THE CORRELATION BETWEEN ASCORBIC ACID EXCRETION AND PLASMA ASCORBIC ACID, LEUKOCYTE ASCORBIC ACID, AND INTAKE OF ASCORBIC ACID IN ELDERLY FEMALES

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TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Adequate ascorbic acid is important in the elderly as well as younger adults. However, past studies indicate that the elderly have lower leukocyte ascorbic acid levels and plasma ascorbic acid levels than do younger adults (Bowers and Kubick, 1965);(Burr, Elwood, Hole, Hurley, and Hughes, 1974);(Vir and Love, 1978).

Low plasma and leukocyte levels of ascorbic acid may contribute to the generalized aches and pains, skin disorders, and the fragility of blood vessels commonly found in the elderly (Bowers et. al., 1965). Wilson (1979) states the aging process alone may initiate these low plasma and leukocyte ascorbic acid levels. In addition, the stress of infection, illness and trauma, the ingestion of certain medication, and poor nutritional intake in the elderly may also cause lower plasma and leukocyte ascorbic acid levels.

In past research, ascorbic acid intake, plasma ascorbic acid level, and leukocyte ascorbic acid level were usually the only variables considered. Since excretion of ascorbic acid was not measured, several questions were raised. In comparison to younger adults, do the elderly excrete more ascorbic acid with a given intake? What is the relationship between ascorbic acid excretion and ascorbic acid intake, plasma ascorbic acid level, and leukocyte ascorbic acid level? The information gained from this study aided in determining if changes in excretion of ascorbic acid accounted for the lower plasma and leukocyte ascorbic acid levels often seen in the elderly.

STATEMENT OF THE PROBLEM

Elderly people tend to have lower leukocyte and plasma ascorbic acid values than younger adults. The purpose of this study was to determine if excretion of ascorbic acid accounts for the lower values by answering the following question: What is the relationship of ascorbic acid excretion to intake of ascorbic acid and plasma and leukocyte ascorbic acid levels?

HISTORICAL PERSPECTIVE

Vitamin C or ascorbic acid ($C_6H_6O_8$) is a water soluble vitamin closely related to glucose. In aqueous solution, the molecule exists in ring configuration and contains a double bond that gives ascorbic acid the property of optical absorbance and optical rotation. L-ascorbic acid has been considered the active form of Vitamin C, but its oxidized form, dehydroascorbic acid, also has antiscorbutic properties.

The majority of vertebrates synthesize ascorbate from glucose in the liver or kidneys. Man, guinea pigs, bats and anthropoid apes, though, are unable to synthesize ascorbate because they lack an enzyme necessary for the conversion of L-gulonolactone to L-ascorbic acid. Lack of this enzyme produces dependence on exogenous L-ascorbic acid in these species (Burns, 1957).

Exogenous ascorbic acid is absorbed actively in the jejunum of man (Mayerson, 1972). After absorption, ascorbic acid enters the systemic circulation to be distributed among various tissues for metabolic and storage purposes. It has not been determined whether ascorbic acid (Hornig, 1975) or dehydroascorbic acid (Okamura, 1979) is the preferred form for uptake by the tissues. In the tissues, ascorbic acid can be oxidized to dehydroascorbic acid and reduced back to ascorbate, or oxidized further to the inactive diketogulonic acid which is metabolized to other compounds and excreted. The only urinary metabolites which have been identified are ascorbate-2-sulfate and oxalate (Baker, Halves,

Johnson, Joyce, Knight, and Tolbert, 1975). The renal turnover of unmetabolized ascorbic acid, ascorbic acid and dehydroascorbic acid is affected by the glomerular filtration rate (normal = 180 liters/day) and tubular reabsorption in the proximal tubule, which normally is almost complete (Berger, Gerson, and Yu, 1977).

Ascorbic acid appears to function in oxidation-reduction reactions such as in protein metabolism (Baker, Hodges, Hood, Sauberlich, March, and Canham, 1971), hydrogen ion transfers, folacin activation, and iron absorption (Sayers, Lynch, Jacobs, Charlton, Bothwell, Walder, and Mayet, 1973). In addition, ascorbic acid is involved in the hydroxylation of dopamine to noradrenaline, the hydroxylation of proline and lysine in collagen formation, and hydroxylation of trytophan to serotonin (Lewin, 1976). Ascorbic acid may also reduce the toxicity of dietary cadmium (Fox, 1975).

One function of ascorbic acid that has been researched extensively is its relationship to atherosclerosis. Several researchers have found increased cholesterol levels in guinea pigs who were deficient in ascorbic acid (Ginter, Babala, and Cerven, 1969);(Fujinami, Okada, Senda, Sugimura, and Kishikawa, 1971). Ascorbic acid may be needed for the transformation of cholesterol to bile acids. If ascorbic acid is deficient, this rate is diminished, causing slower cholesterol turnover rate and increased accumulation of cholesterol in the bloodstream as found in atherosclerosis.

The second effect ascorbic acid has on atherosclerosis is the role it plays in collagen synthesis. Ross and Glomset (1976) described the pathogenesis of atherosclerosis as focal desquamation of the epitheleum

of the vascular wall, which becomes exposed to platelet factors and plasma lipoproteins. Ascorbic acid aids in maintaining the integrity of the vascular wall by its action in collagen synthesis. If the person with atherosclerosis has a continuous good supply of ascorbic acid, it is unlikely that ascorbic acid has been a major factor in causing the disorder. In this circumstance, another factor is responsible for the impaired lipid metabolism and increased ascorbic acid intake would not be beneficial (Peterson, Crapo, Weininger, Ginsberg, and Olefsky, 1975). In addition, Gatenby-Davies and Newson (1974) demonstrated that ascorbic acid does not affect plasma cholesterol levels in subjects with an initial blood cholesterol value of under 200 mg% and may even slightly raise it.

The recommended allowances of ascorbic acid vary widely around the world. FAO-WHO, Australia, Canada and the United Kingdom recommend 30 mg/day of ascorbic acid. The United States recommends 60 mg/day and West Germany recommends 75 mg/day (Report of the International Dietary Allowance of the International Union of Nutritional Sciences, 1975). The wide variance occurs because the lower recommendations are based on allowances to prevent scurvy. The higher recommendations are based on pool size and tissue saturation levels. Ascorbic acid is the only vitamin for which the saturation level in the tissues is felt to be important. This is derived from the idea that the vitamin can improve tissue efficiency and integrity when tissues are saturated (Goldsmith, 1961). Ascorbic acid needs of normal healthy individuals may differ significantly from individual to individual but also the range of ascorbic acid needs within an individual may be extremely wide. Certain circumstances may

necessitate an increased need for ascorbic acid. These include rheumatoid arthritis (Mullen and Wilson, 1976), aspirin usage (Wilson, 1977), high environmental temperature and humidity (Shields, Johnson, Hamilton, and Mitchell, 1945), mineral deprivation (Hodges, Hood, Canham, Sauberlich, and Baker, 1971), bruising or trauma (Burr and Rajan, 1972), usage of alcohol, diethypropion (tenuate) (Wilson, 1977), cigarette smoking (Pelletier, 1975);(Burr et. al., 1974);(Bates, Burr, and St. Leger, 1979), and gastrointestinal operations (Wilson, 1974). Excellent food sources of ascorbic acid include broccoli, brussel sprouts, peppers, turnip greens, and citrus fruits. Ascorbic acid is heat labile and 10 - 15% of ascorbic acid content can be destroyed in cooking procedures.

If intake of ascorbic acid is deficient, the deficiency disease, scurvy, can develop. References to scurvy appeared as early as 1500 B.C. but it was not until 1747 that James Lind, a ship surgeon, successfully treated scurvy with oranges and lemons (Birch and Parker, 1974). Through the combined efforts of King and Waugh (1932), Szent-Gyorgi (1928), and Silva (1923), ascorbic acid was identified as the substance that cured scurvy.

Clinical scurvy can occur within 84 - 97 days in men on an ascorbic acid deficient diet (Hodges et. al., 1971). The symptoms of scurvy, listed chronologically, include petechial hemorrhages, coiled hairs, follicular hyperkeratosis, pain in the joints, swollen gums, and impaired wound healing (Hodges et. al., 1971). In scurvy, ascorbic acid excretion becomes minimal, plasma ascorbic acid levels fall below 0.2 mg/100 ml, and it is speculated that pool size falls to approximately 300 mg

(Hodges et. al., 1971). An intake of 10 mg/day of ascorbic acid is sufficient to cure or prevent scurvy, but the rate of recovery from symptoms is proportional to the repletion dose (Hodges et. al., 1971). In guinea pigs it was found that a scorbutic pig had an increased ability to retain a higher proportion of ascorbic acid than a normal guinea pig and may even catabolize less during repletion (Von Schuching, Enns, and Abt, 1960).

Although clinical scurvy is not prevalent at present, latent ascorbic acid deficiency occurs. Ginter (1979) describes this deficiency as a condition in which the tissue ascorbic acid concentrations are constant and consistently low, but the ascorbic acid deficiency is not manifested outwardly. Since the incidence of foodstuffs rich in ascorbic acid are seasonal, Ginter (1979) believes a large proportion of the population in the cold climates are likely to suffer part of the year from latent ascorbic acid deficiency.

Megadoses of ascorbic acid in the range of 1 - 5 grams have been recommended by the proponents of two theories. The Pauling theory (1971) states that 1 gram of ascorbic acid daily will lead to 45% fewer colds and 60% fewer days of sickness. Anderson (1975), also studying the relationship between ascorbic acid and colds, found that subjects on megadoses experienced 30% fewer days off of work, differences that were statistically significant from the placebo group. On the other hand, although the mean number of episodes of sickness per subject was 7% less in the vitamin supplemented group, this difference was not statistically significant. Wilson and Loh (1973) found no prophylactic effect on the common cold with doses of ascorbic acid ranging from 500 mg to 2000 mg

per day and therefore did not support Pauling's theory (1971). The Stone theory (1966) suggests that the inability to synthesize ascorbate in humans is a genetic disorder that could be cured by an intake of exogenous ascorbic acid in the range of several grams. Stone based his theory on an in vitro study in which the rate of conversion of D-glucuronate to ascorbate was studied in tissue homogenates from ascorbate synthesizing animals. Ginter criticizes Stone's theory because experiments on normal intact rats in which endogenous ascorbate synthesis in vivo was measured by the isotope dilution method yielded much lower values.

Anderson (1975) found no toxic side effects of 2000 mg doses of ascorbic acid, although other researchers have found complications. Hypoxia (Schrauzer, Ishmael, and Kiefer, 1975), diarrhea (Kallner, Hartman, and Hornig, 1977), renal calcification from high urinary oxalate excretion (Briggs, 1973), decreased copper blood levels in the guinea pig (Smith and Bidlack, 1980), and B_{12} destruction by high ascorbic acid intake have been noted (Herbert and Jacob, 1974). Long term ingestion of gram amounts of ascorbic acid may lead to systemic conditioning, decreased ascorbic acid absorption, and eventual lowering of plasma and erythrocyte ascorbic acid levels (Schrauzer and Rhead, 1973). As long as ascorbic acid supplementation is maintained, systemic conditioning is without side effects. Once supplementation is discontinued, rebound scurvy may occur (Schrauzer et. al., 1973). Rebound scurvy may also occur in infants who are exposed to large doses of ascorbic acid in utero (Cochrane, 1965). Schrauzer et. al. (1973) also noted that overdosage of several vitamins together can introduce a new dimension of complexity

to the symptoms that occur.

Present research on ascorbic acid is focused on saturation of tissues and human pool size of ascorbic acid. When ingested, ascorbic acid is transferred through the plasma to tissue sites for storage. The adrenal, pituitary, brain, spleen, testes, and leukocytes are the target tissues of ascorbic acid storage suggesting that ascorbic acid has a physiological role in these tissues (Hornig, 1975). The observation that neither lungs, liver, or kidneys retain labeled material over a long period tends to indicate that these organs have only a metabolizing or excretory function rather than a physiological role.

Kallner, Hartman, and Hornig (1979) estimate the pool size of ascorbic acid in humans to be 20 mg/kg of ascorbic acid at a plasma level of 1.0 mg/100 ml. In addition, Kallner et. al. (1977) state that the rate constant of elimination from plasma appears to be dose dependent, implying that there is an increase in body pool at higher levels of ascorbic acid intake. Baker et. al. (1971), in their radiotracer studies, estimated pool size to be 1500 mg and felt that, at this level, tissues were saturated. Ginter (1979) opposes Baker's theory of saturation and pool size. Ginter states that the term saturation is not well used because it implies that tissues will become saturated and the excess excreted in the urine. Ginter, instead, uses the term steady state level and believes that tissues have different steady state levels that vary according to the tissue. Ginter estimates the combined steady state level of all the tissues would approximate 5 grams. Kallner et. al. (1979) suggests there are three compartments in a pool, the fast exchanging pool, the central

pool, and the slowly exchanging pool. In normal subjects, the rate of degradation of ascorbic acid is expected to be higher because it comes from the central and fast exchanging pools. The rate of degradation in a depleted subject most likely represents the slowly exchanging pool.

In assessing ascorbic acid status, the ascorbic acid levels of the plasma, leukocytes, and urine are considered. Normal plasma levels of ascorbic acid are 0.4 - 1.4 mg/100 ml (Omaye, Turnball, and Sauberlich, 1979). Higher ascorbic acid plasma levels may be seen after ingestion of a meal. The renal threshold for plasma approximates 1.4 - 1.8 mg/100 ml (Lewin, 1976). When the renal threshold is exceeded, urinary excretion of ascorbic acid increases sharply. Persons receiving adequate ascorbic acid (50 mg) excrete in excess of 11 mg/day (Birch, 1974). The normal range of leukocyte ascorbic acid content is 21 - 54 ug/10^8 WBC (Denson and Bowers, 1961). Evans, Currie, and Campbell (1980) feel the standard method of analysis used to measure leukocyte ascorbic acid may overestimate the tissue ascorbic acid content because platelet ascorbic acid is also measured and attributed to the leukocytes. The leukocyte content may also be altered by the leukocyte count. The higher the leukocyte count, the lower the leukocyte content (Vallance, 1979).

Several relationships have been noted between intake, plasma, leukocyte and excretion values of ascorbic acid. A positive relationship exists between intake of ascorbic acid and urinary excretion of ascorbic acid (Harris, 1935);(Omaye et. al., 1979). With normal intake, an estimated 2.6 - 4.1% of the body pool of ascorbic acid pool is excreted per day (Baker et. al., 1971). Plasma ascorbic acid is linearly related to intake

take until renal threshold is exceeded (Kallner et. al., 1977);(Burr et. al., 1974). Bates, Rutishauser, Black, and Paul (1977) also found a positive correlation between leukocytes ascorbic acid and ascorbic acid intake. Leukocytes are known to be indicative of tissue stores and total body pool (Burch, 1961). Bates et. al. (1977) and Vallance (1979) found a positive correlation between plasma ascorbic acid and leukocyte ascorbic acid, but Hodges et. al. (1971) found a poor correlation between these two indicators of ascorbic acid status. The whole blood level of ascorbic acid is related to body pool of ascorbic acid until blood levels fall below 300 mg. If under 300 mg, the whole blood levels are erratic and not indicative of pool size (Baker et. al., 1971).

One population that has received recent attention in surveys of ascorbic acid status is the elderly population. Elderly people have been found to have significantly lower levels of ascorbic acid in the adrenal gland, pituitary, brain, pancreas, cerebral cortex and heart than younger adults (Yarorsky, Almaden, and King, 1934);(Kirk, 1932);(Schaus, 1957). Surveys of populations in Ireland and England have found decreased plasma and leukocyte ascorbic acid levels in the elderly compared to younger adults (Burr et. al., 1974);(Vir et. al., 1978);(McClean, Stewart, Riley, and Beaven, 1977);(Bowers et. al., 1965). Burr et. al. (1974) found leukocyte values of 16.6 ug/10^8 WBC for elderly men and 21.4 ug/10^8 WBC for elderly females. This is in comparison to the values for younger adults of 30.4 ug/10^8 WBC for men and 33.89 ug/10^8 WBC for women (Loh, 1972). Plasma ascorbic acid values of 0.24 mg/100 ml were found for elderly men and 0.37 mg/100 ml for elderly women (Burr et. al., 1974). Plasma levels

in younger adults approximate 0.87 mg/100 ml for men and 0.97 mg/100 ml for women (Loh, 1972). Lower ascorbic acid levels for men than women were also supported by Vir et. al. (1978). Loh and Wilson (1971), however, found no significant difference between males and females. Also, the elderly living in institutions tended to have lower levels than the elderly living at home (McClean et. al., 1977);(Bowers et. al., 1965). McClean et. al. (1977), studying elderly men from a veteran's home, found their plasma levels to be 16 umol/liter, which was significantly lower than elderly men living alone whose plasma value was 26 umol/liter.

Preliminary findings of the first Health and Nutrition Examination Survey in the United States (1971 - 1972) found 48% of the elderly to have intakes less than the RDA of 60 mg of ascorbic acid. McClean et. al. (1977) also found low ascorbic acid intake of 21 mg/day in the subjects he studied. The Ten-State Nutrition Survey (1968 - 1970) concluded that elderly females were of minimal risk while elderly males had a medium risk of ascorbic acid deficiency. In addition, it concluded that low income people and blacks may have an increased risk from ascorbic acid deficiency.

The Canadian Survey (Nutrition Canada, 1975) found 17% of elderly males and 5% of elderly females at high risk of ascorbic acid deficiency since their serum ascorbic acid levels were less than 0.2 mg/100 ml. Thirt-four percent of elderly males and 15% of elderly females were at moderate risks based on serum levels of ascorbic acid between 0.2 - 0.4 mg/100 ml.

The positive correlation between plasma and leukocytes is not as

well defined in the elderly as in younger adults. Loh (1972) found that higher leukocyte ascorbic acid values tended to correlate with lower plasma ascorbic acid values, potentially indicating increased storage as people get older. Vir et. al. (1978) found a linear relationship between plasma ascorbic acid and leukocyte ascorbic acid values for men but not for women. Loh et. al. (1971) found a lower correlation between plasma ascorbic acid and leukocyte ascorbic acid levels in supplemented individuals than in unsupplemented elderly. Results in studies of supplemented elderly individuals suggest that the blood levels of ascorbic acid in the aged can be raised to those of young adults with adequate ascorbic acid intake. It has also been suggested that elderly subjects can metabolize ascorbic acid as efficiently as young adults if intake is adequate (Vir et. al., 1978);(Bowers et. al., 1965).

Since there has been little research on ascorbic acid excretion in the elderly, it is not known whether it is a factor in the low plasma and leukocyte ascorbic acid levels observed in the elderly. Bowers et. al. (1965) suggest that low ascorbic acid levels may aggravate physical and mental disorders and may be a contributory factor to lethargy, generalized aches and pains, skin disorders and fragility of the blood vessels commonly found in elderly persons. It is for this reason that the relationship between ascorbic acid excretion and plasma and leukocyte ascorbic acid levels was studied.

HYPOTHESIS

The following null hypotheses were tested:

1) There is no significant correlation between ascorbic acid excretion and ascorbic acid intake.
2) There is no significant correlation between ascorbic acid excretion and plasma ascorbic acid levels.
3) There is no significant correlation between ascorbic acid excretion and leukocyte ascorbic acid levels.

The minimum level for rejection of the null hypotheses was $p \leq 0.05$. The independent variables were plasma ascorbic acid, leukocyte ascorbic acid and ascorbic acid intake. The dependent variable was ascorbic acid excretion.

RESEARCH DESIGN

Thirteen female volunteers from federally subsidized retirement centers in Pasadena, Texas served as subjects for this study. The retirement center provided individual apartments with cooking centers. The subjects ranged in age from 65 to 74 years old. The study was explained to the subjects by the investigator and a written consent form was obtained from each subject acknowledging willingness to participate in the study.

The study consisted of three, 3-day sessions (Sunday, Monday, and Tuesday). Session one and session two were two weeks apart. Session three took place eight weeks after session one. On the first day of each session, the subject's current height and weight were recorded by the investigator. Information on present diagnosed illnesses, medications, and smoking habits was collected on information forms the subjects completed during the first session.

DIETARY SURVEY

Subjects were given written and oral instructions by the investigator on keeping a three-day diet diary during each session of testing. Subjects were requested to maintain their normal diet and cooking habits during the study, including any vitamin supplements normally taken. Ascorbic acid, protein, and caloric content of the diet diaries was analyzed on the Texas Woman's University's DEC-20 computer using the Ohio State Nutrient Data Base for diet analysis.

BIOCHEMICAL ASSESSMENT

Subjects were instructed to collect a 24-hour urine sample on the third day of each session. Containers were provided by the investigator. Five milliliters of 6N hydrochloric acid were added to the containers as an ascorbic acid preservative. Urine collections were refrigerated during the collection day. The following morning, urine collections were gathered and transported to the lab, where the volume of urine was recorded.

The method of urine analysis was an adaptation of the method by Lowry, Lopez, and Bessey (1945). Three test tubes were prepared for each subject containing 1.5 ml of urine and 1.5 ml of 5% trichloroacetic acid. The test tubes were thoroughly mixed, covered with parafilm, and stored at 0 - 4 degrees Centigrade for approximately 30 days. When thawed, one milliliter of 2,4 dinitrophenylhydrazine-thiourea-copper sulfate solution was added to the sample and mixed. The tubes were then incubated at 37 degrees Centigrade for 4 hours, removed and cooled in ice water. To each test tube, 5 ml of 65% sulfuric acid were added. The test tubes stood at room temperature for 30 minutes and were read in the spectrophotometer at 520 mu. Six standard tubes were read to prepare a standard curve. Ascorbic acid concentration was determined by an equation derived from the standard curve. The correlation coefficient of the standard tubes was 0.997.

A 10 ml and a 1 ml fasting blood sample were obtained by a registered nurse on the morning after completion of the 3-day diet diary. Lithium oxalate was used as an anticoagulant. Samples were kept on ice for approximately 4 hours.

The Lowry et. al. (1945) method was used for analysis of plasma levels. Seven milliliters of blood were centrifuged at 3000 rpm for 15 minutes. Two test tubes from each subject were made containing 1 ml of supernatant and 4 ml of 5% trichloroacetic acid. These were mixed, parafilmed and stored at 0 - 4 degrees Centigrade for approximately 30 days.

When thawed, the test tube mixtures were again centrifuged at 3000 rpm for 15 minutes. Three milliliters of supernatant were removed to test tubes. One milliliter of the 2,4 diniphenylhydrazine-thiourea-copper sulfate solution was added to each tube. The test tubes were then mixed and incubated at 37 degrees Centigrade for four hours. Upon removal, the test tubes were cooled in an ice bath. Five milliliters of 65% sulfuric acid were added to each test tube. The test tubes stood at room temperature for 30 minutes and were then read at 520 mu on the spectrophotometer. Ascorbic acid concentration was determined through use of the standard curve and an equation derived from the curve. The correlation coefficient was 0.998.

The method of Denson and Bowers (1961) was used for analysis of leukocyte ascorbic acid. Three milliliters of blood from each subject were added to a test tube with 12.5 ml of blood diluent. The blood diluent consisted of a mixture of physiological saline, dextran and sequestrene. The tubes stood at room temperature for one half hour, during which time the majority of red blood cells were sedimented out by gravity. Ten milliliters of supernatant were removed and centrifuged at 1500 rpm for 15 minutes. The supernatant was discarded by inverting the tube and allowing it to drain for 30 seconds. To the compact white blood cell

material, 1.3 ml of 5% trichloroacetic acid were added. It was thoroughly mixed, parafilmed and stored at 0 - 4 degrees Centigrade. When thawed, the tubes were centrifuged at 3000 rpm for 15 minutes. One milliliter of supernatant was removed to a test tube. Three-tenths ml of 2,4 dinitrophenylhydrazine reagent was added to the supernatant and the tubes were incubated at 37 degrees for 4 hours. The tubes were cooled in an ice bath and 1.5 ml of 65% sulfuric acid were added. They stood at room temperature for 30 minutes, and were then read at 520 mu on the spectrophotometer. The white blood cell count was obtained from the 1 ml blood sample using a leukocyte Coulter counter.

STATISTICAL ANALYSIS

The intake of ascorbic acid from the three sessions was averaged and used for analysis. Blood values in which more than one sample was analyzed were averaged and the average of the three sessions was recorded for analysis. One-way analysis of variance was used to determine if any of the values were statistically different between sessions. Regression analysis was used for determining correlations between independent variables and dependent variables. All statistics were done using the Statistical Package for the Social Sciences and the DEC-20 computer at Texas Woman's University.

RESULTS AND DISCUSSIONS

There were a total of thirteen subjects in the study. However, two of the subjects were unable to complete all three sessions. No clinical signs of deficiency were observed in any of the subjects during the study.

Although information on diagnosed illnesses, medications and smoking habits was obtained, this information was not statistically analyzed because of the small sample size. This information is documented in Appendix A. By one-way analysis of variance, values of the variables ascorbic acid intake, ascorbic acid excretion, plasma ascorbic acid and leukocyte ascorbic acid measured at different weeks were not significantly different at the .05 level. Therefore, in the following analyses, results from the three sessions were averaged together and treated as one value for each subject.

The average dietary intake of ascorbic acid is presented in Table 1. The average intake was 126 ± 65 mg/day. The range of intake among subjects varied considerably from 54 mg/day to 260 mg/day.

Eight percent of the subjects in session one and eight percent in session three had intakes less than the RDA of 60 mg. In session two, thirty-three percent of the subjects had intakes less than 60 mg. The high percentage of subjects with low intake in session two may be influenced by the timing of collection. Session two was scheduled at the end of the month, prior to the receipt of social security checks. Sessions one and three were scheduled in the middle of the month when money may have been more available for purchasing fruits and vegetables

with higher ascorbic acid content.

TABLE 1

Average Dietary Intake of Ascorbic Acid (mg/day)

Session	$\bar{x} \pm$ SD	Range
1	145 ± 75	52 - 331
2	128 ± 88	17 - 291
3	101 ± 58	38 - 225
Total	126 ± 65	54 - 260

In Table 2, the range of ascorbic acid intake was divided into three levels. Fifty-four percent of the subjects (n = 7) had average ascorbic acid intakes less than 100 mg/day. Twenty-three percent of the subjects (n = 3) had average intakes in the range of 100 - 200 mg/day, and twenty-three percent (n = 3) had ascorbic acid intake greater than 200 mg/day.

Forty-six percent of the subjects (n = 6) normally took vitamin supplements. The amount of vitamin supplements ranged from 50 mg to 500 mg (Table 3). Including the supplemented individuals, the number of subjects whose intake was less than 100 mg/day fell to forty-two percent (n = 6) and the number of subjects with intake in the range of 100 - 200 mg/day of ascorbic acid stayed at twenty-three percent (n = 3). The percent of subjects with ascorbic acid intake greater than 200 mg/day increased to thirty-three percent (n = 4). Even without the vitamin

supplements, the dietary intake of those who took supplements of ascorbic acid still exceeded the recommended dietary allowance of 60 mg. The most common reasons given for taking supplements were 1) to lessen the pain of arthritis, 2) as a cold remedy, and 3) as a supplement to an inadequate diet.

TABLE 2

The Percentage of Subjects at Different Ascorbic Acid Intakes

	Number of Subjects	<100 mg	100-200 mg	>200 mg
Without Supplements	n = 7	54%	23%	23%
With Supplements	n = 6	42%	23%	33%

The ascorbic acid intake of the subjects in this study was higher than recorded values of the Health and Nutrition Examination Survey, (1971-1972). In that study, 48% of the elderly were found to have intakes less than 60 mg, compared to eight percent in this study. These higher intakes may be secondary to the high interest these subjects held in adequate nutrition. The incentive for these subjects to participate in the study was the opportunity to find out if their ascorbic acid status was satisfactory. Since the subjects were interested in nutrition, they may have tended to eat more nutritionally complete meals and use vitamin

supplements. In the Ten-State Nutrition Survey it was stated that women were at a minimal risk of vitamin C deficiency. The results of this study support the findings of the survey.

TABLE 3

Supplements of Ascorbic Acid Taken by Subjects (mg/day)

Subject	Session 1	Session 2	Session 3
77	300	-	-
22	50	50	50
12	60	60	-
42	460	460	200
10	500	500	500
24	60	60	60

The average excretion of ascorbic acid for subjects in this study was 71.23 $\pm$ 48.89 mg/day with a range of 14.28 - 155.54 mg/day (Table 4). Excretion of ascorbic acid was most dependent on ascorbic acid intake. This was indicated by a strong correlation ($r = 0.79$) between the average excretion values and the average total intake (Table 5). This correlation was significant at the .01 level. This high correlation between ascorbic acid intake and excretion may indicate that the elderly cannot reabsorb ascorbic acid as efficiently in the nephron as younger adults and therefore may be excreting a larger proportion of their ascorbic acid intake.

Normally, 97 - 99.5% of the filtered ascorbic acid is reabsorbed in the proximal tubule.

TABLE 4

Average Ascorbic Acid Excretion (mg/day)

Session	$\bar{x} \pm$ SD	Range
1	84.68 ± 65.83	14.71 - 189.76
2	78.21 ± 50.66	8.15 - 145.72
3	47.12 ± 34.33	11.36 - 97.81
Total	71.23 ± 48.89	14.28 - 155.54

TABLE 5

The Correlation between Ascorbic Acid Excretion with Total Ascorbic Acid Intake

$r = 0.79$
$F = 14.6$
$DF = 1,9$
$p \leq .01$

The excretion of ascorbic acid was most indicative of ascorbic acid intake twenty-four hours prior to collection. This relationship occurred

in all three sessions with correlation values of r = 0.72, 0.72, and 0.86 (Table 6). The session one and session two values were significant at the .05 level. The correlation value of session three (r = 0.86) was significant at the .01 level.

TABLE 6

The Correlation between Ascorbic Acid Excretion with Prior Twenty-Four Hour Intake

	Session 1	Session 2	Session 3
r =	0.72	0.72	0.86
F =	10.2	9.9	25.4
DF =	1,9	1,9	1,9
p =	$\leq .05$	$\leq .05$	$\leq .01$

When intake of ascorbic acid was low, excretion of ascorbic acid was minimal. At an ascorbic acid intake less than 100 mg, only 25% of intake was excreted while the other 75% was utilized (Table 7). As intake of ascorbic acid increased to greater than 100 mg/day, 44% of ascorbic acid intake was excreted.

Normal plasma ascorbic acid levels are 0.4 - 1.4 mg/day (Omaye et. al., 1979). The average plasma ascorbic acid value in this study was $1.04 \pm .27$ mg/100 ml (Table 8). It was significantly different at the .05 level from the results of the Ten-State Nutrition Survey (see the t-statistic in Table 9). The average plasma ascorbic acid value in the Ten-

State Nutrition Survey was 0.72 mg/100 ml for low income states such as Texas.

TABLE 7

Ascorbic Acid Excretion at Different Levels of Total Ascorbic Acid Intake

Range of Intake (mg/day)	Number of Subjects	Percentage of Intake Excreted
<100	n = 5	25%
>100	n = 6	44%

Subjects 77 and 85 not included in analysis.

TABLE 8

Average Plasma Ascorbic Acid Level (mg/100 ml)

Session	$\bar{x} \pm$ SD	Range
1	0.98 ± 0.33	0.28 - 1.37
2	0.98 ± 0.27	0.30 - 1.30
3	1.14 ± 0.29	0.48 - 1.33
Total	1.04 ± 0.27	0.35 - 1.34

TABLE 9

t Statistic between Plasma Ascorbic Acid Levels in This Study and Plasma Levels in the Ten-State Nutrition Survey

Sample mean =	1.04
Population mean =	0.72
DF =	36
t =	6.36
Significant at =	$\leq .05$

Only one subject had plasma ascorbic acid levels below the normal range of 0.4 - 1.4 mg/100 ml, as seen in Appendix D. The subject, #28, had an average ascorbic acid level of 0.35 mg/100 ml. According to standards used by the Ten-State Nutrition Survey, plasma ascorbic acid values greater than 0.2 mg/100 ml are still considered acceptable and therefore this person would not be considered deficient.

Plasma ascorbic acid values tended to be low when excretion of ascorbic acid was low (Table 10). However, there was no significant correlation between ascorbic acid excretion and plasma levels. As intake of ascorbic acid increased, plasma levels increased to a degree as seen in Table 11. A correlation of r = 0.63 exists between plasma ascorbic acid and intake of ascorbic acid in session three and was significant at the .05 level (Table 12). Data from session three was used in calculation of this correlation so that the effect of leukocyte ascorbic acid could be considered. Plasma values were most indicative of ascorbic

acid intake twenty-four hours in advance but this relationship did not reach significance. The correlation between ascorbic acid plasma levels and intake of ascorbic acid has been found in other studies (Bates et. al., 1972);(Burr et. al., 1974) and indicates that biochemical status is strongly influenced by dietary intake.

TABLE 10

Mean Plasma Ascorbic Acid Values at Different Excretion Levels

Range of Excretion (mg/day)	Number of Subjects	Mean Plasma Values (mg/100 ml $\pm$ SD)
<50	n = 5	0.82 $\pm$ 0.27
50 - 100	n = 4	1.17 $\pm$ 0.09
>100	n = 4	1.18 $\pm$ 0.14

Not statistically significant.

When considering the effect of leukocyte values on other variables, the leukocyte values of the third session were used. The normal range of leukocyte levels of ascorbic acid is 21 - 54 ug/10^8 WBC. All of the representative values of leukocyte ascorbic acid are in the normal range. The average leukocyte ascorbic value was 39.98 $\pm$ 7.94 ug/10^8 WBC for the twelve subjects. This was substantially higher than the 21.4 ug/10^8 WBC for elderly females that Burr et. al. measured (1974). This is most likely due to the subjects high ascorbic acid intake throughout the study.

Once again, subject #28, who had low ascorbic acid intake, excretion, and plasma levels also had low ascorbic acid stores in the leukocytes. This is logical since the source of ascorbic acid for the leukocyte is the plasma.

TABLE 11

Mean Plasma Ascorbic Acid Values at Different Total Ascorbic Acid Intakes

Range of Intake (mg/day)	Number of Subjects	Mean Plasma Value (mg/100 ml $\pm$ SD)
<100	n = 5	0.88 $\pm$ 0.31
100 - 200	n = 3	1.22 $\pm$ 0.04
>200	n = 4	1.18 $\pm$ 0.16

Subject number 77 not included in analysis.

TABLE 12

The Correlation between Plasma Ascorbic Acid with Average Intake in the Third Session

r =	0.63
F =	5.79
DF =	1,9
p	$\leq$ .05

Table 13 shows that subjects with ascorbic acid intake greater than 120 mg/day could increase their leukocyte ascorbic acid content considerably over people with smaller intakes. However, the relationship of ascorbic acid intake and leukocyte ascorbic acid was not statistically significant.

TABLE 13

The Variation of Leukocyte Ascorbic Acid as Total Intake Increased in the Third Session

Range of Average Intake (mg/ml)	Number of Subjects	Mean Leukocytes ($ug/10^8$ WBC $\pm$ SD)
<100	n = 5	37.60 ± 6.46
100 - 200	n = 3	42.43 ± 5.32
>200	n = 3	45.63 ± 3.37

Not statistically significant.
Subjects 77 and 24 not included in analysis.

Leucocyte ascorbic acid values increased as plasma ascorbic acid levels increased (Table 14). At a plasma ascorbic level less than 0.95, leukocyte values averaged approximately 30.44 $ug/10^8$ WBC. As plasma levels increased to greater than 1.30 mg/100 ml, leukocyte values also increased to approximately 42.96 $ug/10^8$ WBC. The correlation ($r = 0.63$) that exists between plasma and leukocytes is significant at the .05 level (Table 15). The strong correlation between plasma and leukocyte ascorbic acid levels has been found in other studies on elderly subjects (Loh and

Wilson, 1971);(Burr et. al., 1974);(Bates et. al., 1977). These correlations imply a stable relationship between plasma and leukocyte ascorbic acid levels. In addition, the plasma level generally gave the same assessment of status as the leukocyte level. Measurement of plasma levels, therefore, may be adequate enough to measure subjects at risk. No significant correlation was observed between leukocyte ascorbic acid levels and excretion of ascorbic acid.

TABLE 14

Comparison of Leukocyte Ascorbic Acid Levels at Different Plasma Levels in the Third Session

Range of Plasma Levels (mg/ml)	Number of Subjects	Mean Leukocytes ($ug/10^8$ WBC $\pm$ SD)
<0.95	n = 3	30.44 ± 5.57
0.95 - 1.30	n = 4	43.41 ± 6.51
>1.30	n = 5	42.96 ± 5.90

Statistically significant.
Subject 24 not included in analysis.

TABLE 15

Correlation between Leukocyte Ascorbic Acid Values with Plasma Levels in the Third Session

$r = 0.63$
$F = 5.79$
$DF = 1,9$
$p \leq .05$

In general, the subjects in this study had a better ascorbic acid status than the population of elderly people surveyed in the Ten-State Nutrition Survey. There was only one subject who might be considered to be marginally deficient. Through correlation analysis, it was determined that excretion levels of ascorbic acid were most dependent on intake of ascorbic acid. No significant correlation existed between excretion of ascorbic acid and leukocyte and plasma ascorbic acid levels. Plasma ascorbic acid values correlated with intake of ascorbic acid and this was significant at the .05 level. In addition, plasma ascorbic acid, being the only source of ascorbic acid for the leukocytes, was closely correlated with the leukocyte ascorbic acid levels. This correlation indicates that either plasma or leukocyte values may be used to determine people at risk of ascorbic acid deficiency.

CONCLUSIONS

In conclusion, the null hypothesis that stated there was no significant correlation between ascorbic acid excretion and plasma ascorbic acid levels was not rejected. Results from this study showed that plasma levels were more depended on intake of ascorbic acid rather than excretion.

Since there was no correlation between excretion of ascorbic acid and leukocyte levels, this null hypothesis was also not rejected. Leukocyte ascorbic acid levels were significantly correlated to plasma levels. Because there was no relationship between excretion and plasma and leukocyte ascorbic acid levels, excretion can not be used as an indicator of ascorbic acid status.

The null hypothesis that stated there was no significant correlation between ascorbic acid excretion and ascorbic acid intake was rejected. In fact, this correlation was significant at the .01 level. This correlation being higher than expected may indicate that the elderly can not reabsorb the ascorbic as efficiently in the kidneys as younger adults and might therefore account for low biochemical values observed in other studies. The subjects in this study had biochemical values that were similar to younger adults indicating that the problem of decreased reabsorption could be compensated for by higher ascorbic acid intake.

IMPLICATIONS FOR FURTHER RESEARCH

Additional research with a larger sample size is needed to investigate the relationship of ascorbic acid intake and excretion found in this study. A study that includes younger adults and elderly people on a well controlled food intake would yield more accurate food records to compare with excretion values.

Ascorbic acid intake was higher than found in other studies with the elderly. This may have been caused by subjects increasing their vitamin C intake during the study period. One subject admitted she had done this. In addition, subjects were not separated into supplemented and unsupplemented groups. Supplemented individuals may naturally excrete more synthetic ascorbic acid than ascorbic acid from a food source. This may have influenced the correlations found in this study.

Leukocyte ascorbic acid values were analyzed using one sample from each subject. More frequent sampling would assure a more accurate ascorbic acid level.

APPENDIX A

Weight, Caloric Intake and the Occurrence of Factors that may cause Increased Desaturation or Increased Urinary Excretion of Ascorbic Acid

Subject Number	Caloric Intake/RDA	BW/IBW	Illness	Smoking	Medications	Alcohol
77	1285/1545	130/110	-	-	Tenuate	-
96	1095/1473	142/105	Arthritis	-	Anacin Lasix	X
99	1464/1545	127/110	-	-	-	-
22	1666/1545	171/110	Arthritis	-	Dristan Excedrin	-
12	1273/1684	156/120	Arthritis	-	-	-
37	2110/1684	162/120	Arthritis	14/day	Lasix	-
42	1878/1612	112/115	Arthritis	-	Ascriptin	X
28	1355/1616	153/115	Arthritis	-	-	-
63	1297/1755	162/125	-	24/day	Aspirin Lasix	-
51	1599/1684	156/120	-	-	Aspirin Tylenol	-
85	1219/1993	187/142	Arthritis	-	-	X
10	1495/1823	119/130	-	-	-	-
24	1020/1545	156/110	Arthritis	-	-	X

APPENDIX B

Dietary Intake of Ascorbic Acid (mg/day)

Subject	Session 1	Session 2	Session 3	Average
99	98	17	124	80
77	67	-	64	66
22	148	159	78	128
96	132	243	173	183
12	117	134	63	105
37	331	223	225	260
42	245	177	178	200
28	90	29	43	54
63	110	37	75	74
51	209	40	38	96
85	52	106	63	74
10	113	82	85	93
24	167	291	-	227

APPENDIX C

Ascorbic Acid Excretion Levels (mg/day)

Subject	Session 1	Session 2	Session 3	Average
99	17.71	18.40	15.84	17.32
77	15.17	-	20.43	17.87
22	109.22	102.95	55.20	89.12
96	93.49	101.09	69.00	87.86
12	120.73	124.55	31.88	92.39
37	189.76	43.16	79.28	104.07
42	153.41	116.90	110.00	126.77
28	15.01	16.22	19.00	16.74
63	27.78	29.33	11.36	22.82
51	14.71	8.15	19.98	14.28
85	37.28	122.68	35.70	65.22
10	141.19	109.32	97.81	116.11
24	165.36	145.72	-	155.54

APPENDIX D

Ascorbic Acid Plasma Levels (mg/100 ml)

Subject	Session 1	Session 2	Session 3	Average
99	0.85	0.83	0.92	0.87
77	0.65	-	1.33	0.99
22	1.17	1.16	1.21	1.18
96	1.24	1.14	1.27	1.22
12	1.26	1.20	1.31	1.26
37	0.96	0.74	1.29	0.97
42	1.17	1.14	1.41	1.24
28	0.28	0.30	0.48	0.35
63	0.95	1.09	1.40	1.15
51	0.50	0.89	0.79	0.73
85	1.11	0.95	1.11	1.02
10	1.17	1.03	1.30	1.17
24	1.37	1.30	-	1.34

APPENDIX E

Ascorbic Acid Leukocyte Levels (ug/10^8 WBC)

Subject	Session 3
99	35.47
77	51.70
22	48.57
96	39.62
12	39.12
37	49.27
42	42.63
28	24.46
63	36.35
51	31.40
85	36.21
10	45.00
24	-

LITERATURE CITED

(1) Anderson, T.W.: Large-scale trials of vitamin C. Ann. N. Y. Acad. Sci. 258:498, 1975.

(2) Baker, E.M., Hodges, R.E., Hood, J., Sauberlich, H.E., March, S.C., Canham, J.E.: Metabolism of C^{14} and H labeled L-ascorbic acid in human scurvy. Am. J. Clin. Nutr. 24:444, 1971.

(3) Baker, E.M., Halves, J.E., Johnson, D.O., Joyce, B.E., Knight, M.K., Tolbert, B.M.: Metabolism of ascorbic acid and ascorbic-2-sulfate in man and the subhuman primate. Ann. N. Y. Acad. Sci. 258:72, 1975.

(4) Bates, C.J., Rutishauser, I.H., Black, A.E., Paul, A.A.: Long-term vitamin status and dietary intake of healthy elderly subjects. Brit. J. Nutr. 42:43, 1977.

(5) Bates, C.J., Burr, M.K., St. Leger, A.S.: Vitamin C, high density lipoproteins and heart disease in elderly subjects. Age and Aging 8:177, 1979.

(6) Berger, l., Gerson, C., Yu, T.: The effect of ascorbic acid on uric acid excretion with a commentary on the renal handling of ascorbic acid. Am. J. Med. 62:71, 1977.

(7) Birch, G.G., Parker, K.J.: Vitamin C John Wiley and Sons, N.Y. 1974.

(8) Bowers, E.F., Kubick, M.M.: Vitamin C levels in old people and the response to ascorbic acid and to juice of the acerola. Brit. J. Clin. Prac. 19(3):441, 1965.

(9) Briggs, M.H.: Side effects of vitamin C. Lancet 2:1439, 1973.

(10) Burch, H.S.: Methods for detecting and evaluating ascorbic acid deficiency in man and animals. Ann. N. Y. Acad. Sci. 92:268, 1961.

(11) Burns, J.J.: Biosynthesis of L-gulonic acid in rats and guinea pigs. J. Amer. Chem. Soc. 74:1257, 1957.

(12) Burr, R.G., Rajan, K.T.: Leucocyte ascorbic acid and pressure sores in paraplegia. Brit. J. Nutr. 28:275, 1972.

(13) Burr, M.L., Elwood, P.C., Hole, D.J., Hurley, R.J., Hughes, R.E.: Plasma and leucocyte ascorbic acid levels in the elderly. Am. J. Clin. Nutr. 27:144, 1974.

(14) Cohrane, W.A.: Overnutrition in prenatal and neonatal life- a problem. Can. Med. Ass. J. 93(17):893, 1965.

(15) Denson, K.W., Bowers, E.F.: The determination of ascorbic acid in white blood cells. A comparison of WBC ascorbic acid and phenolic acid excretion in elderly patients. Clin. Sci. 21:157, 1961.

(16) Evans, R.M., Currie, L., Campbell, A.: Effect of platelets on apparent leucocyte ascorbic acid content. Ann. Clin. Biochem. 17: 252, 1980.

(17) Fox, S.M.R.: Protective effects of ascorbic acid against toxicity of heavy metals. Ann. N.Y. Acad. Sci. 258:144, 1975.

(18) Fujinami, T., Okado, K., Senda, K., Sugimura, H., Kishidawa, M.: Experimental atherosclerosis with ascorbic acid deficiency. Jap. Circulat. J. 35:1559, 1971.

(19) Gatenby Davies, J.D., Newson, J.: Ascorbic acid and cholesterol levels in pastoral peoples in Kenya. Am. J. Clin. Nutr. 27:1039, 1974.

(20) Ginter, E., Babala, J., Cerven, J.: The effect of chronic hypovitaminosis C on the metabolism of cholesterol and atherogenesis in guinea pigs. J. Atheroscler. Res. 10:341, 1969.

(21) Ginter, E.: Chronic marginal vitamin C deficiency: Biochemistry and pathophysiology. Wld. Rev. Nutr. Diet. 33:104, 1979.

(22) Goldsmith, G.: Human requirements for vitamin C and its use in clinical medicine . Ann. N.Y. Acad. Sci. 92:230, 1961.

(23) Harris, L.J.: Diagnosis of vitamin C subnutrition by urine analysis. Lancet 1:71, 1935.

(24) Herbert, V., Jacob, E.: Destruction of B_{12} by ascorbic acid. J. A. M. A. 230(2):241, 1974.

(25) Hodges, R.E., Hood, J., Canham, J.E., Sauberlich, H.E., Baker, E.M.: Clinical manifestions of ascorbic acid deficiency in man. Am. J. Clin. Nutr. 24:432, 1971.

(26) Hornig, D.: Distribution of ascorbic acid, metabolites and analogues in man and animals. N.Y. Acad. Sci. 258:102, 1975.

(27) Kallner, A., Hartman, D., Hornig, D.: Determination of bodypool size and turnover rate of ascorbic acid in man. Nutr. Metab. 21:31, 1977.

(28) Kallner, A., Hartman. D., Hornig, D.: Steady-state turnover and body pool of ascorbic acid in man. Am. J. Clin. Nutr. 32:530, 1979.

(29) King, C.G., Waugh, W.A.: Chemical nature of vitamin C. Science 75:357, 1932.

(30) Kirk, J.E.: Vitamins and Hormones Academic Press, N.Y. 20:83, 1962.

(31) Lewin, S.: Vitamin C: It's molecular biology and medical potential. Academic Press, N.Y., 1976.

(32) Loh, H.S., Wilson, C.W.M.: Relationship between leucocyte and plasma ascorbic acid concentrations. Brit. Med. J. 3:733, 1971.

(33) Loh, H.S.: The differences in metabolism of ascorbic acid between the sexes at different ages. Internat. J. Vit. Nutr. Res. 42:86, 1972.

(34) McClean, H.E., Stewart, A.W., Riley, C.G., Beaven, D.W.: Vitamin C status of elderly men in a residential home. NZ Med. J. 86:379,1977.

(35) Mayerson, M: Ascorbic acid absorption in man- pharmacokinetic implications. Eur. J. Pharmacol. 19:140, 1972.

(36) Mullen, A., Wilson, C.W.M.: The metabolism of ascorbic acid in rheumatoid arthritis. Proc. Nutr. Soc. 35:8A, 1976.

(37) Nutrition Canada, The Ontario Survey Report Bureau of Nutritional Sciences, 1975.

(38) Okamura, M.: Uptake of L-ascorbic acid and L-dehydroascorbic acid by human erythrocytes and HeLa cells. J. Nutr. Sci. Vitaminol. 25:269, 1979.

(39) Omaye, S.T., Turnball, J.D., Sauberlich, H.E.: Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods in Enzymology Vol 62, Academic Press, 1979.

(40) Pauling, L.: Letter to the editor. Can. Med. Ass. J. 105:448, 1971.

(41) Pelletier, O.: Vitamin C and cigarette smokers. Ann. N.Y. Acad. Sci. 258:156, 1975.

(42) Peterson, V.E., Crapo, V.A., Weininger, J., Ginsberg, H., Olefsky, J.: Quantification of plasma cholesterol and triglyceride levels in hypercholesterolemic subjects receiving ascorbic acid supplement. Am. J. Clin. Nutr. 28:584, 1975.

(43) Report of the Committee on International Dietary Allowances of the International Union of Nutritional Sciences. Nutr. Abtr. Rev. 45: 89, 1975.

(44) Ross, R., Glomset, J.A.: The pathogenesis of atherosclerosis. N. Eng. J. Med. 295:369,420, 1976.

(45) Sayers, M.H., Lynch, S.R., Jacobs, P., Charlton, R.W., Bothwell, T.H., Walder, R.B., Mayet, F.: The effects of ascorbic acid supplementation on the absorption of iron in maize, wheat and soya. Brit. J. Haematol. 24:209, 1973.

(46) Schaus, R.: The ascorbic acid content of human pituitary, cerebral cortex, heart, and skeletal muscle and its relation to age. Am. J. Clin. Nutr. 5:39, 1957.

(47) Schrauzer, G.N., Rhead, W.J.: Ascorbic acid abuse: Effects of long term ingestion of excessive amounts on blood levels and urinary excretion. Internat. J. Vit. Nutr. Res. 43:201, 1973.

(48) Schrauzer, G.N., Ishmael, D., Kiefer, G.W.: Some aspects of current vitamin C usage: Diminished high-altitude resistance following overdosage. Ann. N.Y. Acad. Sci. 258:377, 1975.

(49) Shields, J.B., Johnson, B.C., Hamilton, T.S., Mitchell, H.H.: The excretion of ascorbic acid and dehydroascorbic acid in sweat and urine under different enviromental conditions. J. Biol. Chem. 161:351, 1945.

(50) Silva, S.S.: The influence of reaction of the oxidation of the anti-scorbutic factor in lemon juice. Biochem. J. 17:410,416, 1923.

(51) Smith, C.H., Bidlack, W.R.: Interrelationships of dietary ascorbic acid an iron on the tissue distribution of ascorbic acid, iron, and copper in female guinea pigs. J. Nutr. 110:1398, 1980.

(52) Stone, I.: Hypoascorbemia, the genetic disorder causing the human requirement for exogenous ascorbic acid. Perspect. Biol. Med. 10: 133, 1966.

(53) Szent Gyorgyi, A.: Observations on the function of peroxidase systems and the chemistry of the adrenal cortex. Description of a new carbohydrate derivative. Biochem. J. 22:1399, 1928.

(54) U.S. Dept. of Health, Education and Welfare, Preliminary Findings of the First Health and Nutrition Examination Survey, U.S., 1972.

(55) U.S. Dept. of Health, Education and Welfare. Ten State Nutrition Survey, 1968-1970.

(56) Vallance, S.: Leucocyte ascorbic acid and the leucocyte count. Brit. J. Nutr. 41:409, 1979.

(57) Vir, S.C., Love, A.H.G.: Vitamin C status of institutionalized and non-institutionalized aged. Internat. J. Vit. Nutr. Res. 48:274, 1978.

(58) Von Schuching, S., Enns, T., Abt, A.F.: Connective tissue studies. IV: L-ascorbic-I-C^{14} acid excretion in intact and wounded guinea pigs on varying vitamin C intakes. Ann. J. Physiol. 199(3):423, 1960.

(59) Wilson, C.W.M., Loh, H.S.: Common cold and vitamin C. Lancet 1:638, 1973.

(60) Wilson, C.W.M.: Tissue saturation, metabolism and destruction. Practioner 212:418, 1974.

(61) Wilson, C.W.M.: Ascorbic acid metabolism and the clinical factors which affect tissue saturation with ascorbic acid. Acta Vitamin. Enzymol. 31:35, 1977.

(62) Yarorsky, M., Almaden, P., King, C.G.: The vitamin C content of human tissues. J. Biol. Chem. 106:525, 1934.