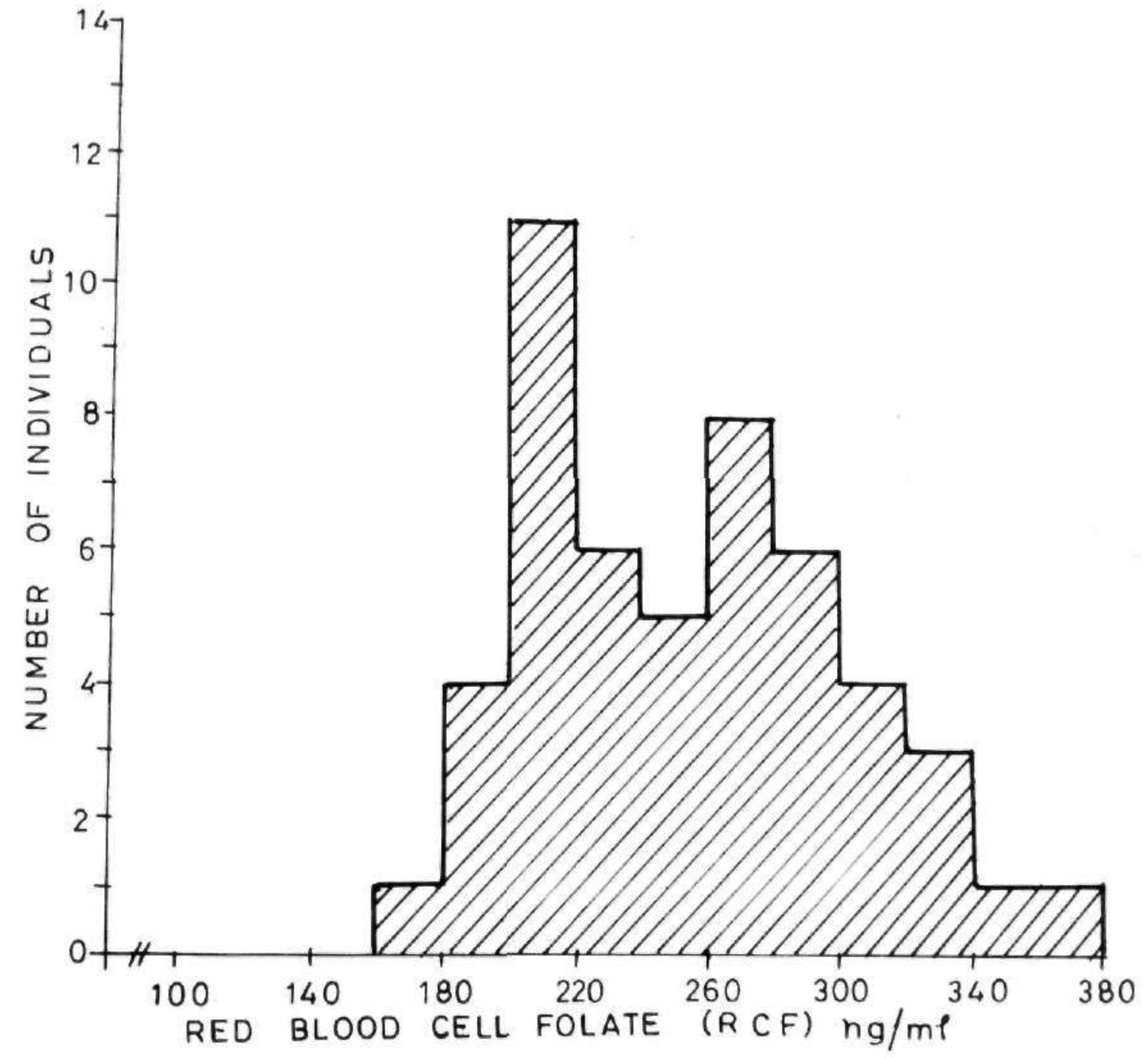
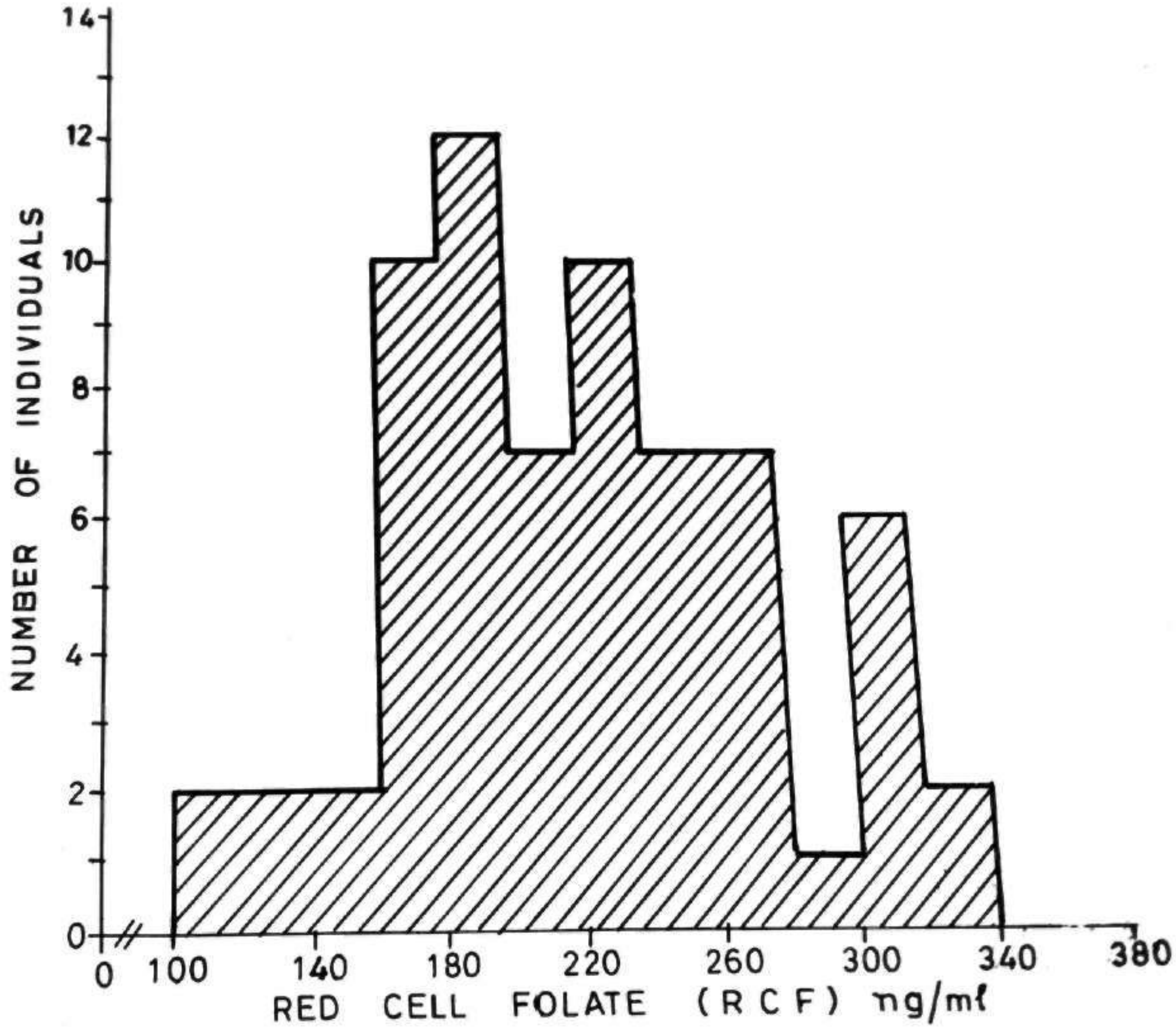


FIG 5 RED BLOOD CELL FOLATE LEVELS IN A SELECTED FULANI POPULATION* AROUND ZARIA



* These red cell folate levels are irrespective of sex and age.

FIG 6 RED BLOOD CELL FOLATE LEVELS IN A SELECTED FULANI POPULATION AROUND ZARIA

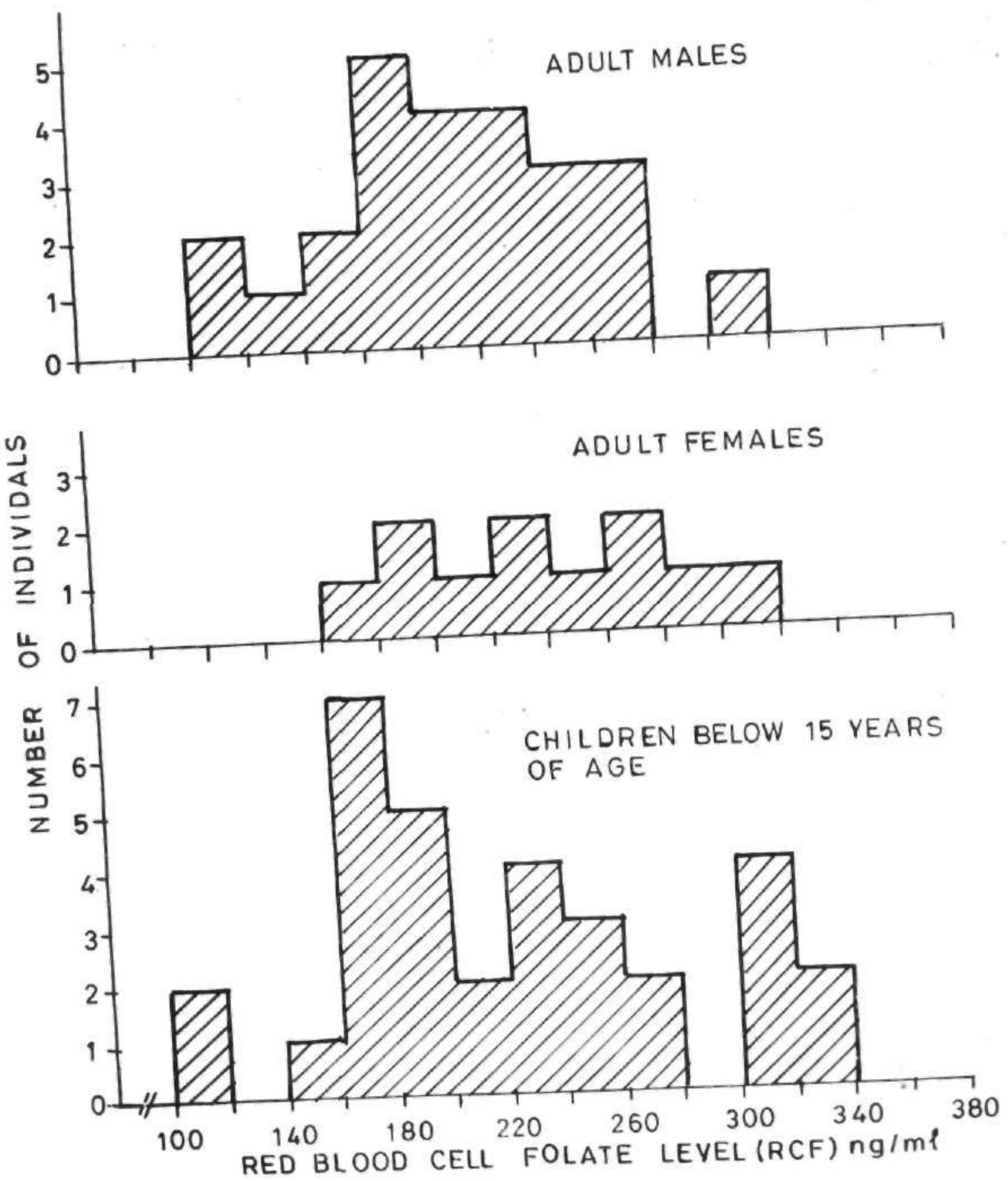


TABLE 1

AGE, PCV[1] VALUES, AND FOLATE RESULTS IN 74 FULANIS AND 50 HEALTHY BLOOS DONORS

		AGE		PCV		SFA[3]			RCF[4]		
	df[2]	MEAN	95 % C.I[5]	MEAN %	95 % C.I	MEAN ng/ml	95 % C.I	RESULT	MEAN ng/ml	95 % C.I	RESULT
Healthy blood donors*	49	a	-	43.8	37.5-51.0	3.42	1.48-7.87	b	249.9	173.3-360.0	-
Fulanis (Total)	73	-	-	41.8	29.0-54.4	2.76	1.28-5.96	N. S.[6]	211.7	124.7-359.0	N.S.
Adult Fulani males	27	-	-	45.8	34.4-57.2	3.01	1.42-6.38	N. S.	205.9	128.1-330.8	S[7]
Adult Fulani females	13	-	-	38.6	28.3-48.9	2.81	1.31-6.05	N. S.	231.9	155.2-346.7	N.S.
Fulani children	31	9	3-15	38.8	21.0-55.0	2.57	1.19-5.56	N. S.	209.7	114.7-383.6	S

$\alpha = 0.0025$

1 PCV = Packed cell volume

2 df = degrees of freedon

3 SFA = Serum folic acid

4 RCF = Red blood cell folate

5 C.I = Confidence interval

6 N. S. = Not significant

7 S = Significant.

a. = Age values were not available.

* Donors only adult males.

TABLE 2.

AVERAGE FOLATE CONTENTS OF SOME RAW FOOD ITEMS AVAILABLE AROUND ZARIA.

Food item	µg folate/g food (dry wt.)
Rice	0.44
Cowpeas	0.49
Guinea corn	0.45
Millet	0.41
Maize	0.46
Popcorn	0.43
Egusi	0.60
Black pepper	0.31
Dried red pepper	0.51
Kuka (Baobab leaves)	0.40
Kulikuli	0.50
Dry okra	0.20
Fresh okra	0.25
Tomato	0.20
Red pepper	0.69
Locust bean cake	0.95
Carrot	0.18
Bread	0.18
Spinach	0.42
Sweet potato	1.94
Cocoyam	0.50
Mango	0.50
Orange	0.11
Milk	0.45

S. D. = + 0.03

TABLE 3.

SEASONAL VARIATION OF FOLATE IN RAW LOCAL FOODSTUFFS[a]

FOOD ITEM	ug FOLATE/g FOOD (DRY WT.)								
	JAN	FEB.	MAR.	MAY	JUN.	AUG.	SEP.	NOV.	DEC.
Guinea corn	0.47	0.05	0.56	0.49	0.45	0.49	0.48	0.48	0.49
Rice	0.42	0.45	0.40	0.45	0.39	0.46	0.45	0.45	0.44
Millet	0.42	0.45	0.43	0.40	0.40	0.40	0.41	0.42	0.41
Bread	0.39	0.45	0.47	0.43	0.39	0.39	0.42	0.36	0.41
Locust bean cake	0.88	0.80	0.79	1.00	0.80	0.84	0.94	0.82	0.91
Kuli-kuli	0.55	0.52	0.50	0.50	0.54	0.52	0.54	0.51	0.52
Spinach	1.86	1.96	1.92	1.97	1.92	1.97	1.82	1.88	1.93
Tomato	0.18		0.18			0.19	0.16	0.17	0.19
Pepper		0.68	0.67			0.62	0.64	0.62	
Okra (Fresh)	x	x	x	x	0.24	0.27	x	x	x
Okra (Dry)	x	0.16	0.18	0.19	0.16	0.19	0.16	x	x
Sweet potato	x	x	0.59	x	x	0.59	0.53	0.58	x
Coco yam	0.53	x	0.55	x	x	0.51	0.54	x	x
Mango	x	x	0.12	0.10	0.12	x	x	x	x
Kuka	0.44	0.46	0.46	0.39	0.42	0.40	0.42	0.41	0.43

S.D. = $\pm$ 0.03

[a]These raw foodstuffs were examined at Giwa at intervals of six weeks.

[x]The food stuff was not available at the given time.

TABLE 4.

FOLATE CONTENTS[a] OF A VARIETY OF COOKED MEALS CONSUMED BY FULANIS IN THE THREE SELECTED AREAS AROUND ZARIA IN THE NORTHERN PART OF NIGERIA.

FOOD ITEM	WET WEIGHT (g)	MOISTURE (% WET WEIGHT)	DRY MATTER (g)	FOLATES (ug/g DRY WEIGHT)	FOLATES/ MEAL (ug)
Tuwo Dawa + Okro soup	521	79.24	108	0.24	26.14
Tuwo Dawa da Taushe	207	78.50	45	0.14	6.18
Sweet potato + Pumpkin soup	550	70.62	162	0.41	66.42
Tuwo Masara (Maize) + soup	300	74.24	77	0.27	20.87
Gero	500	76.67	117	0.17	19.89
Tuwo Dawa + Kuka soup	269	77.07	62	0.27	12.40
Fura da Nono	418	77.53	94	0.21	19.74
Tuwo Masara (Maize) + Okro soup	977	59.65	394	0.20	78.80
Steamed Guinea corn + Cow-peas	401	56. 6	174	0.35	60.90
Tuwo Shinkafa (Rice) + soup	344	70.84	100	0.17	17.00
Boiled Cassava + soup	341	50.55	172	0.04	6.88
Pate	900	84.23	142	0.23	32.66
Tuwo Millet + Spinach soup	275	57.46	117	0.21	24.57
Boiled Yam + Palm Oil	275	70.62	81	0.14	11.34
Kunun Tsamiya (Tamarind)	311	87.90	38	0.14	5.32
Fura da Nono with Guinea corn	751	80.90	143	0.14	20.02
Rice + Chicken soup	394	69.97	118	0.17	20.06
Rice + Meat soup	391	69.10	121	0.19	22.99
Tuwo Dawa + Chicken soup	481	76.04	115	0.39	44.85
Danbu (Butter + Groundnut + Hemp + Jugale + Maize)	125	58.40	52	0.22	11.44

S.D. = $\pm$ 0.05

[a]These values were obtained during rainy season.

TABLE 5.

FOLATE CONTENTS[b] OF A VARIETY OF COOKED MEALS CONSUMED BY FULANIS IN THE THREE SELECTED AREAS AROUND ZARIA IN THE NORTHERN PART OF NIGERIA.

FOOD ITEM	WET WEIGHT (g)	MOISTURE (% WET WEIGHT)	DRY MATTER (g)	FOLATE (ug/g DRY WEIGHT)	FOLATE/ MEAL 1 (ug)
Tuwo Millet + Palm oil	239	61.40	92	0.25	23.00
Steamed Cassava + Groundnut cake	430	27.80	310	0.04	12.40
Tuwo Dawa + Kuka soup	348	72.13	97	0.28	27.26
Tuwo Millet + Kuka soup	266	72.73	73	0.23	16.79
Fura da Nono	252	81.87	46	0.15	6.90

S.D. = $\pm$ 0.03

"These values were obtained during the dry season."

CHAPTER 4

RESULTS AND DISCUSSION

As stated earlier, there is need for establishing the normal range for a parameter for a particular population by determining the frequency distribution of values. This can be accurately characterized by mean and standard deviations of the mean. Ideally, the whole Fulani population should have been studied to obtain true representation of the whole community. However, this was not possible and the investigator had to study the population most accessible to her.

Figures 1-3 and 4-6 summarize the results of estimations of serum folate and red blood cell folate levels respectively. Most of the figures showed two peaks except for the RCF levels of adult male Fulanis and the SFA levels of Fulani children (Fig. 3). The first peak in all cases was more prominent which implied that, as expected, fewer people in the population have a higher content of serum and red blood cell folate on the distribution curve. The position of the peaks for the general Fulani population appeared to be the same as those for the healthy control subjects but as shown by the breakdown of the peaks (Figs. 3 and 6), the children had a lower mode.

PCV results were available on the subjects (Table 1). The mean PCV values of Fulani women and children was found to be lower than the other groups. The same pattern was reflected in the SFA values although the difference did not reach formal significance. The normal range for the SFA levels of blood donors (1.48-7.8 ng/ml) was lower than that obtained by 17 different laboratories (4.6-15.8 ng/ml) (Rodriguez, 1978). However it agreed with the range reported (2.39-10.72 ng/ml) for 95 female nurses and laboratory technicians (Fleming, 1971). Two of the control subjects showed indications of folate deficiency with RCF levels of 174 and 191 ng/ml and SFA values of 2.25 and 1.72 ng/ml respectively. Seven men, 6 women and 17 children had SFA values towards the lower limits of the 95 % confidence interval; 2 men and 2 children had SFA levels in the "danger zone" (that is, levels lower than the lowest levels of 95 % confidence limit) while 9 men, one woman and 13 children had RCF levels below the population mean and 2 men and 2 children were deficient. (Fig. 3). When compared with the control subjects, 15 men, 11 women and 23 children had SFA levels below the mean of the control subjects; 2 men and 2 children were deficient in SFA (Figs. 1 and 3). Seventeen men, 6 women and 13 children had RCF values towards the lower limits of the 95 % confidence interval of the control subjects and 3 men, one woman and 8 children were RCF deficient. (Figs. 4 and 6). The RCF levels in Fulani men and children were found to be significantly lower than those of the control subjects. Because man obtains his folate supplies from food, it was necessary to look at the food folate results. The folate content of uncooked food items usually eaten by local Fulanis as available in the markets in the three villages under study, have been shown in table 2. Each value was constructed from the mean of three samples. Comparison with published figures (Hurdle et al. 1968) showed higher than mean values. This might be attributed to the fact that in earlier investigations carried out by Hurdle, Barton, and Searles, (1968) the authors autoclaved the food products. It has been shown in this investigation that such a treatment caused a 60-70 % loss in the activity by Lactobacillus casei. In place of the heat treatment, membrane sterilization coupled with application of ascorbic acid as a preservative was employed. Such latter treatments often gave higher folate values than autoclaving. Hurdle, Barton, and Searles (1968) also confirmed this observation when they recorded higher folate levels following preservation with ascorbic acid than values obtained after use of conjugase.

Among the food items analyzed, sweet potato has the highest level of folates (1.94 μg folate/g dry weight) [Table 2] but it is not very commonly eaten by the people under study. Locust bean cake, a food-flavouring agent employed in almost all soups had a high folate content (0.95 μg/g dry weight). Cereals fell about mid-scale and contained almost uniform levels of folate. Millet and guinea corn are the two cereals most commonly consumed as staples. Maize is grown around the houses and is eaten boiled and roasted when fresh and it is used in powdered form for food (tuwo) throughout the year. However, it is used much less than millet or guinea corn. Rice seems to be reserved for festive occasions and it is eaten boiled or as mashed rice (tuwo shinkafa) accompanied by meat or chicken and is something of a **luxury.** Other sources of carbohydrate are rare.

Carrot and coco yam are not too important in the Fulani diet. Leaves which are called the "protective foods" of the human race (McCulloch, 1929) are consumed by the Fulanis. Fresh or dried kuka leaves are added to almost every soup. Fruits, for example, mangoes and oranges are eaten only occasionally when they are in season.

Table 3 shows the result of an investigation conducted in order to find out the variations in folate levels in food items available at various times of the year in the chosen villages. In an earlier work, Fleming (1960) reported that the folate levels in a selected population in Southern Nigeria was generally higher in mid-dry season than in the mid-wet season. The variation in folate levels in food items did not achieve statistical significance at the 95 % confidence interval ($P > 0.025$). In the case of fresh okra, sweet potato, coco yam and mango, the absence of figures indicates non-availability of the food item at that particular time of the year. Locust bean cakes had a higher than usual value in May but this is to be expected as it is a combination of ingredients whose proportions cannot be constant every time. The same is true for bread.

Tables 4 and 5 show the result of a similar investigation conducted on cooked meals frequently consumed by the Fulanis in the selected villages. This investigator measured the folate levels in these locally prepared foods during the rainy (wet) season (Table 4) and during the dry season (Table 5). Unfortunately the data from the dry season (Table 5) could not be statistically reliable since it was not possible to obtain commonly cooked foods for both seasons. Under this situation, no firm conclusion can be drawn on the effect of season on the folate contents of cooked foods in these villages.

In addition, Tables 4 and 5 would enable one to estimate the amount of folate lost during the process of cooking and the amount of folates being taken in by an individual. Only approximate estimates can be made because there are discrepancies in the data available. Attempts were made to obtain individual servings for analysis but probably keeping up of appearances in some cases, and generosity in others, as well as individual appetites caused too much deviation in the quantity of food consumed by a person. For instance, 200g of fura da nono was supplied at one time while 900g of it was given at another. The folate level might again depend on the proportions of fura and nono in the meal. Moreover, it is very difficult to define a serving in actual terms as the meal for a family is served in one container, Customarily the children consume these meals after males have fed stomach-full, while women usually contend with the left-overs. If any food remains it is kept against the next meal-time and consumed again.

The effect of cooking or processing of food varies with the type of food, the duration and severity of the process, and the size and condition of the portion of food (Malik, 1967). However, one certain thing about folic acid is that it is destroyed by cooking to a lesser or a greater degree, depending upon the temperature and time of the water and heat treatments, that the food undergoes. As a matter of fact it has been found to be the most susceptible of all vitamins (Malin, 1975). The loss is aggravated when the food is finely divided. A much larger quantity of food, therefore, is required to supply the same amount of folic acid than raw ingredients would. Like other ethnic groups of people, the Fulanis value their habits, tastes and their ancestors' experiences and this is reflected in the traditionality of their food. The recipes for the preparation of some of their food items are given in Appendix 4. Cooking is thorough and this would suggest that most of the folic acid is destroyed in the process of cooking.

Carbohydrate forms the bulk of the meal in most cases where the soup is added as a sauce rather than as a main item of a meal. However, the moisture content of all except one meal was found to be more than 50 %. The solid material, therefore, consumed by a person would be relatively very low compared with the bulk of the food. As expected, considerable losses were found in cooked meals. Boiling of the rice caused about 70 - 75 % loss of folate while about 65 - 70% of the folate was lost when the rice was processed into tuwo (Table 4). As explained earlier, it is very difficult to ascertain the amount of food ingested by an individual from the population under investigation. However, the amount of folate consumed by an individual from these villages in a day could be estimated from values in (Tables 4 and 5) to be about 80 - 120 µg, which, according to the Expert Groups of FAO/WHO (Malin, 1975) is not enough for adults, pregnant and lactating women. The intake of folate recommended by them is 200 µg for adults and 300 - 400 µg for pregnant and lactating women.

No significant difference was found in the folate levels due to age (Table 1). Although by observation the men had the first choice and a greater share of food, they had significantly lower RCF values than women. This might be due to a greater folate requirement for males than females as suggested by some other workers (Rodriguez, 1978).

It is demonstrated that even with a restricted diet, specific diseases associated with folate deficiency are not always immediately observable. However, such diets impair health and diminish the resistance of an individual to infection. Therefore one has to look into the possible causes of malnutrition both immediate and remote. In this investigation they are:-

(1) The households had few earning members with a large number of dependants so that an economic use of food was necessary. The children, especially, were apparently malnourished.

(2) The women involved in the cooking of the foods were illiterate who caused destruction of most of the folates in the food by wrong preparation of the food, such as washing and soaking food in large quantities of water, or over-cooking food.

(3) Over-dried foods need a longer time in order to be edible. Poor storage also leads to great losses, in the fields, market places and houses, of food which could otherwise be eaten.

(4) The wrong choice of foods, for example, cheap and stomach-filling starch, is preferred to vitamin-rich foods.

(5) In most families there is an uneven distribution of foods so that not all members of the family get a fair share of the foods.

(6) Some people are influenced by taboos and prejudices.

(7) The availability of folate varies from food to food. Individuals also differ in their ability to absorb the folacin from different foods.

Folate deficiency is related to some other deficiencies and diseases, such as malaria. The interrelationship between iron and folic acid is complex and there are contradictions in the literature (Rodriguez, 1978). Some investigators suggest that the folic acid requirement of an individual may depend on his iron status. However, iron deficiency has been indicated to interfere with the utilization of folic acid. The relationship of folic acid with ascorbic acid is still uncertain but there are reports of anaemia being corrected by ascorbic acid (Rodriguez, 1978). The relationship between vitamin B_{12} and folic acid is complex. However, vitamin B_{12} deficiency interferes with the metabolism and storage of folic acid (Rodriguez, 1978). Zinc depletion contributes to lack of absorption of polyglutamates as the intestinal conjugase is zinc-dependant (Tamura et al., 1978). The incidence and effect of the deficiencies will be clear on completion of the work of another investigator from this department.

The success of any work aimed at improving the nutritional status of a community depends on its acceptance by the people of that community. However, such an advice is usually good in communities that can afford a great variety of foods to obtain from one item what is lacking in another but in poor communities, restricted by poverty and traditions, such an advice is not very practicable.

5. An individual in the community under study ingests between 80-120 µg folate per day and this is not enough for adults and pregnant or lactating women according to the standard of the Expert Group of FAO/WHO (Malin, 1975) which recommends 200 µg for adults and 300 - 400 µg for pregnant and lactating women.
6. No significant difference was found in folate levels due to age differences.

The following recommendations and suggestions can be made:

Folates are essential for man and attempt should be made to ensure their maximum retention while cooking or processing food items. As the various folate derivatives vary in their resistance to different treatments, it is important to know the types and distribution of folate compounds in food items. Attempts were made to identify such derivatives in course of this investigation but these were unsuccessful due to lack of available experimental materials.

One unresolved question, however, is the utilization of available folates from different food items as well as the effect of folate inhibitors in foods consumed regularly.

<u>Contribution to knowledge</u>: The present study has made the following important contributions to knowledge especially in Nigeria:

1. The normal ranges for serum and red blood cell folate levels in a Fulani community in the Northern part of Nigeria have been determined.

2. Attempts have been made to study the variation in food folate levels at different times of the year even though a clear picture has not emerged because of the unco-operative attitude of human subjects.

3. Attempts have been made to study the effect of cooking on food folate levels and the amount of folates ingested in a day by an individual from the population under study. This level of folates clearly falls short of the recommended level by authorities in this area.

Appendices

APPENDIX I

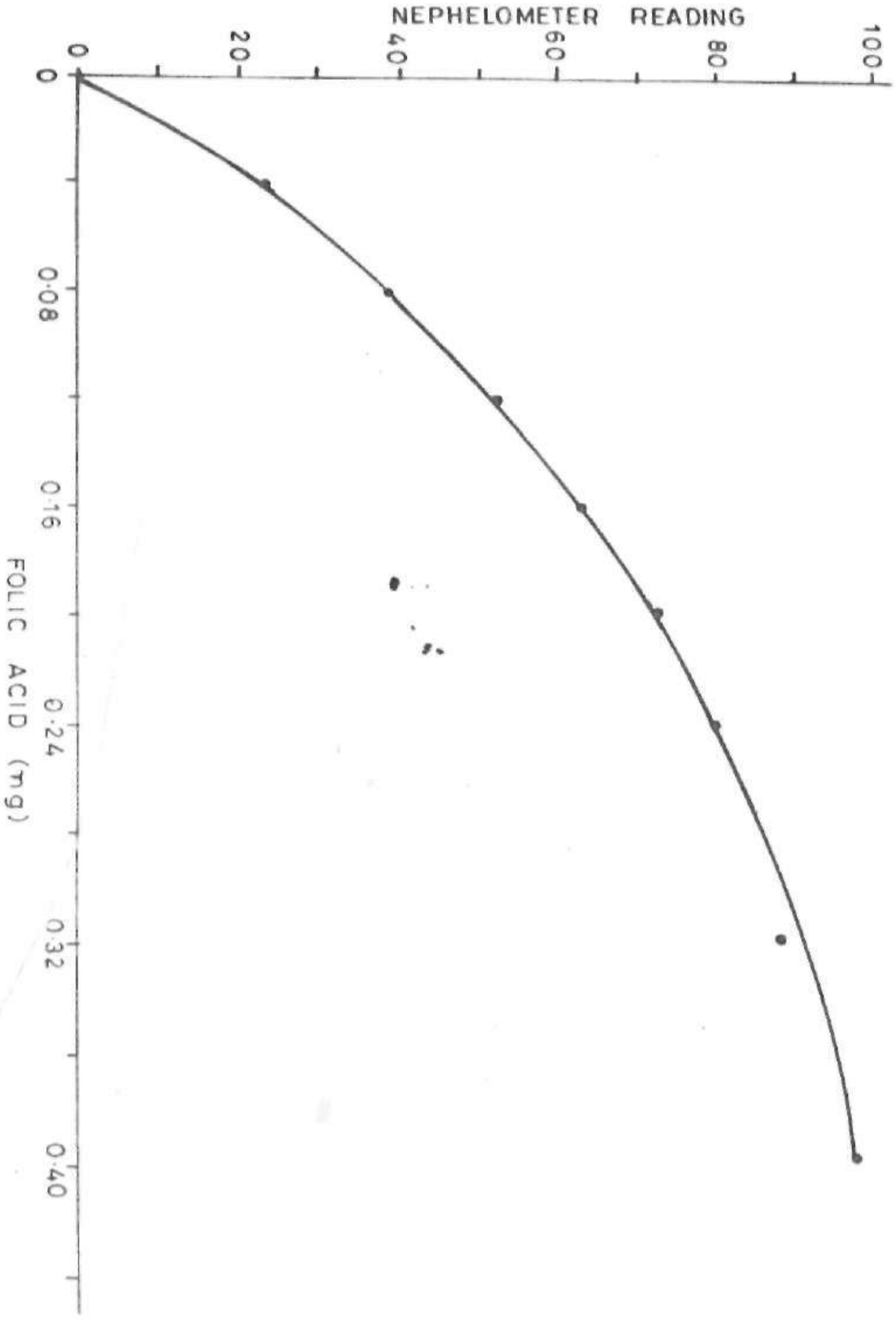
Determination of Moisture Content (A.O.A.C. 1975)

Apparatus: Oven

Crucibles with lids.

Procedure:-

1. Five g of sample was weighed in a weighed crucible and kept in a laboratory oven at 80°C for 24 hours.
2. The crucible was removed, cooled in a descicator and weighed.
3. The drying was continued until two consecutive readings differed by not more than 1 - 3mg.
4. The loss in weight represents the moisture content.



STANDARD CURVE OF NEPHELOMETER READING Vs FOLIC ACID CONCENTRATION

APPENDIX 3(a)

BACTO MICRO ASSAY CULTURE AGAR

Approximate Formula/litre purified water

Proteose Peptone No. 3, Difco.... 5g.

Bacto-Yeast Extract ....20g.

Bacto-Dextrose ....10g.

Monopotassium Phosphate .... 2g.

Sorbitan Monooleate Complex.....0.1g.

Bacto-Agar ....10g.

Final pH 6.8 at 25°C.

Manufactured by DIFCO Laboratories, Detroit, Michigan, U.S.A.

APPENDIX 3(b)

BACTO B_{12} INOCULUM BROTH USP.

Approximate Formula/Litre Purified water.

Tomato Juice	100ml.
Proteose Peptone No. 3, Difco.	7.5g.
Bacto-Yeast Extract	7.5g.
Bacto-Dextrose	10g.
Sorbitan Monooleate Complex	0.1g.
Monopotassium Phosphate	2g.

Final pH 6.7 at 25°C.

Manufactured by DIFCO Laboratories, Detroit, Michigan, U.S.A.

APPENDIX 3(c)

FOLIC ACID ASSAY PGA BROTH

Approximate Formula/litre purified water

Acidicase No. 2

Low salt peptone ...... 5.0g.

L-Tryptophane 1.1

Adenine 0.005

Guanine 0.005

Uracil 0.005

Xanthine 0.01

Asparagine 0.03

L-Cysteine HCl 0.25

p-Aminobenzoic acid ... 0.001

Pyridoxine 0.002

Dextrose 20.00

Maganesium sulphate .. 0.2

Sodium chloride ....0.01g.

Manganese sulphate 0.11

Sodium acetate 20.00

Potassium phosphate 1.00

Ferrous sulphate 0.01

Riboflavin 0.5mg

Thiamine 0.2

Glutathione 2.5

Calcium Pantothenate 0.4

Nicotinic acid 0.04

Biotin 0.010

Final pH 6.6 + 0.02

Manufactured by BBL, Div. Becton, Dickinson and Co. Cockeysville, MD 21030, U. S. A.

APPENDIX 4.

Recipes for the preparation of some traditional meals.

(McCulloch, 1929; Personal Communication)

Tuwo: After husks have been removed by slight grinding, the millet or guinea corn grains are washed, dried and made into flour. A quantity of the flour is added to boiling water and stirred continuously with a long wooden stick until it becomes nearly solid. A little salt is added and stirred into it. It is then taken off the fire. A portion of the cooked tuwo is scooped out from the pot with a small piece of Calabash, placed in an eating bowl and eaten hot with an accompanying soup. In times of scarcity bran is not separated.

Kunun: This is a thin porridge used as a drink. It is prepared as tuwo but with less flour. Millet is more popular than guinea corn.

Fura: This is a solid preparation of flour of grains, chiefly millet, and is usually served in round balls. The coarse flour is prepared by pounding. Sugar is boiled in water. Then pepper and the prepared flour are mixed with the water to make a stiff paste. This is moulded into small balls which are covered with cold water and brought to the boil. After cooling, the cooked balls are broken up into the mortar, pounded, and the paste is again molded into larger balls, which are coated with corn flour to avoid sticking. Fura is usually served with milk (nono) or yoghurt.

Nono (Sour milk): The milk is kept until sour. A few hours are enough for this as the milk is collected under dirty conditions. The pulp of pods of Baobab trees is added to the nono to increase its acidity and viscosity. It is eaten with fura alone or some other grain preparation.

Miya (soup or light stew): Meat (if used) is cut into rough cubes. Tomatoes, onions and fresh pepper are ground into a paste or chopped finely. Salt and daddawa (the black fermented seeds made from locust beans (*Parkia fillicoidea*) are added. The meat is fried in oil, water added and then cooked until tender. Finally, the meat is added to the tomato paste and boiled. However, meat is usually for feasts.

Okra soup: The washed and finely grated okra is added to boiling water. When soft, salt, ground pepper and a little oil are added. After cooking for a few minutes, it can be served with rice or some other grain preparation.

Miya kuka: Tomatoes, onions and fresh pepper are ground into a paste or chopped finely. Then they are boiled in water. The kuka is normally added in a powdered form after the other ingredients or approximately ten minutes before serving.

Yakuwa soup: Salt, pepper and daddawa (locust bean-cake) are boiled in water. Dried yakuwa is added and cooked till soft. Groundnuts are added and mixed to a soft consistency. After cooking for a further few minutes, yakuwa soup can be served hot or cold with rice or tuwo.

Taushe: This is a richer miya. Onions, pumpkin (Cucurbita pepo) and leaves and calyx of yakuwa (Hibiscus sabdariffa) are added. Butter or palm oil are added when afforded. Taushe can be served with fura or tuwo and is usually a diet for the rich.

Kuli kuli: Roasted shelled groundnuts are pounded in a mortar with a pestle until a smooth mixture is formed, This is removed and placed on a tray, kneaded and squeezed, adding a little warm water after each squeeze, until all the oil has been extracted. The groundnut cake is then shaped into even sized balls or rings which are fried in groundnut oil, with a few slices of onion for flavour, until golden brown.

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