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Title: FOLIC ACID AND ASCORBIC ACID STATUS OF ELDERLY SUBJECTS

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Folic acid and ascorbic acid status have been assessed in twenty non-institutionalized elderly subjects with respect to both hematological and dietary aspects.

The hemoglobins, hematocrits, and serum ascorbic acid concentrations were within the normal range. A few subjects had total serum protein concentrations which were less than the acceptable level. More than half of the subjects had serum folic acid concentrations below the acceptable level. Mean dietary nutrient intakes were comparable to the Recommended Dietary Allowances with the exception of calories and folic acid.

In general, the diets of the men were higher in calories, protein and iron while diets of the women were higher in ascorbic and folic acids. The men tended to have higher hematocrits, hemoglobin and serum folates while the women had higher serum

concentrations of ascorbic acid. No significant effect due to vitamin C supplementation was observed. Although there was no significant correlation between the serum concentrations of ascorbic and folic acids, examinations of group means suggested that these parameters were inversely related.

Multiple regression analyses of hematological values and dietary nutrient intakes revealed no significant effects.

Folic Acid and Ascorbic Acid Status of Elderly Subjects

by

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To my parents

TABLE OF CONTENTS

LIST OF TABLES

FOLIC ACID AND ASCORBIC ACID STATUS OF ELDERLY SUBJECTS

INTRODUCTION

The population of elderly persons in the western world has increased markedly within the last few years. As the number of older people increases, more attention is being directed to the functional and structural changes associated with aging. Many factors may influence the health and longevity of older people. Proper nutrition has been suggested as one of the best means of minimizing degenerative changes. However, because of socioeconomic, psychological, physiological and physical factors (Exton-Smith, 1972; Mann, 1973; Schlenker et al., 1973; Corless, 1973), suboptimal nutrition is not an uncommon problem in the elderly. A number of investigations into the extent and degree of folic acid and ascorbic acid deficiencies in the aged suggest that the incidence of deficiency of these two vitamins is higher in elderly people than in younger groups (Forshaw, 1965; O'Sullivan et al., 1968). It is generally accepted that inadequate diet is the major cause of deficiency in the elderly.

It has been long believed that ascorbic acid has a protective effect on folic acid metabolism by enhancing the conversion of folic acid to tetrahydrofolic acid (King, 1968; Bird et al., 1965; Bakerman,

1961). This would suggest that a high concentration of ascorbic acid would be associated with a high concentration of folic acid. However, Loh and Dempsey (1974) demonstrated that the serum folic acid was inversely related to serum ascorbic acid concentration. This is contrary to what has been suggested. The role of ascorbic acid in folic acid metabolism is still unclear and further investigations are required.

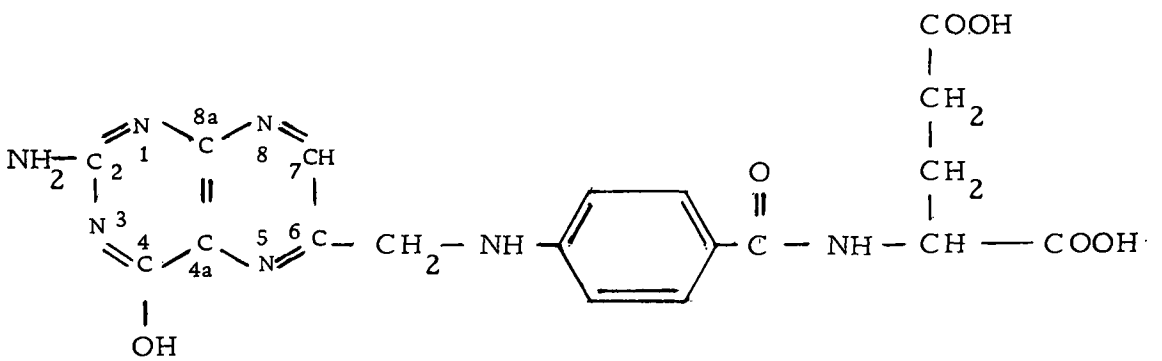
The object of this study is to assess the folic acid and ascorbic acid status of twenty non-institutionalized elderly subjects, in terms of dietary intakes and serum concentrations of these vitamins.

REVIEW OF LITERATURE

Folic Acid

Biochemistry of Folic Acid

Folic acid (folacin, folate, pteroylglutamic acid, PGA) consists of three characteristic building blocks: 1) a derivative of pterine, 2) p-amino benzoic acid, and 3) glutamic acid (Angier et al., 1946).



Pteridine nucleus | p-Aminobenzoic acid | Glutamic acid

Pteroioc acid

Pteroylglutamic acid

The folates naturally occurring in food are a mixture of free folic acid and folate conjugated with glutamic acid. The glutamic acid conjugates are broken down by conjugases in the intestine. During

absorption the free folic acid is reduced by the action of enzymes (reductases) and NADH or NADPH to tetrahydrofolic acid (THF), which is the physiologically active coenzyme form. Ascorbic acid could reinforce this reaction by preventing reoxidation of the reduced folates in the body but there is little evidence to show that ascorbic acid is required for or will facilitate the formation of THF by dehydrofolic acid reductase (Bird et al., 1965; Bakerman, 1961). The folate coenzymes, functioning in the transfer of one-carbon units, are extremely important in biological catalysis. The formyl, hydroxymethyl, methyl, methenyl, methylene, and formimino groups are one carbon units, which may be linked at the N^5, N^{10}, or N^5-N^{10} positions of THF. Six known folate coenzymes which are metabolically active are listed in Table 1. These coenzymes are involved in many important metabolic reactions (reviewed by Huennekens, 1968; Blakely, 1969; Stokstad and Koch, 1967), such as:

1) synthesis of purines and pyrimidines
2) formation of methionine from homocysteine
3) metabolism of other amino acids including interconversion of serine and glycine, hydroxylation of phenylalanine to form tyrosine, catabolism of histidine, and formylation of glutamic acid
4) synthesis of choline from ethanolamine
5) other reactions, such as the interconversion of imidazole

Table 1. Folate Coenzymes (Stokstad and Koch, 1967).

Form	One carbon unit	Oxidation state
N^{10}-formyltetrahydrofolic acid (10-CHO-THF)	-CHO	Formate
N^{5}-formyltetrahydrofolic acid (5-CHO-THF)	-CHO	Formate
N^{5},N^{10}-methenyltetrahydrofolic acid (5, 10=CH-THF)	-CHO	Formate
N^{5}-formiminotetrahydrofolic acid (5, 10-CHNH-THF)	-CH=NH	Formate
N^{5},N^{10}-methylenetetrahydrofolic acid (5, 10-CH_2=THF)	-CH_2	Formaldehyde
N^{5}-methyltetrahydrofolic acid (5-CH_3-THF)	-CH_3	Methanol

carboxamide and inosinic acid, and the interconversion of creatine and guanidoacetic acid; methylation of nicotinamide to N^1-methylnicotinamide

Physiology of Folic Acid

N^5-methyltetrahydrofolate appears to be a predominant form in serum, liver and other tissues. As noted above, food folate exists mainly in conjugated form. In 1971, Rosenberg and Godwin reported that there is no conjugated folate response in the serum after the oral administration of conjugated folate, but there is a rise in free folate activity. Butterworth et al. (1969), using ^{14}C-labeled polyglutamates, demonstrated the intestinal deconjugation of ingested PGA. It is believed that these glutamate conjugates are deconjugated before release into the blood stream. Both clinical observations (Cox et al., 1958; Sheehy et al., 1961) and experimental evidence (Rosenberg and Godwin, 1971) support the belief that the hydrolysis of conjugated folate occurs on the mucosal surface of the intestinal cell by action of conjugases. Hepner et al. (1968), using perfusion techniques, demonstrated that the free folates or monoglutamic folates are absorbed in the proximal intestine, duodenum and jejunum. The mechanisms of transport of PGA and its derivatives are: 1) passive transport or diffusion (Rosenberg and Godwin, 1971), and 2) active transport or energy dependent transport (Hepner et al., 1968).

Reduction and methylation have to be completed before the folates enter into the blood stream. It is agreed that the reduction may need to precede methylation (Zakrzewski and Nichol, 1960; Bernstein et al., 1970). Conflicting evidence leaves the mechanism of methylation unclear. It has been suggested that 5-methylation of reduced PGA may take place in the liver or during passage through the gut wall (Chanarin and Perry, 1969; Whitehead and Cooper, 1967; Bernstein et al., 1970).

The serum folates are delivered to bone marrow cells where hematopoiesis takes place. Folic acid is one of the vitamin coenzymes necessary for cell proliferation, since it is required for DNA synthesis. If a patient is deficient in folic acid, then all forms of folate are in reduced supply, and the conversion of deoxyuridylate to thymidylate occurs with reduced speed. One of the signs of disturbance of nucleoprotein metabolism during lack of folic acid is the increase in the ratio of RNA/DNA. At the same time, this is accompanied by the characteristic "nuclear-cytoplasmic dissociation." The maturation of the red blood cell is disturbed. The nucleus appears immature and cytoplasm appears mature. It is called megaloblastic anemia.

The total body storage of folate is about 5 to 12 mg, primarily in the liver. The daily excretion of folate is approximately 0.1 mg in bile and 4 to 5 μg in urine (FAO-WHO, 1970).

Assays of Folic Acid

The folic acid activity in serum, plasma, red blood cell or whole blood has been the subject of a great number of studies aimed at providing a convenient and practical means of detecting folate status in human subjects. Even though a radioisotopic assay was developed in 1970, the most frequently used procedure is still the microbiological method for measuring tiny quantities of folic acid activity, as in serum (nanograms per milliliter of serum). Three organisms are commonly used to assay folic acid activity: Lactobacillus casei, Streptococcus faecalis, and Pediococcus cerevisiae (Leuconostoc citrovorum). The relative activity of the various organisms is given in Table 2. Among these organisms, L. casei responds to essentially all forms of folate. S. faecalis is active only with the monoglutamate or diglutamate, either in the oxidized or reduced form. P. cerevisiae is apparently active only with folinic acid, N^5-formyl THF. In the human body, serum and plasma contain similar amounts of N^5-methyl THF. Evidence has been presented that the folic acids in red blood cells are predominantly polyglutamates or conjugated derivatives of N^5-methyl THF, which become microbiologically active after conjugase treatment (Usdin, 1959). Since N^5-methyl THF is the preodminant form of folate in serum and tissues, and only L. casei responds to

Table 2. Relative Activity of Assay Organisms for Various Forms of Folic Acid (Stokstad and Koch, 1967).

Compound	*Lactobacillus casei*	*Streptococcus faecalis*	*Pediococcus cerevisiae*
Folic acid (PGA)	+	+	-
Pteroyldiglutamic acid	+	+	-
Pteroyltriglutamic acid	+	-	-
Pteroylheptaglutamic acid	-	-	-
H_4PGA (THF)	+	+	-
5-CHO-THF	+	+	+
10-CHO-THF	+	+	-
5, 10-CH_2=THF	+	+	-
5-CH_3-THF	+	-	-

N^5-methyl THF, the only organism which can adequately measure folates in man is L. casei.

The concentration of folate in serum is usually used to detect folic acid deficiency. However, this test is not totally reliable because of the difficulty of defining the normal range of serum folate levels. As shown in Table 3, there can be a four-fold variation in the estimates of the lower limit of a normal range, which is the critical level of folic acid deficiency. Even though the serum folate concentration was extremely low in some of these studies, no sign of megaloblastic anemia was found. Leevy et al. (1965) suggested that a prolonged period of folate deficiency is required to produce megaloblastic bone marrow, which induces megaloblastic erythropoiesis. It is concluded that the serum folate level is a poor index of the degree of folate deficiency but it does indicate an early stage of folate deficiency.

Few studies have been done on the content of folates in red blood cells. Two difficulties are present in using the red cell folate level as an index of folate deficiency: 1) a low level of red cell folates is also a common indication of pernicious anemia, which is due to vitamin B_{12} deficiency (Cooper and Lowenstein, 1964), and 2) the red cell folate level does not correlate satisfactorily with folate deficiency even when vitamin B_{12} is adequate. A longer period of folate deficiency is necessary to produce depletion of red

Table 3. Serum Concentrations of Folate Derivatives in Normal Subjects.

Reference	No. of Subjects	Serum Folate	
		Range (ng/ml)	Mean (ng/ml)
Cooper and Lowenstein, 1964	33	-	8.1
Spray, 1964	94	2.1-28.0	7.8
Herbert, 1966	2161	7.0-15.9	-
Girdwood et al., 1967	62	2.2-13.6	6.1
Girdwood, 1969	72	2.0-25.0	6.4

cell folates than to produce depletion of serum folates. By the same token, a subnormal level of red cell folates indicates a more serious deficiency than does a subnormal level of serum folates.

A few other methods are used for detecting folate deficiency: the plasma clearance test; determination of folate derivatives in liver biopsy and in leucocytes; and test of urinary excretion of formiminoglutamate (a product of histidine degradation). A general drawback among these methods is in the differentiation between megaloblastic and pernicious anemia. None of the above methods appears successful in detecting a mild or a developing deficiency. Determination of folates in serum appears to be a reasonably satisfactory method of detecting folate deficiency and of differentiating it from vitamin B_{12} deficiency.

Folic Acid in Serum

The variation in results obtained in different laboratories causes a difficulty in defining a normal level of serum folates (Table 3). Generally speaking, a serum folate level above 6.0 ng/ml is considered normal. A serum folate level between 6.0 and 3.0 ng/ml is considered an indication of folic acid deficiency (Sauberlich et al., 1974). Many factors may influence the serum folate level, among them dietary intake (Varadi and Elwis, 1964; Leevy et al., 1965; Forshaw et al., 1964); diseases such as steatorrhea, tropical sprue,

liver disease and hemoglobinopathies; pregnancy (Cowan et al., 1966); body storage of folic acid (Varadi and Elwis, 1967); age (Girdwood et al., 1967; Varadi and Elwis, 1967; Batata et al., 1967; Girdwood, 1969); drugs such as antibiotics (Shojania and Hornady, 1969), anticonvulsants (Gerson et al., 1972; Maxwell et al., 1972; Pritchard et al., 1971; Roe, 1971), oral contraceptives (Roe, 1971; Pritchard et al., 1971), and folic acid supplements (Pritchard et al., 1971; Bernstein et al., 1970). It has not been determined whether or not sex is a factor in the serum folate level (Girdwood et al., 1967); serum folate alters so easily. According to Herbert's experimental results (1963), when an extremely low folate diet (5 mg daily) was given, the concentration of serum folate rapidly fell to 1 ng per ml serum at the end of approximately three weeks. After a supplementary dosage was given, the serum folate level gradually rose toward normalcy over a period of 2 to 4 weeks. Many studies have shown that half of the cases of folic acid deficiency were caused by dietary deficiency (Batata et al., 1967; Forshaw et al., 1964).

Folic Acid in Food

Folic acid is present in nearly all natural food. Yeast, glandular meat, fresh vegetables, mushrooms, and some fresh fruits are foodstuffs with the highest folate content per unit of dry weight. Only a little total folate is present in egg white, meat and

poultry. Much of the folate in food is in the form of conjugates. Jandl and Lear (1956) reported that folate conjugates in an orally administered yeast extract preparation were 25% available. When the conjugates in the same preparation were first hydrolyzed by conjugase treatment, the availability increased to 60%. Hurdle's experiment in 1968 confirmed that incubation with conjugase produced increases in the folate value of cooked and uncooked food. It is believed that conjugase is needed to split conjugates to free and/or pteroylmonoglutamate, which are available for absorption.

It has been reported that cooking and processing may cause up to 95% or more destruction of the total food folate content (Cheldelin et al., 1943; Hanning and Mitts, 1949; Herbert, 1963; Hurdle, 1968). The more finely the food is divided and the longer it is heated (especially in water), the greater the percentage of folate that is destroyed.

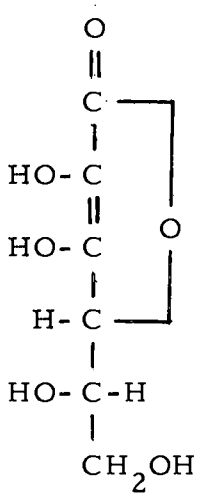
There is evidence that the principle form of folate in orange juice is monoglutamate (Streiff, 1971; Tamura and Stokstad, 1973). However, the availability of folate in orange juice was low. It has been suggested that inhibitory factors may be present in orange juice. Gerson et al. (1971) reported that glucose may enhance folic acid absorption. Thus, not only the presence of conjugase and the method of cooking but also some other inhibitory and enhancing factors may affect the availability of food folate in man.

It is generally accepted that the minimum daily requirement for folate is about 50 μg (Herbert, 1968a). The requirement may be increased by a rise in the metabolic rate or cell turnover rate. The FAO-WHO Expert Group (1970) recommends a daily intake of 200 μg of free folate for adults, 50 μg for infants, 100 μg for children, 400 μg for pregnancy, and 300 μg during lactation. It has been reported that a balanced American diet contains approximately 600 μg (range 379-1097 μg) by L. casei assay, with 10 to 15% of the activity in the free form (Butterworth et al., 1963).

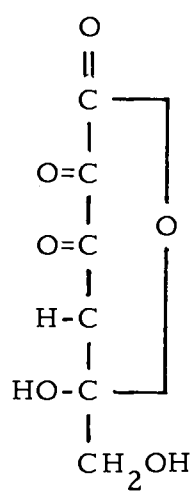
Ascorbic Acid

Biochemistry of Ascorbic Acid

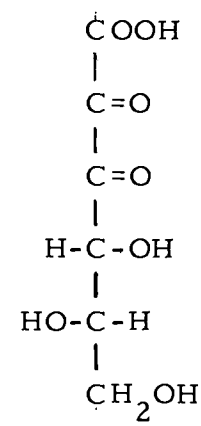
Ascorbic acid (ascorbate, vitamin C) is a rather simple compound, a hexose derivative, with the empirical formula $C_6H_8O_6$.



Ascorbic acid



Dehydroascorbic acid



Diketogulonic acid

Only the L-isomer is active. It exists in nature in both the reduced and oxidized forms, ascorbic and dehydroascorbic acid. Both forms have the same biological activity and remain in a reversible equilibrium state. It may lose vitamin activity by further oxidation of dehydroascorbic acid to diketogulonic acid. The mechanism by which ascorbic acid acts in biological systems remains to be determined. Because ascorbic acid has strong reducing properties, it is believed that most biological functions depend on this property. Also, ascorbic acid plays an important role in hydroxylation. Many hydroxylation reactions have been reported to require this vitamin.

Some of the biological functions, in which ascorbic acid may be involved, are summarized as follows:

1) function in the electron transport chain between NADH and cytochrome b_5 (Staudinger et al., 1961).
2) function as a reducing agent, for instance, reduction of ferric ion to ferrous, protection of the oxidase in tyrosine metabolism (Sealock and Silberstein, 1939), and perhaps maintenance of reduced folic acid coenzymes.
3) function in hydroxylation, such as formation of hydroxyproline from proline (Stone and Meiser, 1962), formation of 5-hydroxytryptophan from tryptophan (Cooper, 1961), metabolism of 3, 4-dihydroxyphenylethylamine to norepinephrine (Levin et al., 1960; Friedman and Kaufman, 1965), and metabolism of cholesterol to bile acids (Ginter, 1972).

4) function as primary antioxidant for vitamin A and polyunsaturated fatty acids, and in the protection of other antioxidants such as vitamin E.

Physiology of Ascorbic Acid

Ascorbic acid is rapidly absorbed from the gastrointestinal tract and distributed to various tissues of the body. Woodruff (1956) demonstrated that a load dose of ascorbic acid equilibrated with the tissues within 4 hours of administration. The distribution of ascorbic acid in the human body appears to be similar to that of the experimental animal (Kuether et al., 1944). The highest concentration is in the adrenal glands, and following in order are pancreas, thymus, spleen, liver, pituitary, kidney, lung and muscle. According to Baker et al. (1971), the body pool of ascorbate is about 1500 mg. It is still not possible to relate the distribution of this vitamin in the tissues to specific functions.

The metabolism of ascorbic acid is very complicated. Isotopic techniques have been used to study the ascorbate turnover. Ascorbic acid is metabolized to diketogulonic acid or oxalic acid which appears in the human urine (Hellman and Burns, 1958; Baker et al., 1966). Recently, the demonstration of ascorbate 3-sulfate in human urine has stimulated interest in the biological role of this metabolite (Mumma, 1968; Chu and Slaunwhite, 1968). Because of

these complexities, the metabolism of ascorbic acid remains obscure. Ascorbic acid is a water soluble vitamin. Once the tissues are saturated, any excess will be excreted in the urine. The renal threshold level is stated to be about 1.4 mg/100 ml plasma, because a rapid increase in the rate of plasma clearance of ascorbic acid occurs when plasma concentrations reach about 1.4 mg/100 ml (Friedman et al., 1940).

Analysis of Ascorbic Acid

Ascorbic acid has a dienol group on carbons 2 and 3, which is easily reduced by losing H atoms. Determination of ascorbic acid is usually based on oxidation-reduction reactions. Two types of analytical method are used in determining ascorbic acid: 1) the method based on measurement of the extent to which the dye, 2, 6-dichlorophenolindophenol, is decolorized by ascorbic acid in biological fluids, and 2) the method based on measurement of the colored 2, 4-dinitrophenylhydrazone formed by coupling oxidized ascorbic acid with 2, 4-dinitrophenylhydrazine in a strong acid solution (György and Pearson, 1967). The former method can measure only reduced ascorbic acid but the latter can measure both the reduced and oxidized forms of the vitamin (total vitamin C). Since the reduced form of ascorbic acid in serum usually represents about 80% of the total, results from the two methods may be

compared. A micromodification of the dinitrophenylhydrazine method has also been developed that is particularly useful for dealing with very small blood and urine samples.

Serum (or plasma) and white blood cell-platelet levels are the most common methods of determining ascorbic acid status. Some investigators have tried to relate the intake of ascorbic acid with the urinary excretion, either by the rate of clearance of ascorbic acid (Friedman et al., 1940) or by the ascorbic acid/creatinine ratio (Lowry, 1952). However, the results showed that the urinary ascorbic acid level is not as informative as is the plasma level.

A number of loading tests have been devised for diagnosing ascorbic acid deficiency in the absence of clinical signs and symptoms. A large amount of ascorbic acid is administered after a fasting serum ascorbic acid determination. Blood is then drawn at hourly intervals during a specified period of time. The responses of serum concentration to the test dose can be plotted. A healthy individual will have a gradually rising curve. It reaches the maximum level three hours later, afterwards a decline in response occurs. The flat responses found in scurvy patients were interpreted as evidence of greatly diminished tissue stores. A normal rise in serum concentration may be found even in patients who have zero fasting serum levels of vitamin C. This indicates the presence of appreciable tissue stores (Dutra de Oliveira et al., 1959). Thus,

this method can be used to differentiate the biochemically deficient state from a partial saturation state.

Urinary excretions after ascorbic acid administration are informative, too. In the study by Lowry et al. (1946), a large amount of ascorbic acid (500 to 2000 mg) was administered on each of four consecutive days. By the last day, it was assumed that no retention occurred. The difference between the amount given and that excreted indicated the metabolic destruction. The total retention by the body, after a correction for destruction, should equal the tissue deficit. Beaton and McHenry (1966) appraised this method as superior to other loading tests, because it gives a rather exact measure of tissue unsaturation. Although these loading tests provide some basis for estimating the adequacy of ascorbic acid intake, they do not provide quantitative information about requirements. Furthermore, because they are time consuming, loading tests are not very practical for use in large population studies.

Ascorbic Acid in Serum

A positive correlation has been found between dietary intake and serum or plasma ascorbic acid levels. An increase in dietary intake corresponds to an increase in plasma ascorbic acid (Loh, 1972a; Lowry et al., 1946; Loh and Dempsey, 1974). A liberal intake of ascorbic acid (75 to 100 mg per day) usually results in a

plasma level greater than 0.7 mg per 100 ml. Crandon et al. (1940) reported that when a person consumed an ascorbic acid-free diet, his serum level approached zero in four to six weeks. In animal studies, a plasma ascorbic acid level of around zero is compatible with tissue stores which may range up to 50% (Lowry, 1952). Thus, the serum or plasma levels reflect recent dietary intake but do not necessarily indicate the amount of ascorbic acid stored in the tissues. At the lower levels of intake, the plasma ascorbic acid concentration is not as precise an index of the state of nutrition as is the white cell-platelet level.

In healthy persons, the ascorbic acid concentration is about 0.4 to 1.0 mg/100 ml serum (ICNND, 1963). Scurvy has not been reported at serum levels of 0.2 mg/100 ml or more. Table 4 presents some guidelines for assessing ascorbic acid status on the basis of serum levels and dietary intake. The Interdepartmental Committee on Nutrition for National Defense (ICNND, 1963) designated a serum level above 0.2 mg/100 ml as acceptable. Hodges and Baker (Goodhart and Shils, 1973) proposed somewhat more stringent guidelines (Table 4).

The serum level of ascorbic acid may be affected not only by dietary intake but also by sex (Brook and Brimshaw, 1968; Dodds, 1969); stress such as bone fracture, surgery, and paraplegia (Barker, 1967; Bourne, 1946; Burr and Rajan, 1972; Jonec, 1964);

Table 4. Guidelines for Judging Ascorbic Acid Status.

	ICNND (1963)		Goodhart and Shils (1973)
	Diet (mg/day)	Serum (mg/100 ml)	Serum (mg/100 ml)
Deficient	< 10	< 0.10	< 0.10
Low	10 - 29	0.10 - 0.19	0.10 - 0.39
Acceptable	30 - 50	0.20 - 0.40	0.40 - 0.59
High	> 50	> 0.40	> 0.60

smoking (Brook and Brimshaw, 1968; Pelletier, 1968; Calder et al., 1963). A low serum ascorbic acid level has often been associated with age, but this is believed to be attributable to low dietary intakes rather than solely to the aging process (Brook and Brimshaw, 1968). A decline in serum levels usually occurs when the metabolic rate is increased, and in tuberculosis and rheumatic fever (Getz and Koëmer, 1941; Wilson and Lubschez, 1948). Mobilization of ascorbic acid from the blood into the area of need may explain the decline. As noted earlier, a renal threshold exists at a plasma level of about 1.4 mg/100 ml. Thus, even massive intakes of ascorbic acid may fail to maintain the plasma level much above 1.5 to 2.0 mg/100 ml, although individual differences may occur.

Ascorbic Acid in Food

Plant products are the major sources of ascorbic acid in the diet. The vitamin occurs predominantly in the reduced form. The distribution of ascorbic acid within an individual vegetable or fruit may be variable. It may be influenced by the variety, degree of ripeness, season or geographic source. In general, the active part of the plant contains appreciable amounts whereas the resting seeds (for example, grain or cereal) are devoid of the vitamin. Handbook No. 8 (Watt and Merrill, 1963) supplies a complete table of ascorbic acid content of food.

Fresh fruit, such as oranges, grapefruit, strawberries, cantaloupe, and guavas, are excellent sources, as are fresh vegetables, like broccoli, brussels sprouts, spinach, kale, and green pepper. The use of potatoes as a staple food in low economic groups can provide a substantial amount of ascorbic acid, although potato is not an excellent source of ascorbic acid.

A warm environment, exposure to air, solubility in water, heat, alkali, metal (especially copper) and dehydration are detrimental to retention of ascorbic acid in foods. Cooking loss may depend on length of cooking time, amount of water added, pH of the medium in which food is prepared, and holding time before serving. It has been found that the average cooking loss of vitamin C in leafy vegetables and potatoes is about 60%. The stability of ascorbic acid in acid medium is demonstrated by the fact that loss of ascorbic acid in tomato during cooking is only about 15% (Teply and Derse, 1958). Processing food may reduce some of ascorbic acid content. On the other hand, ascorbic acid is sometimes added as an antioxidant.

The minimum dietary requirement of ascorbic acid to prevent scurvy is about 10 mg daily (Hodges et al., 1969). The recommended daily allowance of ascorbic acid is 20 mg in the United Kingdom and 30 mg in Canada, Australia and Norway. Forty-five milligrams daily is recommended for the adult in the United States (National Academy of Sciences, National Research Council, 1974). The

average intake per day in an American diet has been estimated at about 117 mg (King, 1968). Scurvy is rarely seen in the United States except for occasional cases of scurvy among infants, small children, and elderly people as a result of consuming severely restricted diets.

Nutritional Status of Geriatric Subjects

Hematological Aspects

A number of investigators have examined hematological values in evaluating the nutritional status of elderly persons. Table 5 shows data from some of the reported studies. Also presented in Table 5, are the values which were considered acceptable by the U. S. Dept. of Health, Education and Welfare in the Ten-State National Nutrition Survey (U. S. HEW, 1972). These values give guidelines for evaluating the nutritional status of the elderly.

Of the various hematological measurements, the volume of packed red cells (Hematocrit) and the hemoglobin concentration are most often used to detect generalized anemia. It has been demonstrated that the hematocrit is highly correlated with the hemoglobin in both sexes (Myers et al., 1968; Elwood et al., 1971). Hematocrit measurements of 38% and 44% have been accepted as satisfactory for adult women and men, respectively (U. S. HEW, 1972).

Table 5. Hematological Values of Elderly.

Reference	Age	Total No. of Subjects	No. of Females	No. of Males	Hemoglobin (gm/100 ml)	Hematocrit (Percent)	Serum Protein (gm/100 ml)	Serum Ascorbic Acid (mg/100 ml)	Serum Folic Acid (ng/ml)
Morgan et al. (1955)	> 50		293	232				1.07(F) 0.83(M)	
Brin et al. (1965)	71					42.3		0.68 0.84(F) 0.55(M)	
Read et al. (1965)	> 59	15							3 - 6
Hurdle & Williams (1966)	> 70	72	46	26					1.6-32.0
Batata et al. (1967)	> 60	14							7.4
Girdwood et al. (1967)	> 65	72	40	32					6.4 2 -25
Myers et al. (1968)	> 65	202	121	81	13.3 13.11(F) 13.62(M)				
Elwood et al. (1971)	> 65	533	304	229	13.4(F) 14.6(M)	41.8(F) 45.7(M)			6.7(F)[a] 7.0(M)[a]
Loh and Wilson (1971)	54-87	45	29	16	13.93 13.5(F) 14.7(M)				

Table 5. Continued.

Reference	Age	Total No. of Subjects	No. of Females	No. of Males	Hemoglobin (gm/100 ml)	Hematocrit (Percent)	Serum Protein (gm/100 ml)	Serum Ascorbic Acid (mg/100 ml)	Serum Folic Acid (ng/ml)
Loh (1972a)	51-57		22	13				0.42[a] 0.41(F)[a] 0.44(M)[a]	
Loh (1972b)	51-88		29	16				0.37(F)[a] 0.41(M)[a]	
	65-92		18	9				0.35(F)[a] 0.49(M)[a]	
U.S. HEW (1972)	> 59	1748	1024	724	13.7(F) 14.7(M)	42.4(F) 45.1(M)	7.2(F) 7.3(M)	1.08(F) 0.84(M)	6.9-7.8(F) 5.9-7.2(M)
Burr et al. (1974)	> 65		429	202				0.37(F)[a] 0.24(M)[a]	
Loh and Dempsey (1974)	68-79	40	27	13	13.54(F) 14.81(M)			0.38(F) 0.34(M)	4.99(F) 1.99(M)
U.S. HEW (1974)	> 60	1938			14.69	43.46	7.06		
Acceptable Values U.S. HEW (1972)					≥12.0(F) ≥14.0(M)	≥38(F) ≥44(M)	≥6.5(F) ≥6.5(M)	≥0.2(F) ≥0.2(M)	≥6.0(F) ≥6.0(M)

[a]Plasma values.

Acceptable hemoglobin concentrations for women and men are 12 and 14 gm/100 ml blood. These standards apply to all adults, regardless of age. The cell components of healthy elderly persons follow the usual pattern observed in young adults, and advancing age is not normally associated with diminution of erythrocyte life span or with compensatory failure (Thomas and Powell, 1971). Nevertheless, there is some evidence that the hemoglobin level in old age, although acceptable, may be significantly lower than in young adulthood (Loh and Wilson, 1971; Myers et al., 1968). However, Myers et al. (1968) concluded that any fall in hemoglobin in the elderly must be attributed to an abnormality of health or environment and not to age alone.

The incidence of anemia in the elderly has been studied in many surveys. Myers et al. (1968) found that only 2% of 202 fit elderly people had hemoglobin concentrations below the WHO recommended normal range. In South Wales, Elwood et al. (1971) obtained a figure of 10%. In a similar study, Batata et al. (1967) found 33% of the patients over the age of 60 had hemoglobin below 11.7 gm/100 ml blood. In 1973, Morgan et al. reported that 25% of the elderly people in their study had hemoglobin levels below the WHO recommended normal range. There was no difference in incidence between the sexes. It is apparent that the incidence of anemia in the elderly is considerable and that its prevalence increases as age advances.

Anemia may be caused by blood loss, malnutrition, and malabsorption. Iron deficiency is the major cause of anemia, but depletion of vitamin B_{12} and folate is particularly prevalent among the elderly (Thomas and Powell, 1971; Parsons et al., 1965). However, since multiple deficiencies are often seen in the elderly, extensive hematological studies are usually necessary to differentiate between the types of deficiency.

Protein fractions in the plasma were studied by Haferkamp et al. (1966). There was no significant difference in the total protein content between different age groups. Thomas and Powell (1971) measured the total protein in 293 elderly persons. In the majority of subjects the serum protein level ranged from 6.1 to 8.0 gm/100 ml. No sex variation was found. A concentration of 6.5 gm/100 ml is considered acceptable for all adults (U.S. HEW, 1972).

The incidence of low serum folate concentration in the elderly has been reported by many investigators (Table 5). Serum folate concentrations of 6 ng/ml are accepted as satisfactory (U.S. HEW, 1972). In the Read et al. (1965) study, 40 out of 50 patients in an old people's home had serum folate levels below 6 ng and ten of them were below 3 ng/ml. Hurdle and Williams (1966) also found that one third of the unselected geriatric patients in their study had low folic acid levels (less than 5 ng/ml). Elwood et al. (1971) confirmed that folic acid deficiency is common in old age in South

Wales. In a community-based survey, 252 out of 553 elderly persons had folate levels below 5 ng/ml. Some investigations designed to compare serum folate concentrations of geriatric subjects living at home and in hospital showed that there is no significant difference between young and geriatric subjects living at home, but that people living in the hospital had a significantly lower level of folate than did those living at home (Batata et al., 1967; Girdwood et al., 1967). Although it is not uncommon to have low serum folate in the elderly, it is not due simply to physiological lowering in old age but chiefly to dietary deficiency (Hurdle and Williams, 1966; Read et al., 1965).

Plasma or serum ascorbic acid falls significantly as age increases. Brook and Grimshaw (1968) stated "The calculated mean annual fall in vitamin C concentration is 0.008 mg/100 ml for each sex." However, use of this guide would still not account for the very low plasma concentrations of vitamin C observed in many elderly. Therefore, these investigators concluded that the low ascorbic acid levels were probably attributable more to low dietary intake than to the aging process. Some reported values for serum or plasma ascorbic acid concentrations of geriatric subjects are shown in Table 5. Sex differences found in the elderly are inconsistent and seem unlikely to be explained entirely by differences in food consumption (Morgan et al., 1955). Loh (1972a) has stated "...there is a

metabolic difference between the sexes in the metabolism of vitamin C when the ascorbic acid tissue status is unstable as in adolescence and in old age but this difference does not manifest itself when the metabolic needs of the vitamin reach equilibrium as in adult males and females." Probably physiological old age and dietary deficiency both play a role in lowering serum ascorbic acid in the elderly.

Dietary Aspects

Nutritional requirements may change with age owing to change in 1) muscular efficiency, 2) physical activity, and 3) body composition. There is a striking decrease in food intake with age (Exton-Smith, 1972). Although there is a considerable difference in nutrient intake between young and aged people, the proportion of calories contributed by carbohydrate, protein, and fat still remains fairly constant (Chope and Breslow, 1956; Steinkamp et al., 1965; Ferber et al., 1973).

With advancing years, several phenomena in the normal aging process might adversely affect digestion. Such conditions as atrophy of the tongue epithelium, impairment of salivary gland secretion, loss of taste, loss of teeth, achlorhydria, deficiency of intrinsic factor, and gallbladder dysfunction may restrict the selection of food to a bland and easily masticated diet. Other factors, such as social isolation and low income may limit the enjoyment of

preparing and consuming food. Any deterioration either in quantity or quality of food may lead to malnutrition or a subclinical deficiency.

Numerous surveys have been undertaken to evaluate the dietary intake of the elderly. Table 6 presents the reported dietary intakes of several groups of elderly persons. Only dietary ascorbic acid and folic acid will be discussed here. According to several reports, ascorbic acid intakes of most elderly persons were greater than 30 mg per day (Table 6). However, the intake of vitamin C has been reported to be very low in some groups of elderly. For example, Woodhill (1970) found that elderly Australians who received meals-on-wheels consumed an average of 19 mg per day. The intake of vitamin C by low income Irish was only 25.4 mg per day (O'Sullivan et al., 1968). Choice of food, cooking loss, and growing seasons may have some influence on the availability of ascorbic acid. There was no difference in the mean intake of elderly males and females (O'Sullivan et al., 1968; Loh, 1972a; Loh and Dempsey, 1974). Since the minimum daily intake of ascorbic acid to prevent scurvy is slightly less than 10 mg, scurvy in the elderly is not often seen.

A high incidence of megaloblastic anemia due to dietary deficiency or folic acid has been reported in persons over 65 years of age (Varadi and Elwis, 1964; Forshaw et al., 1964). Since most elderly patients are unable to give a precise dietary history, a detailed analysis of the diets is usually not possible, but a general

Table 6. Daily Dietary Intake of Elderly.

Reference	Age	Total No. of Subjects	No. of Females	No. of Males	Calorie (Kcal)	Protein (gm)	Folic Acid (μg)	Ascorbic Acid (mg)	Iron (mg)
Morgan et al. (1955)	> 50		293	232				86(F) 99(M)	
Read et al. (1965)	59-95	51	34	17	1643	51.9	52.7(F) 52.6(M)		7.25
O'Sullivan et al. (1968)	60-85	100						26.7	
Dymock (1970)	65-87							31.8 (5-106)	
Pekkarinen and Roine (1970)	71		30		1720			30.0 (12-68)	
	74			18	2214			20.0 (10-38)	
Wirths (1970)		250			2400 (1970-2680)			43.0 (21-63)	
Woodhill (1970)	67-76		6		2214	59.5		51.0	10.62
Evans and Stock (1971)	77 (61-94)	39	25	14	1560	61.0		40.0	8.5
Loh (1972a)	51-87	35	22	13				36.23	

Table 6. Continued.

Reference	Age	Total No. of Subjects	No. of Females	No. of Males	Calorie (Kcal)	Protein (gm)	Folic Acid (μg)	Ascorbic Acid (mg)	Iron (mg)
U.S. HEW (1972)	> 60		1233	895	1412(F) 1949(M)	59.5(F) 80.1(M)		67.0(F) 59.0(M)	9.6(F) 13.1(M)
Ferber et al. (1973)	> 50	102	79	23	2333	69.9		89.6	14.0
U.S. HEW (1974)	> 60	1938			1641	68.41		95.69	11.23
Recommended Dietary Allowance (1974)	> 50				1800(F) 2400(M)	46(F) 56(M)	400(F) 400(M)	45.0(F) 45.0(M)	10(F) 10(M)

description of the diets suggests a folic acid deficiency. Forshaw et al. (1964) calculated a mean folic acid content of only 22 μg per day in the diets of 76 patients with megaloblastic anemia. According to Read et al. (1965), the folic acid intake of people living in an old people's home was significantly lower than that of elderly persons living at home. These authors recommended the use of a regular supplement of folic acid (25 to 50 μg per day) for people living alone.

EXPERIMENTAL PROCEDURE

Description of Subjects

The study comprised 4 male and 16 female subjects, ranging in age from 60 to 87 years. These subjects were participants in a larger study of food acceptance and nutrition of 100 non-institutionalized elderly persons living in or near Corvallis, Oregon. Ten of these twenty subjects were randomly selected from those who were known to take a vitamin C supplement, the other ten from those who did not take any vitamin C supplement. None of them received a folic acid supplement. Most of the individuals ate at home, in a restaurant, or occasionally with relatives. Subjects 9, 11, 17, and 20 had their major meal at the retirement community where they lived. A description of the subjects appears in Table 7.

Preparation of Blood Samples

Thirty milliliters of fasting venous blood were drawn from each subject into three vacutainers. Two vacutainers contained powdered EDTA as anticoagulant (12 mg/10 ml blood). The third vacutainer was untreated. The anticoagulated blood was packed in ice and the hematocrit and hemoglobin were determined within an hour of drawing. Blood in the untreated vacutainer was allowed to

Table 7. Description of Subjects.

Subject No.	Sex	Age	Vitamin C Supplement (mg)
9	M	87	50
11	F	76	0
17	F	80	0
20	F	80	800
24	F	77	0
28	F	72	0
34	F	68	300
36	F	71	250
38	F	75	90
42	F	75	50
45	F	69	600
47	F	80	250
61	M	68	50
63	F	75	0
77	F	85	0
81	F	69	0
84	M	60	0
85	F	63	50
90	F	60	0
91	M	67	0

clot at room temperature. Then, plasma and serum were separated by centrifugation at 3000 rpm for 15 minutes at 0°C. Plasma was stored at -10°C for other investigations. Total serum protein was determined at once. A portion of serum was acidified and deproteinized for later ascorbic acid analysis. The remaining serum was stored in screw-capped vials at -10°C until analyzed.

Blood Analysis

Hematocrit

The hematocrit (packed cell volume) is a measure of the volume of red cells in the whole blood. The microhematocrit was determined in duplicate as described by Richterich (1968, p. 331) and expressed as the percent of red cells in the blood. A MSE microhematocrit centrifuge and microhematocrit reader were used in the determination.

Hemoglobin

The cyanomethemoglobin method (Oser, 1965, p. 1096) was used to determine hemoglobin. Hemoglobin is converted to methemoglobin by oxidizing the ferrous ion of hemoglobin to the ferric ion. Methemoglobin then reacts with cyanide to form cyanomethemoglobin. The intensity of this colored compound is

measured in a Bausch and Lomb Spectronic 20 colorimeter at 540 nm. Because the absorbance of cyanomethemoglobin is directly proportional to concentration, the assay values can be determined by comparing the absorbance of the unknown with that of the standard. Hycel[1] cyanomethemoglobin reagent (No. 116 C) and standard (No. 117) were used. Duplicate samples of blood (20 μl) were analyzed and the results expressed as grams of hemoglobin per 100 ml whole blood.

Total Serum Protein

Total serum protein was determined by the Biuret reaction (ICNND, 1963, p. 133). A violet color is formed when peptide bonds of serum protein react in alkaline solution with the copper salt, which is a component of Biuret reagent. The color intensity of the unknown is measured in a Bausch and Lomb Spectronic 20 colorimeter at 540 nm, and compared with that of standard. Full strength Versatol[2] was used as a standard. The volumes used were one tenth of those used in the original procedure. Samples were analyzed in duplicate and the results expressed as grams of protein per 100 ml of serum.

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[1]Hyland, Division Travenol Laboratories, Cosla Mesa, California.

[2]General Diagnostics Division, Warner-Chilcott Laboratories, Morris Plains, New Jersey.

Serum Ascorbic Acid

A modification of the dinitrophenylhydrazine method of Lowry et al. (1945) was used to determine the serum ascorbic acid (György and Pearson, 1967). Ascorbic acid is oxidized by copper ion to dehydroascorbic acid, and if further oxidation occurs, to diketogulonic acid. Both of the oxidized forms produce stable colored derivatives when coupled with 2, 4-dinitrophenylhydrazine and dissolved in 85% H_2SO_4. The absorbance of the phenylhydrazones was measured with the Beckman DU Spectrophotometer at 520 nm.

The procedure was modified as follows: all measurements were increased by a factor of 10; 10% trichloroacetic acid was used instead of 5%; oxidation was carried out in two hours at 60°C instead of four hours at 37°C (Schaffert and Kingsley, 1955).

On the day of drawing blood, duplicate samples of serum (200 µl) were deproteinized with 800 µl of 10% trichloroacetic acid. Four 300 µl samples of the supernatant were stored at -10°C until analyzed. Analysis of serum ascorbic acid was done in triplicate. The fourth tube was read only when the triplicate results were not reliable. Preliminary studies in this laboratory confirmed the finding of Bradley et al. (1973) that serum ascorbic acid values stablized after a week's storage and could be held for 45 days or so. Samples of this study were analyzed between one and six weeks after

deproteinization. The results are reported as mg of ascorbic acid per 100 ml of serum.

Serum Folic Acid

A modification of the aseptic addition microbiological assay (Herbert, 1966) was used to determine serum folic acid (Brineman, 1974). Lactobacillus casei is the organism used in this study. Two levels of serum, 50 μl and 100 μl, were assayed in this study. Each level was analyzed in duplicate. A standard dose-response curve was plotted against optical density on a semilogarithmic scale and assay values were determined by interpolation. The concentrations of standards used in this study are $1x10^{-10}$, $3x10^{-10}$, $1x10^{-9}$, $3x10^{-9}$, $1x10^{-8}$, and $3x10^{-8}$ grams pteroylmonoglutamic acid per milliliter solution. The results are reported as nanograms of folic acid per milliliter of serum.

Dietary Analysis

A three day diet record was submitted by each subject for evaluation of nutrient intakes. Dietary calories, protein, ascorbic acid, and iron were calculated from data published by Watt and Merrill (1963), Home and Garden Bulletin No. 72 (1971), and Bowes and Church (1970). Folic acid values were derived from data given by The Ohio State University Nutrition Data Base (1973), Hurdle et al.

(1968), Herbert (1963), Toepfer et al. (1951), and Streiff (1971). For some specific foods no published data on food folate content are available. Values for these foods were derived from basic recipes or by correcting for cooking losses (Cheldelin et al., 1943; Hanning and Mitts, 1949).

The average daily intake of each nutrient is reported for each individual. Mean nutrient intakes of the twenty subjects are also reported.

Statistical Treatment

Statistical analyses of experimental data have been completed by computer. The major statistical tests used in this study are the Student's t test and the F test.

Statistically significant correlations among tested parameters have been examined at the $\alpha_{0.10}$ and $\alpha_{0.05}$ levels with 18 degrees of freedom. The Student's t test was used to determine if there was a significant difference in response between supplemented and unsupplemented groups for each parameter. The F test was used in all regression analyses. Both the Student's t test and the F test were made at the $\alpha_{0.05}$ level.

RESULTS AND DISCUSSION

Hematological Values

The blood values of individual subjects are presented in Table 8. The hemoglobin and ascorbic acid concentrations of all subjects were within acceptable limits as defined by the U. S. Dept. HEW (1972), and only one person had a low hematocrit. On the other hand, the serum protein concentrations of five women and one man were below acceptable levels. Even more striking was the finding that eight subjects had low serum folate levels (< 6 ng/ml) and two persons were in the deficiency range (< 3 ng/ml). This finding supports the observations of others that low serum folate concentrations are not uncommon in the elderly (Read et al., 1965; Hurdle, 1966; Elwood et al., 1971).

The mean blood values of subjects, by groups, are given in Table 9. The mean hematocrits, hemoglobin and serum folic acid concentrations are similar to those which have been reported for other aged people (Table 5). The mean total serum protein concentrations are lower than those reported by U. S. HEW 1972 and 1974 (Table 5). The average serum concentrations of ascorbic acid are much higher than those observed in the earlier studies (Table 5).

As might be expected, the mean hematocrit and hemoglobin

Table 8. Blood Values of Individual Subjects.

Subject Number	Hemoglobin (gm/100 ml)	Hematocrit (Percent)	Total Serum Protein (gm/100 ml)	Serum Ascorbic Acid (mg/100 ml)	Serum Folic Acid (ng/ml)
9[ab]	15.8	50	7.2	1.18	2.2
11	15.2	48	6.0	1.31	4.4
17	13.9	43	5.8	1.13	5.2
20[b]	12.8	38	5.9	1.58	10.3
24	14.0	43	6.4	1.73	2.4
28	14.1	44	7.0	1.90	7.3
34[b]	12.1	38	7.0	1.83	9.6
36[b]	13.4	41	7.3	1.80	4.2
38[b]	13.8	42	7.0	2.02	4.4
42[b]	12.5	38	6.0	2.00	6.4
45[b]	14.7	45	6.6	2.46	3.2
47[b]	12.0	37	7.0	1.14	4.1
61[ab]	16.5	51	6.3	1.22	18.9
63	14.8	44	7.0	1.98	24.0
77	14.4	42	7.4	0.52	3.3
81	16.0	47	6.6	1.04	6.9
84[a]	15.4	46	6.9	0.93	3.0
85[b]	13.0	38	6.2	1.69	3.2
90	14.5	42	6.5	1.85	7.9
91[a]	15.8	44	6.0	1.00	7.9
Mean	14.2	43	6.6	1.52	6.9
	$\pm$ 1.3	$\pm$ 4	$\pm$0.5	$\pm$0.48	$\pm$ 5.5
Acceptable values	F$\geq$12.0	$\geq$38	$\geq$6.5	$\geq$0.2	$\geq$ 6.0
	M$\geq$14.0	$\geq$44	$\geq$6.5	$\geq$0.2	$\geq$ 6.0

[a]Male subjects.

[b]Took supplemental vitamin C.

Table 9. Mean Blood Values of Subjects by Groups.

	Hemoglobin (gm/100 ml)	Hematocrit (Percent)	Total Serum Protein (gm/100 ml)	Serum Ascorbic Acid (mg/100 ml)	Serum Folic Acid (ng/ml)
Female	13.8	42	6.6	1.62	6.7
Male	15.9	48	6.6	1.08	8.0
Supplemented	13.7	42	6.6	1.69	6.6
Unsupplemented	14.8	44	6.5	1.34	7.2
All Subjects	14.2	43	6.6	1.52	6.9

concentration of the men was higher than that of the women, and the serum protein concentration was the same in both sexes. Because of uneven subject numbers in the two sex groups, statistical treatment of sex differences was not possible. However, the men, as a group, had higher serum folate and lower serum ascorbic acid concentrations than did the women.

Ten subjects were taking supplemental ascorbic acid. Although the effects of vitamin C supplementation on blood parameters was not statistically significant, the supplemented subjects had a higher mean concentration of ascorbic acid and a lower mean hematocrit, hemoglobin, total protein and folic acid concentration than did the unsupplemented subjects (Table 9). This difference was noticeable in individual values as well (Table 8).

Correlation coefficients among the five blood parameters have been determined. A highly significant ($p \leq 0.05$) linear relationship existed between hemoglobin and hematocrit. This relationship is consistent with the findings of Myers et al. (1968) and Elwood et al. (1971). No other significant correlation was found in this study. However, there was a trend toward an inverse relationship between mean serum folic acid and mean serum ascorbic acid values (Table 9). In other words, high ascorbic acid concentrations tended to be associated with low serum folic acid concentrations and vice versa. Although this result was not statistically significant and was not

apparent in the individual values, it agrees with the report of Loh and Dempsey (1974). These authors suggested that the protective effect of ascorbic acid on serum folate may be questioned and that further investigation of the role of ascorbic acid in folic acid metabolism is necessary.

Multiple regression analyses have been examined by the t test. The results showed that the serum folic acid concentrations, serum ascorbic acid concentrations, and vitamin C supplements had no bearing on either hemoglobin or hematocrit values.

Dietary Intakes

The calculated nutrient intakes of these elderly subjects are given in Table 10 and the mean intakes, by groups, appear in Table 11. The average dietary nutrient intakes were comparable to those obtained in the U. S. Dept. HEW survey of 1974, and even higher than some of those reported in other studies (Table 6).

Caloric intakes were generally lower than the recommended allowances for persons over 50 years (Table 10). This finding was not unexpected. In the first place, the age range of the subjects in this study was 60 to 87 years and their requirements may be lower. The National Research Council suggests that caloric allowances be reduced by 5% per decade, from ages 55 to 75, and that an additional decrease of 7% is appropriate after the age of 75

Table 10. Average Daily Nutrient Intakes of Individual Subjects.

Subject Number	Energy (Kcal)	Protein (gm)	Ascorbic Acid (mg)	Folic Acid (μg)	Iron (mg)
9[a]	1533	67.8	21 (71)[b]	68.3	10.6
11	1352	65.0	150 (150)	181.0	11.6
17	1408	48.7	53 (53)	51.7	7.4
20	983	61.1	116 (916)	109.5	9.4
24	2091	78.1	119 (119)	142.7	13.1
28	1384	62.8	75 (75)	105.0	9.5
34	1773	74.2	202 (502)	123.0	7.6
36	1537	50.4	53 (303)	86.6	8.9
38	1516	57.9	152 (242)	109.9	10.8
42	2368	104.0	145 (195)	231.0	14.6
45	983	69.4	83 (683)	125.2	11.1
47	1113	61.6	18 (268)	49.8	9.1
61[a]	2025	95.2	106 (156)	122.6	16.6
63	1538	71.7	164 (164)	181.1	10.7
77	2087	81.9	73 (73)	112.3	13.9
81	2262	90.0	80 (80)	123.4	11.5
84[a]	2145	81.7	97 (97)	101.4	12.6
85	1612	76.8	104 (154)	147.5	15.8
90	1742	83.5	119 (119)	175.2	11.6
91[a]	2421	91.1	110 (110)	135.2	20.7
Mean	1694 ± 441	73.7 ±14.8	102 (226) ± 225	124.1 ± 44.9	11.9 ± 3.2
Recommended Dietary Allowance (1974) F	1800	46.0	45	400.0	10.0
M	2400	56.0	45	400.0	10.0

[a]Male subject.

[b]Ascorbic acid values in parentheses refer to total intake, including supplements.

Table 11. Mean Daily Nutrient Intakes of Subjects by Groups.

	Energy (Kcal)	Protein (gm)	Ascorbic Acid (mg)	Folic Acid (μg)	Iron (mg)
Female	1609	71.1	107 (256)[a]	128.4	11.0
Male	2031	84.0	84 (108)	106.9	15.1
Supplemented	1544	71.9	100 (349)	117.4	11.4
Unsupplemented	1843	75.5	104	130.9	12.3
All Subjects	1694	73.7	102 (226)	124.1	11.9

[a]Ascorbic acid values in parentheses refer to total mean intake, including supplements.

(National Academy of Sciences. National Research Council, 1968). Secondly, nine of the subjects were more than 25% overweight and six of them claimed to be following a special diet, either low in calories or modified in fat.

The protein intake of all subjects met or exceeded the recommended dietary allowances, but the dietary iron of six subjects was lower than recommended.

On the average, the dietary ascorbic acid was liberal and was derived from fruits, fruit juices and leafy vegetables. Subjects 9 and 47, whose ascorbic acid intake from food was extremely low, were both taking vitamin C supplements (Table 10). This is reflected in their high serum concentrations of ascorbic acid (Table 8).

The estimated dietary folic acid of all individuals was far below the recommended allowances (Table 10). It ranged from 49.8 to 231.0 μg per day. The daily requirement of folic acid for an adult is about 50 μg of pure PGA. However, only 25% of the dietary folic acid is in the free form, the polyglutamates making up most of the dietary folic acid. For this reason, the recommended dietary allowance of folic acid has been set at 400 μg/day for the adult (National Academy of Sciences. National Research Council, 1974). The calculated folic acid values shown in this study may not be truly representative of the actual nutrient content. Only a few

reports have been published on the folic acid content of foods. As a result, many adjustments have been necessary to the calculations. Nevertheless, it seems unlikely that the food folate of any of these subjects approached the recommended 400 μg/day, much less the dietary intake of 600 μg, as estimated by Butterworth et al. (1963). Folic acid is widely distributed in nearly all natural foods. In this study, the dietary sources were mainly orange juice, green vegetables, milk, and cottage cheese. Potato and wheat products supplied moderate amounts of folic acid. However, folate deficiency could easily occur in the elderly, who tend to use cooked rather than raw foods.

Differences in nutritent intakes due to sex have been compared in Table 11. Male subjects tended to have higher calories, protein, and iron intakes than female subjects, but their intakes of ascorbic acid and folic acid were lower. The mean dietary nutrient intakes of different sex groups were comparable to those reported in the U. S. HEW survey of 1972 (Table 6). Although not statistically significant, the unsupplemented group had higher nutrient intakes from food than did the supplemented group (Table 10).

Correlations among dietary parameters have been estimated. Highly significant correlations ($p \leq 0.01$) between intakes of calories and protein, calories and iron, protein and iron, protein and folic acid, and food ascorbic acid and folic acid have been found.

These relations could be explained in terms of the food groups which contribute both nutrients in a fair amount. For instance, both ascorbic acid and folic acid contents are high in dark green vegetables, and fruit juices; folic acid and protein are high in milk and cottage cheese on the basis of a serving.

Correlations of Hematological Values with Dietary Intakes

A significant relationship ($p \leq 0.10$) existed between the concentration of hemoglobin and dietary iron. There was a significant correlation between ascorbic acid intake (with or without supplements) and serum ascorbic acid. This relationship has also been found in many investigations (Loh, 1972a; Lowry, 1946; Loh and Dempsey, 1974). No significant correlation was found between serum folic acid concentration and dietary folic acid intake, even at the ten percent level of confidence. A significant correlation between serum ascorbic acid concentration and dietary folate was observed. A possible explanation of this finding is the fact that the food sources of ascorbic acid also provided a major portion of the dietary folate. When a multiple regression analysis was applied to the dietary and blood parameters, no significant result could be found.

SUMMARY

The folic acid and ascorbic acid status of 4 male and 16 female non-institutionalized elderly subjects have been assessed. Serum levels for folic acid, ascorbic acid, total protein, and hemoglobin and hematocrit have been determined. The average daily nutrient intakes of calories, protein, ascorbic acid, folic acid, and iron were calculated from three day dietary records.

The hemoglobin concentrations, hematocrits, and serum ascorbic acid concentrations were within the normal range. Six subjects had total serum protein concentrations which were less than the acceptable level. Eleven subjects had serum folic acid concentrations below the acceptable level, and three of them were even in the deficiency range. Mean dietary nutrient intakes of protein, ascorbic acid and iron were comparable to the Recommended Dietary Allowance for the age group. Mean daily calories and folic acid intake were low, in general.

Because of the uneven sex distribution, no difference in any of the parameters has been measured between sexes. However, higher hematocrits, hemoglobin and serum folic acid concentrations were observed in male subjects than in female subjects, but the serum ascorbic acid concentration was higher in females than in males. Male subjects generally had higher dietary intakes of

calories, protein, and iron than did female subjects. Female subjects had higher folic acid and ascorbic acid intakes. Correlations among both dietary and hematological parameters have been estimated. There was a significant correlation between serum ascorbic acid and ascorbic acid intake, with or without supplements. No other significant effect of vitamin C supplementation was found. Hemoglobin concentrations were significantly correlated with dietary iron. No correlation has been found between serum folic acid and dietary folic acid intake. In individuals, there was no significant relationship between the serum concentrations of folic and ascorbic acids. However, examination of group mean values indicated an inverse relationship between serum ascorbic acid concentration and serum folic acid concentration. No significant results were found in multiple regression analyses of hematological values and dietary nutrient intakes.

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