AN EVALUATION OF THE ASCORBIC ACID NUTRITION OF A GROUP OF RURAL MANITOBANS AS INDICATED BY FOOD INTAKE AND BLOOD LEVELS

A Thesis
Presented To
The Faculty of Graduate Studies and Research
University of Manitoba

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Vivian M. Bruce
1960

ACKNOWLEDGMENTS

The author wishes to express her thanks to Mrs. Elizabeth Feniak, Associate Professor of Foods and Nutrition for her direction, encouragement and assistance during the course of this study.

The willingness of Dr. A.D. Robinson, Professor of Biochemistry, to assist the author is gratefully acknowledged.

Thanks are tendered to the subjects who volunteered for the study. Without their continued co-operation the study would not have been possible.

The use of equipment in the Department of Dairy Science, University of Manitoba and the Carman Memorial Hospital, Carman, was greatly appreciated.

An Evaluation of the Ascorbic Acid Nutrition of a Group of Rural Manitobans as Indicated by Food Intake and Blood Levels

by

Vivian M. Bruce

Forty-five food records and 45 micro blood serum samples from a selected group of rural Manitobans were analysed for ascorbic acid and total ascorbic acid respectively. The average intake of ascorbic acid was 91.0 $\pm$ 41.6 mg. per day. This figure is well above the Canadian Dietary Standard and the Recommended Allowance of the U.S. National Research Council. Two persons consumed amounts below the Canadian Dietary Standard of 30 mg. per day.

Citrus fruits and tomatoes accounted for 38 per cent and potatoes 21 per cent of the ascorbic acid intake of the group.

The average serum level of total ascorbic acid was 1.25 $\pm$ 0.74 mg. per cent. A majority of daily intakes above 30 mg. corresponded to serum concentrations which were above 0.8 mg. per cent; approximately 28 per cent of the group had serum levels below this figure. There was no evidence of a direct relationship between the dietary intake and the serum concentration.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

In the fall of 1958, a family food consumption study was undertaken by the School of Home Economics with the co-operation of a group of rural Manitobans. The study was continued the following spring with a second series of food consumption records. The nutritional status of the family unit was calculated from the food records obtained. It was felt that an evaluation of the nutritional status of individual members as indicated by intake of a specific nutrient and related biochemical findings would be a valuable supplement to this study.

Protein consumption was known to be above recommended allowances. Blood levels of Vitamin A fluctuate greatly with intake. Calcium intakes were low in some of the families. However biochemical tests are not known to be indicative of intake and X-rays were not practical in this situation. Preliminary findings in the fall and spring surveys revealed that fruit and vegetable consumption was generally below the amount recommended by Canada's Food Rules. In the food groups as listed, the percentages of families below the recommended intakes in the fall were as follows: tomatoes and citrus fruits, 44, other fruits, 37, leafy, green and yellow vegetables, 42. The spring survey reported similar findings. The percentages of families below the recommended intakes were as follows: tomatoes and citrus fruits, 31, other fruits, 41, leafy, green and yellow vegetables, 50.

Analysis of the food records, however, indicated that the ascorbic acid intakes of the family groups in the fall survey exceeded the minimal allowance of the Canadian Dietary Standard (30 milligrams per person per day).

Because of the low consumption of the foods rich in ascorbic acid, a study of the ascorbic acid intake of individuals, appeared to be worthwhile. Blood levels of this vitamin are claimed to be related to previous dietary intake. Micromethods of analyses have been perfected and there was reason to believe that co-operation could be expected from the group if small blood samples were adequate for analyses. In the spring of 1960, 56 individual food records and 53 micro blood samples were obtained from the group previously studied.

REVIEW OF LITERATURE

Chemistry and Physiological Function of Ascorbic Acid

Ascorbic acid is a hexose derivative. When synthetically prepared, it is a white crystalline compound which is soluble in water and sensitive to oxygen and heat in solution. It may be distinguished from other sugars by titration with oxidizing agents in acid solution. Acid solutions of silver nitrate, dichlorophenol, 2,4 dinitrophenylhydrazine are reduced by ascorbic acid. Methylene blue is reduced by ascorbic acid on irradiation with light. In the absence of light, there is no reaction (18).

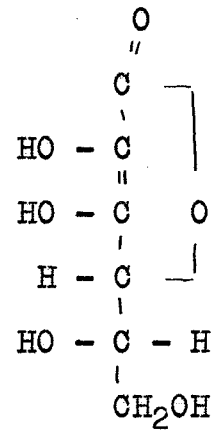
Ascorbic acid contains four hydroxyl groups, two of which are enolic in character. This gives ascorbic acid its acidic properties (57).

Ascorbic acid displays some of the color reactions typical of carbohydrates. In the presence of strong acids furaldehyde is produced (57).

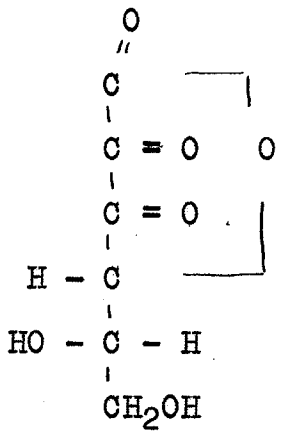
Compounds which contain the group —CH(OH)-CHO are reducing agents and precipitate cuprous oxide from Fehling's solution (21). Another characteristic reaction is the formation of phenylosazones when heated with phenylhydrazine. These compounds have decreased solubility. The 2,4 dinitrophenylhydrazones of saturated aldehydes and ketones are yellow in color and those of unsaturated carbonyl compounds are orange or red (12). Ascorbic acid forms osazones with phenylhydrazine which show maximum absorption in the spectrum

at 245 mμ in acid solutions and at 265 mμ in neutral solutions.

Ascorbic acid is readily oxidized in aqueous solutions by oxidizing agents because of its endiol structure. The first product of oxidation is dehydroascorbic acid.



L-Ascorbic Acid



Dehydro-L-Ascorbic Acid

Dehydroascorbic acid is one of the important derivatives of ascorbic acid because it retains the antiscorbutic properties of the vitamin (57). Unlike ascorbic acid, dehydroascorbic acid does not show absorption in the ultraviolet region of the spectrum.

The deficiency disease associated with ascorbic acid is known as scurvy. According to reports of the early explorers, scurvy was a common disease. James Lind, a young Scottish physician, is credited with conducting the first controlled experiments which indicated that ascorbic acid was of prime importance in the control of scurvy. Around 1747 when he was attached to the Royal Navy, he observed that citrus fruits possessed curative powers in greater amounts than other cures used during that time (21).

Recently, Whelan et al (67) reported clinical findings on a group of children suffering from symptoms typical of scurvy who had been admitted to the Hospital for Sick Children in Toronto. Up to 1954, there had been an average of 7 cases of scurvy per year. In 1954 there were 46 cases and in 1955, 25 cases. The authors attributed the increased incidence of scurvy to the lack of a food source of ascorbic acid. They found that 80 per cent of the admissions, which ranged in age from 6-12 months, had not received any source of ascorbic acid 6 weeks prior to diagnosis. At the same time similar rises in the incidence of infantile scurvy were being reported in Winnipeg.

Observations on 11 adults admitted to two London hospitals during 1951-1957 were made by Cutforth (7). The diets fell into 3 classes, one consisted mainly of tea, toast, bread and butter, a second group followed diets for the treatment of duodenal ulceration for extended periods of time, the third consisted of an individual who was a vegetarian on a diet which consisted mainly of bread, fat, milk, honey and eggs. Characteristic features of scurvy, e.g. bruising, purpura, hyperkeratotic hair follicles, responded favorably to treatment of a dosage of 600 mg. of ascorbic acid in a 24 hour interval. Nine out of eleven subjects reported improvement.

By observing experimental cases of scurvy, the physiological role of ascorbic acid has been studied. Humans and

guinea pigs have been found to be susceptible to deficiencies of the vitamin. The Sheffield Experiment conducted by H.A. Krebs (22) observed symptoms of ascorbic acid deficiencies in the human. Nineteen men and women volunteered for the study. No definite changes were observed during the first 17 weeks. The first changes in all subjects in a 17-26 week period were enlargement and keratosis of the hair follicles. Main areas affected were upper arms, backs, buttocks, back of thighs, calves and shins. The enlarged hair follicles eventually became hemorrhagic. Changes in the gums were also observed. Experimental scars made earlier, which had healed normally, became red and livid. As the scurvy progressed, wounds showed a reduced tendency to heal. Changes in knee joints and the development of heart murmur were also reported.

The oxidation-reduction properties of ascorbic acid are believed to be responsible for the role of ascorbic acid in enzyme systems (57). Ascorbic acid may be a co-enzyme in one step of tyrosine metabolism; it may be involved in the hydroxylation of aromatic compounds and the conversion of folacin into the citrovorum factor. Ascorbic acid appears to be involved in the failure to deposit collagen, which leads to a tendency to hemmorhage, to the failure to form dentine which results in abnormal tooth development and in the failure of osteoblasts to form osteoid. As a result, the scorbutic bone is weak and fractures easily.

There are many gaps in our knowledge about the human re-

quirement for ascorbic acid. Studies have involved the dosages needed to prevent clinical signs of scurvy. It has been suggested that there is a wide range between the minimum and optimum requirements. There may be cases of subclinical deficiencies which cannot be detected by the tests currently used.

Krebs (22) found that clinical signs of scurvy were not evident on an intake of 10 mg. daily of ascorbic acid. Twenty healthy adult subjects were involved in the study, part of which served as controls on a daily intake of 70 mg. The group ingesting 10 mg. did not exhibit any signs of scurvy during a 424 day period on the diet. Blood levels were determined by titration with a dye. There are many errors in this method so the author did not consider the results to be of value. In view of this experimental evidence, Krebs postulated that a requirement three times as great as the amount required to prevent clinical symptoms of scurvy would be logical.

Kyhos and co-workers (23) observed the effect of ascorbic acid dosage on the response of oral disease in 71 adult men. They found that 50 mg. was not adequate to prevent the recurrence of oral disease during a 17 month period and estimated the ascorbic acid requirement to be around 75 mg. daily.

Uhl (65), reviewing literature on ascorbic acid requirements, postulated that 70 mg. was an adequate amount for the human. Plasma levels on this intake approached those of breast fed infants who are known to be in a state of good ascorbic acid nutrition.

Saturation tests are also used for the determination of requirements. The metabolic role of ascorbic acid is not completely understood and therefore the data from experiments of this nature will vary.

Lowry, Lopez and Bessey (29) reported that 78 mg. of ascorbic acid resulted in a 90 per cent saturation of 30 male subjects. Corresponding serum levels were found to be 0.76 mg. per 100 ml. From a study of 2 male subjects, Storvick and Hauck (60) recommended an intake of 75-160 mg. daily in order to maintain tissue saturation. Four preschool children were maintained at a saturation level, as designated by plasma levels of 0.7 mg. per cent on a daily intake of 31 mg. of ascorbic acid.

It has been established that serum levels reflect previous intake of ascorbic acid. The level of serum ascorbic acid known to represent adequate intake has yet to be defined. In reviewing the literature published in 1942 on ascorbic acid, Gyorgy (15) recommended an intake of 75 mg. daily. A corresponding fasting plasma level was thought to be 0.7 mg. per cent. Storvick and Hauck (62) found high plasma levels corresponded in general to high intakes. The range of values at each level of intake was very wide. For an intake of 65 grams, fasting plasma levels were reported from 0.84-1.32 mg. per cent. Total ascorbic acid in blood serum corresponding to intakes rated as "good" by Davey et al (10) were 0.6-0.8 mg. per cent. Potgeiter (48) et al reported average ser-

um values of total ascorbic acid to be 1.02 mg. per cent for subjects on an average daily intake of 45 mg. Merrow et al (35), studying a group of children, found high intakes of ascorbic acid produced serum levels from 0.2-3.1 mg. per cent and low intakes from .02-1.1 mg. per cent. An estimated dietary intake of 198 mg. produced a range of total ascorbic acid serum levels from 1.13-2.58 mg. per cent in a group of women investigated by Roderuck et al (47). Mayer et al (31) in evaluating the nutritional status of children found intakes which corresponded to o.7 mg. per cent to be adequate. In better nourished children serum values from 0.7 mg. per cent to more than 1.2 mg. per cent were reported. Serum ascorbic acid values were found to range from 0.2-2.4 mg. per cent in a group of 384 boys and girls.

Roe, Keuther and Zimler (53) stated that an adequate whole blood level of ascorbic acid is approximately 0.6 mg. per cent. Below 0.6 mg. per cent they suggested that the body might be in negative balance.

The Nutrition Division, Department of National Health and Welfare, Ottawa (33), reported serum ascorbic acid levels ranging from 1.6-2.1 mg. per cent over a two year period. No attempt to evaluate dietary intake with serum levels was made. It should be noted that nutritional surveys are made by this department to discover deficiencies rather than to evaluate nutritional status and, therefore, groups which are suspected of consuming low intakes are studied.

Miller (37), while conducting a nutritional study at the Residence of the University of Manitoba, found that ascorbic acid intakes averaged 90 mg. per day while 71.8 per cent of the subjects had serum levels below 0.96 mg. per 100 ml.

The Canadian Dietary Standard for ascorbic acid has been established at a daily intake of 30 mg. while the U.S. National Research Council recommends an intake of 75 mg. daily. To date, there is no experimental evidence to support the hypothesis that a man is in better health on 70 mg. of ascorbic acid than 30 mg. In view of the findings reported, a serum level of 0.8 mg. per cent in a fasting human subject can be considered to represent adequate intake.

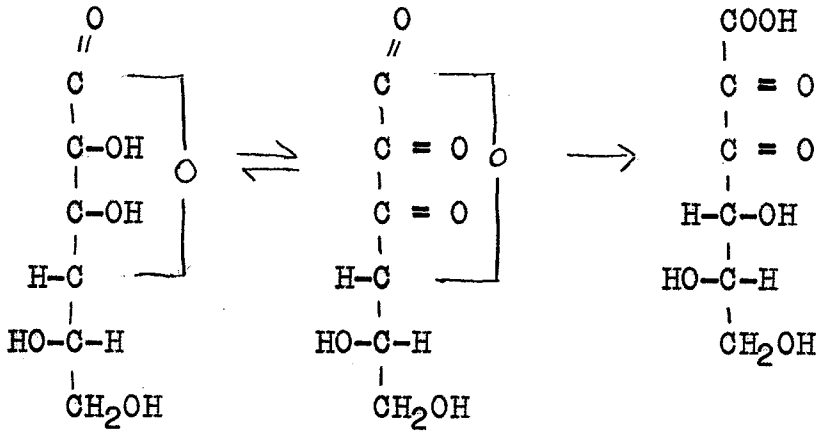
Ascorbic Acid and Dehydroascorbic Acid in Blood

Ascorbic acid is found in greatest concentration in tissues of high metabolic activity. This includes tissues in the pituitary gland, adrenal cortex, brain and spinal fluid, white blood cells, whole blood and plasma.

Ascorbic acid and dehydroascorbic acid are interconvertible in plant and animal tissues. Dehydroascorbic acid is the only ascorbic acid derivative which retains antiscorbutic activity.

Penny and Zilva (45) studied the behavior of dehydroascorbic acid in 3 guinea pigs by determining dehydroascorbic acid in the tissues, urine, and contents of the digestive tract. After the ingestion of dehydroascorbic acid none could be found in the blood. They postulated that the de-

hydroascorbic acid was reduced in the liver. The reversible oxidation-reduction was represented as follows:



L-Ascorbic Acid | Dehydro-L-Ascorbic Acid | 2,3 diketo-L-Gulonic Acid

Reduction of dehydroascorbic acid in the tissues rather than the blood was demonstrated by Borsook et al (2). Following the ingestion of ascorbic acid and dehydroascorbic acid in the form of orange juice, ascorbic acid in the plasma and urine increased. However there were insignificant changes in the concentration of oxidized ascorbic acid. Further evidence was observed in vitro when dehydroascorbic acid which was added to minced or intact isolated tissues was rapidly reduced. Glutathione was thought to be active in the reduction of dehydroascorbic acid under physiological conditions.

As a result of work by Stewart et al (61), blood was found to contain dehydroascorbic acid. The antiscorbutic activity of dehydroascorbic acid was thought by Roe and Barnum (50) to be due to the conversion to the reduced form by a substance in blood.

Regardless of the natural occurence of dehydroascorbic acid in blood, there is a possibility that ascorbic acid may be oxidized to dehydroascorbic acid during the interval between collection of the sample and the addition of acid which is known to stabilize ascorbic acid prior to analysis. It becomes important to compare studies where similar blood constituents have been analysed for similar forms of ascorbic acid.

Ascorbic acid was thought to pass from the plasma to the red blood cells by Butler and Cushman (3). In persons with adequate intake, they reported that the ascorbic acid concentration of the red blood cells increased from 0.7 to 1.4 mg. per 100 c.c. of blood as the ascorbic acid ingested increased from 250 to 500 mg. The plasma and white cell platelet concentration did not show a significant change. Studying 7 subjects with ascorbic acid deficiency after supplementation, they observed a rise in the reducing substance of the white layer.

Heinemann (19) was able to show that red cells in vitro were permeable to ascorbic acid at a slow rate. Ascorbic acid was measured in terms of mg. per litre. The highest concentration was 1.0 mg. per litre. After the ingestion of ascorbic acid, the concentration of the vitamin in the serum tended to rise above the concentration in the red cells. After 4 hours, the ascorbic acid had gained access to the red cells. It was observed that the ascorbic acid had pen-

etrated the red cell provided there was no hemolysis. Heinemann reported that the concentration of ascorbic acid in the red cells was greater than in serum or plasma when calculated for equal volumes.

Further work by Heinemann and Hald (20) confirmed that ascorbic acid passed from the serum to the cells. They added ascorbic acid to whole blood and observed a decrease in the serum which had been in contact with the red cells until the time of analysis as compared to serum which had been separated from the red cells immediately. This decrease was not observed at low temperatures (7°C) which may indicate that this change was due to metabolic activity rather than diffusion. The authors also reported that agitation accounted for some of the permeability of the cell wall.

A study of 7 subjects by Davey et al (10) revealed that plasma ascorbic acid levels for both total and reduced ascorbic acid were lower than serum ascorbic acid concentrations. the authors could not offer any explanation for this. This observation might be interpreted as impermeability of the cell wall, depending on temperature and physical conditions.

Similarily, the impermeability of the cell wall was reported in results obtained by Pijoan and Eddy (47). They studied 100 individuals and found plasma ascorbic acid levels were higher than the red cell concentrations. Borsook (1) observed that ascorbic acid was most stable in whole blood. He stated that the bulk of ascorbic acid supplements did not

enter the red corpuscles.

Stephens and Hawley (60) observed 30 subjects. The ascorbic acid content of the red blood cells was lower but the differences were not great.

Differences reported in the permeability of the cell may be due to method of measurement. Davey et al measured both total ascorbic acid and ascorbic acid. If part of the ascorbic acid was oxidized to dehyroascorbic acid, then decreases would be observed in ascorbic acid if total ascorbic acid was not measured.

Heinemann (19) also observed that, by increasing ascorbic acid intakes, there appeared to be a rise in serum concentration which approached equilibrium with that of the red blood cells. As the concentration of the ascorbic acid in whole blood decreased, the ratio of the concentration of ascorbic acid in serum increased. Therefore the distribution depended to some extent on the ascorbic acid content of whole blood.

Similar findings were reported by Roe, Keuther and Zimler (53) on the plasma concentration of ascorbic acid. In guinea pigs and humans, whole blood levels below 0.6 mg. per 100 ml. resulted in plasma concentrations which were below this level. At whole blood levels between 0.6 and 0.9 mg. per 100 ml. the plasma concentration of ascorbic acid ranged from values which were slightly higher than that of the whole blood content. At whole blood

levels above 0.9 mg. per 100 ml. the plasma concentrations were consistently higher than the whole blood ascorbic acid levels. It appears that the distribution of ascorbic acid in blood constituents is relative to total concentration. Serum, plasma and red cell levels vary from project to project and individual to individual.

Steele (59) et al found a direct relationship between ascorbic acid concentrations of serum and white blood cells. They reported results on 23 fasting subjects. Serum values of less than 0.4 mg. per cent included only one cell value greater than 14 mg. per cent. When serum values were greater than 0.4 mg. per cent, there were no values for white cells under 14 mg. per cent. Both high and low white cell concentrations occured at serum levels of 0.4 mg. per cent. Again, it appears that ascorbic acid distribution in the blood is related to total concentration.

The white blood cells of normal individuals are rich in ascorbic acid, but ascorbutic individuals have low white blood cell concentrations of the vitamin. Lowry et al (29) also observed a relationship between the ascorbic acid concentration of the serum and the ascorbic acid concentration of the white cells. In 39 subjects, serum values greater than 0.4 mg. per cent were associated with only one white cell value less than 20 mg. per cent. When the concentration of serum ascorbic acid of 42 subjects was less than 0.3 mg. per cent, the ascorbic acid concentration in the white cells exceeded 20 mg. per cent in only one subject. Between

0.3 mg. per cent and 0.4 mg. per cent, both high and low white cell concentrations were encountered.

If the white cells are rich in ascorbic acid in normal persons, and there is a definite relationship between the concentration of the vitamin in the white cells and the serum then it would appear that serum can be used to evaluate ascorbic acid nutrition.

Chemical Analyses of Blood for Ascorbic Acid

Roe and Keuther (51) developed a method for the determination of ascorbic acid in whole blood and urine by the formation of 2,4 dinitrophenylhydrazine derivatives of dehydroascorbic acid. The derivative forms a reddish colored product which absorbs maximally from 500 to 550 mμ and 350 to 389 mμ. The color is proportional to dehydroascorbic acid concentration and is in agreement with Beer's Law. The color is comparatively stable. After standing 18 hours at room temperature, fading of 2.25 galvanometer units was reported. Norit clarified the solution and oxidized the ascorbic acid to dehydroascorbic acid. Trichloroacetic acid or acetic acid was necessary in the oxidation reaction. Thiourea produced a mild oxidizing medium. Stronger oxidizing agents produced coloration which interfered with the colorimetric determination. The color was produced by sulfuric acid. To prevent charring of sugars and other organic matter, which could result from the heat of the chemical reaction, the temperature of the solution was

lowered by cooling in an ice bath. As an added precaution, the acid was added slowly.

The same authors (52) found that the method was specific for dehydroascorbic acid. Blood and urine from guinea pigs suffering from acute scurvy gave negative results for ascorbic acid. They were able to identify possible interfering substances and determine their relationship to color reaction. Aldehydes and ketones couple with 2,4 dinitrophenylhydrazine but do not react with sulfuric acid nor absorb light in the 540 mμ region. Pentoses and hexoses could not be considered to be interfering substances unless the concentrations were above those ordinarily found in blood. The presence of glucuronic acid up to concentrations of 50 mg. per cent caused a plus error of 0.04 mg. per 100 c.c Thiourea did not produce any interference with color determinations. Roe and Oesterling (54) adapted the method for the determination of dehydroascorbic acid in the presence of ascorbic acid. Norit was omitted and the extracting solution contained a 1 per cent solution of thiourea for the purpose of stabilizing the ascorbic acid.

In 10 analyses on the same sample, Roe and Keuther (52) found the maximum deviation was 0.04 mg. per 100 c.c. It was possible to obtain 96-104 per cent recovery of the ascorbic acid. This satisfied the requirements of precision and accuracy of the method.

The red compound produced was thought to be a new product