

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



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as Indicated by Food Intake and Blood Levels

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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 ± 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 ± 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.

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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).

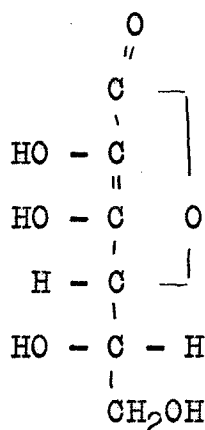
Ascorbic acid contains four hydroxyl groups, two of which are enolic in character. This gives ascorbic acid its acidic properties (57).

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

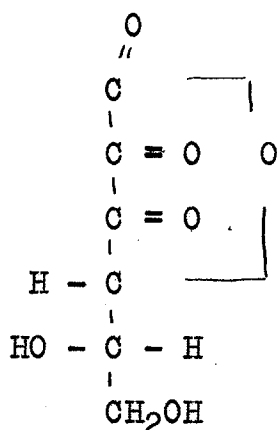
Compounds which contain the group $-\text{CH}(\text{OH})-\text{CHO}$ are reducing agents and precipitate cuprous oxide from Fehling's solution (21). Another characteristic reaction is the formation of phenylosazones when heated with phenylhydrazine. These compounds have decreased solubility. The 2,4 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 $m\mu$ in acid solutions and at 265 $m\mu$ 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 hemorrhage, 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 sub-clinical 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 0.7 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.

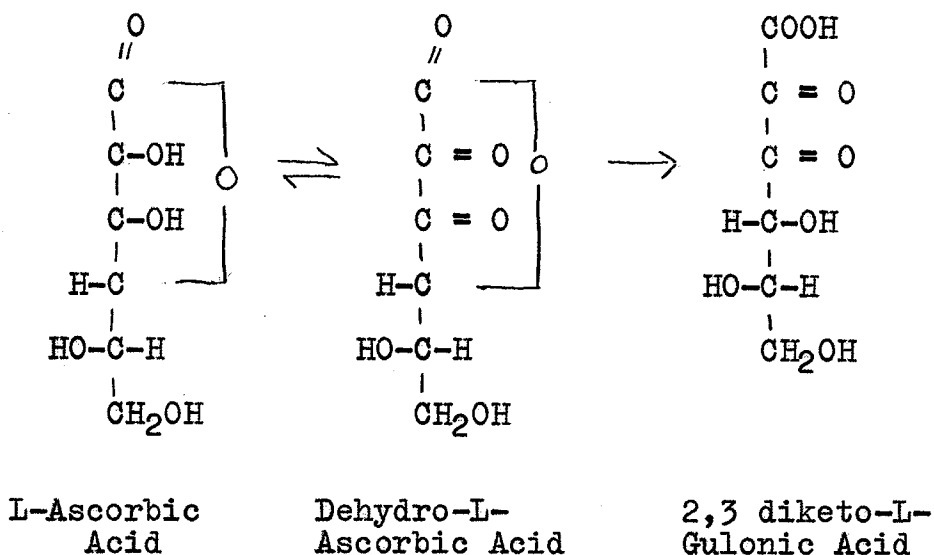
Ascorbic Acid and Dehydroascorbic Acid in Blood

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:



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 occurrence 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.

Similarly, 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 dehydroascorbic 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 occurred 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 $m\mu$ and 350 to 389 $m\mu$. 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 $m\mu$ 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

for the following reasons-

1. Solutions of 2,4 dinitrophenylhydrazine derivatives of dehydroascorbic acid in alcohol, glacial asetic acid and dilute sulfuric acid were brown. When 60-85 per cent sulfuric acid was used, a red product was formed.

2. When excess water was added to the solution, a brownish compound separated which had the same absorption spectrum as the original derivative and yielded a red solution when mixed with 85 per cent sulfuric acid. The reaction was reversible and the mechanism was believed to be dehydration.

Lowry, Lopez and Bessey (28) modified the 2,4 dinitrophenylhydrazine method to measure ascorbic acid in small amounts (.01 ml.) of blood serum. Such a method is desirable for use in nutritional surveys where it is necessary to make several biochemical determinations on the same sample. This method was found to be the most practical for use on small volumes of fluid. The methylene blue method (Butler and Cushman) required an irradiation step which was inconvenient with small volumes. It was difficult to prevent prior oxidation of the ascorbic acid when the dichlorophenolindophenol method was used with large numbers of samples. The 2,4 dinitrophenylhydrazine method oxidized the ascorbic acid to dehydroascorbic acid. Dehydroascorbic acid was found to be stable in serum after

trichloroacetic acid was added. No differences were observed due to the presence of the protein extract. The concentration of sulfuric acid was reduced from 85 to 65 per cent and chilled. This offset the heat produced on mixing and the acid could be added in one operation. After the trichloroacetic acid was added to the serum, the ascorbic acid remained unchanged for several weeks at -20°C . The authors emphasized the importance of preventing evaporation with small samples. Samples were analysed after storage periods ranging up to 13 days. There was no loss of ascorbic acid at room temperature with acidified samples or of serum stored at 4°C . It is important that blood or serum should be kept cool until acidified. At 38°C , there was a loss of ascorbic acid in one hour and an appreciable loss in 4 hours. Hemolysis tended to accelerate destruction. Findings by Golden and Garfinkel (14) with plasma further confirm these results. At room temperature, changes in ascorbic acid in blood plasma were insignificant after 3 hours while significant changes occurred after 6-24 hours at this temperature. No changes were observed in ascorbic acid content at refrigeration temperatures up to 24 hours.

Absorption spectrophotometry measures the intensity of absorption exerted at different wave lengths by molecules in the path of the light (42). All colored solutions absorb some light. Lambert (16) investigated the relationship between the intensity of the incident light and the transmitted

light in terms of the thickness of the layer of the solution through which it passes. This can be expressed as

$$\log_{10} \frac{I_0}{I} = kt$$

I_0 - Intensity of incident light
 I - Intensity of transmitted light

Beer (16) studied the relation between the concentration of a colored solution and the amount of monochromatic light absorbed by it. His law can be formulated as

$$\log_{10} \frac{I_0}{I} = kc$$

By combining the two laws the following formula is obtained:

$$\log_{10} \frac{I_0}{I} = kct$$

As applied to photoelectric colorimetry, c is expressed as moles per litre, t is the thickness of the layer in centimeters and k becomes the molar extinction coefficient. This coefficient is characteristic of the solution for incident light of any one wave length.

In practice, accuracy can be achieved by comparing the light transmitted by a standard solution to that of an unknown.

After passing light through the chosen optical filter, the emergent light is somewhat more homogeneous in wave length. Upon traversing the solution which is placed in tubes or cuvettes of constant thickness, some light is absorbed. Whatever is transmitted falls upon the photocell which generates an electrical current. The magnitude of this current

is measured by a galvanometer. The spectrophotometer contains a diffraction grating or prism which produces a spectrum. The spectrum is used for incident light. The filter in the spectrophotometer reduces the incident light to a comparatively narrow band.

Lowry and Bessey (27) adapted the Beckman DU spectrophotometer for measurements in small quantities of biological materials. The cuvettes are approximately 2 x 10 mm, 25 mm. high and can be filled to a volume of 50 ccm. The light path has been reduced with a diaphragm to less than 2 x 2 mm. so that the light can pass through the liquid without touching the cuvette walls or the liquid meniscus. The optical density of micro volumes compared favorable with expected values as shown by the following readings:

Standard Solutions (mg. per cent)	Density Readings	
	Micro	Theoretical
0.2	0.013	0.017
0.4	0.034	0.034
0.8	0.069	0.069
1.6	0.133	0.138
3.2	0.276	0.276

If whole blood is allowed to clot, the serum separates from the red cells. Turbidity and hemolysis can be expected in serum left in contact with cells for 24 hours (30).

Serum may be stored at -20°C in small volumes. Red cells may be kept as long as 2-3 weeks at 4°C . An anticoagulant must be added if longer storage is required. Red cells may be frozen at -20°C in citrateglycerol mixtures (9).

Mindlin and Butler (36) and Lowry, Lopez and Bessey (28)

found that capillary blood did not vary markedly from venous blood in plasma ascorbic acid concentration. Collection of micro samples did not appear to be accompanied by oxidation of ascorbic acid. This was demonstrated by a comparison of finger and venous blood samples.

Subject	Dichlorophenol-indophenol (macro)	Roe and Kuether Vein (micro)	Roe and Keuther finger (micro)
1		1.92	1.84
2	1.31	1.34	1.34
3	1.33	1.33	1.35
4	1.01	0.93	1.03
5	1.14	1.11	1.23
6	0.24	0.13	0.20
7	1.10	1.05	1.12
8	0.32	0.27	0.28
9	1.64	1.63	1.65
10	1.26	1.21	1.28

Total ascorbic acid and ascorbic acid measurements of plasma were compared by Sabry and Dodds (55). Fingertip blood samples were collected in oxalated vials. The Bessey-Lowry method was used to determine total ascorbic acid and the Mindlin-Butler method was used for ascorbic acid determinations. Both methods were modified for use with plasma samples of 0.1 ml. A total of 61 measurements were made in triplicate and duplicate. The difference between the total ascorbic acid and ascorbic acid was 0.18 mg. per 100 ml. This was a significant difference. Differences were attributed in part to oxidation during the time between collection and precipitation of the protein. This does not necessarily indicate that dehydroascorbic acid is present in circulating blood but direct comparisons between values for total ascorbic acid

and ascorbic acid should not be made.

Human Blood Levels of Ascorbic Acid

The response of blood serum and white cells to the ingestion of ascorbic acid and dehydroascorbic acid has been investigated by several workers. The methods most commonly used for the measurement of total ascorbic acid were those of Roe and Keuther and micro analyses were carried out by the method developed by Bessey and Lowry.

Morse, Potgeiter and Walker (40) studied a group of 19 women consuming increased intakes of ascorbic acid for 22 weeks. Venous blood samples were analysed for total ascorbic acid by the method of Roe and Keuther. Serum ascorbic acid levels were found to be good indicators of white cell saturation from intakes of 33 to 83 mg. Higher intakes of 133 mg. did not produce an increase in the white cell concentration of ascorbic acid. Serum ascorbic acid levels ranged from 0.47-1.69 mg. per cent. Serum levels continued to rise in 15 of the 19 subjects at the high level of intake. These workers concluded that serum ascorbic acid concentrations were indicative of the general nutritional status but not of the degree of tissue saturation.

The effect of supplementation with ascorbic acid at levels 6-8 times greater than the intake of the control group (47-60 mg.) was studied over a 3 year period on a group of women attending college. Lamden (24) and co-workers observed an increase in both serum and white cell ascorbic acid levels. Analyses were made on blood samples from fasting subjects

obtained from a finger prick. The micro method developed by Bessey and Lowry was used for the total ascorbic acid determinations. The subjects with the greatest increases in the serum ascorbic acid content tended to be the subjects with the greatest increases in the ascorbic acid in the white cells. No significant correlations were found between the ascorbic acid intakes and the white cell concentrations of the vitamin. These observations were thought to be the result of intakes which were excessively higher than the control group.

Morse, Potgeiter and Walker (41) obtained similar findings in a study on the response of serum and white blood cells to ascorbic acid supplements. They increased the daily dietary intake to 107 mg. over a 10 week period. This represented a smaller level of supplementation than in the above studies. The groups consisted of 28 women divided into two age groups with average ages of 31 and 64 years. Venous blood samples were analysed for total ascorbic acid by the method developed by Roe and Keuther. Serum levels of ascorbic acid exhibited a steady rise at each level of supplementation. Each rise was significant at every level except for 57 mg. in the younger group. Serum levels rose from 0.33 to 1.76 mg. per 100 ml. in the younger group and from 0.24 to 1.42 in the older group. The correlation between serum and white cell ascorbic acid was significant at two levels of intake, 32 and 47 mg., in the younger group. The average white cell concentration of the younger group reached a

peak 2 weeks earlier than the older group on the same intake. A t-test indicated there was no significant difference due to age.

Thus it seems that supplementation of ascorbic acid over intakes considered to be normal resulted in detectable rises in the serum levels of ascorbic acid. White cell concentrations do not appear to show a continuous response to increased intakes of ascorbic acid. Therefore, serum ascorbic acid levels probably relate more closely than the concentration of the white cells to previous intake.

Steele and co-workers (59) observed a rise in the ascorbic acid content of serum in 13 adults who had previously been depleted. The study was carried out over a 78 day period. During the first 38-42 days, the subjects were given a diet containing 10 mg. of ascorbic acid. The ascorbic acid was increased at intervals of 20 mg., 30 mg., and 40 mg. Venous blood samples were analysed for total ascorbic acid by the 2,4 dinitrophenylhydrazine method of Roe and Keuther. At the 40 mg. level of intake, significant differences were reported for serum and white cell concentrations.

Lowry, Bessey and Lopez (29) studied the response of serum and white cells depleted of ascorbic acid to supplementation. They reported on 30 subjects who had been on controlled intakes of 8 mg. daily for 8 months. Daily intakes were increased for 500 to 2000 mg. Serum and white cell concentrations responded quickly. In 1 day with a dosage of

2000 mg., 2 days with 1000 mg., and 2-4 days with 500 mg., the recovery of the depleted serum and white cells was nearly complete. The samples were analysed by the micro method developed by the author for the determination of total ascorbic acid.

Thus from studies using depleted subjects, there is evidence of correlation between the rise of ascorbic acid serum and the increased concentration of the vitamin in the white cells.

Relationship between serum concentrations and dietary intake was observed on a total of 23 subjects by Morrow (35) ranging in ages from 8-15 years. In a group considered to have a high intake, 70 per cent consumed 40 mg. of ascorbic acid or more and 71 per cent had serum values of 0.6 mg. per cent or more. In a group considered to have low intakes, 33 per cent of the children had intakes of 40 mg. or more and 49 per cent had serum levels of 0.6 mg. per cent or more. There were more serum levels of 0.6 mg. per cent in the high group than in the low group.

Bryan and co-workers (1) were able to correlate dietary intake and plasma levels of ascorbic acid. They correlated 65 weighed dietaries with the plasma ascorbic acid levels of 56 fasting subjects ranging in ages from 12-35 years. The diet was recorded from 10-14 days. Slightly more males than females were studied. It was observed that increases at high levels of intake did not cause a direct increase in plasma level above 1.0 mg. per

cent. Intakes ranged from 1.7-1.9 mg. per kilo, e.g., a 150 lb. man would consume approximately 105 mg. of ascorbic acid.

There appears to be some relationship between serum ascorbic acid and the dietary intake although correlations have not been reported by all investigators, e.g., Mirsky (38). Factors contributing to the lack of correlation were summarized by Bryan et al (1). Some of these factors are the variation found in plasma determinations, the irregular eating habits of the subjects in respect to ascorbic acid and the fact that the value assigned to the ascorbic acid in the food is only an estimate. In these cases, it is questionable whether the plasma attains complete equilibrium with the dietary intake of ascorbic acid. Bryan concluded that the determination of ascorbic acid in a fasting subject afforded an objective method of evaluating the diet of the normal individual in regard to previous ascorbic acid intake.

Evidence indicates that serum levels of ascorbic acid reflects previous intake. If average serum levels are to be obtained, blood samples from fasting subjects should be analysed.

The rate of increase in blood plasma after ingestion of ascorbic acid was studied by Todhunter, Robbins and McIntosh (64) on 5 subjects. When studying large population groups it is not always convenient to obtain a blood sample

from a fasting subject. Reduced ascorbic acid was measured by the micro method of Farmer and Abt. The maximum increase in the plasma was found to occur 1 1/2 hours following the ingestion of 50 mg. of ascorbic acid. Blood levels returned to normal 3-4 hours later.

Linkswiler (26) reported that 20 per cent of the total ascorbic acid in the blood is in the dehydro form. Therefore, it is important to know something about the human utilization of dehydroascorbic acid. Micro samples were used for the analyses. Total ascorbic acid was determined by the Lowry-Bessey method and ascorbic acid was measured using the method of Mindlin and Butler. This author also observed an increase in the concentration of dehydroascorbic acid 1 1/2 hours following the intake of either form of the vitamin.

Excretion of both forms of the vitamin, using 2 male subjects, was measured by Clayton et al (6). Similar excretions of ascorbic acid were observed, regardless of the form consumed, thus indicating that dehydroascorbic acid was comparable to ascorbic acid. Ascorbic acid was determined by titration with 2,6 dichlorophenolindophenol and dehydroascorbic acid was measured by the method developed by Roe and Keuther.

Utilization of dehydroascorbic acid was investigated by Sabry, Fisher and Dodds (56) on 12 young men at two levels of intake. Total ascorbic acid was measured in the

blood plasma and urine using the Lowry-Bessey method of analysis after the ingestion of 110 and 75 mg. of total ascorbic acid. Ascorbic acid was determined by the Mindlin-Butler method. Although there were differences in the total ascorbic acid measurements of the blood and urine, the utilization of dehydroascorbic acid maintained comparable blood plasma levels of ascorbic acid on both forms of intake. Micro blood samples were used.

Todhunter et al (63) used orange juice treated with norit as a source of dehydroascorbic acid. Ingestion by human subjects produced levels of reduced ascorbic acid in blood serum comparable to those obtained when the reduced form of the vitamin was fed. This indicated that the utilization of dehydroascorbic acid was satisfactory. Micro blood samples were analysed for total ascorbic acid using the Lowry-Bessey method of analysis.

There is reason to believe that dehydroascorbic acid is utilized by the human in a manner similar to that of ascorbic acid. It appears that the preferred method of analysis in the evaluation of ascorbic acid should measure total ascorbic acid.

Nutritional Studies of Population Groups

Canadian nutritional studies to date have been confined to the evaluation of the nutritional status of groups, primarily the low income group. All groups studied in Saskatchewan, British Columbia, Winnipeg, Toronto, Halifax,

and Edmonton revealed similar findings in regard to adequacy of nutritional intake. Blood serum levels of Saskatchewan school children ranged from 0.01-2.0 mg. per cent, with 56 per cent of the group below 0.4 mg. per cent (46). A corresponding group in British Columbia reported serum ascorbic acid values from 0.04-2.4 mg. per cent, with 40 per cent of the group below 0.4 mg. per cent. Dietary intakes were estimated for the remainder of the groups and were found to be inadequate in the majority of diets.

The American population has been studied more extensively. Leverton (25) found that one of the greatest lacks of the diets of the farm families in Nebraska was citrus fruits. As a result only 42 per cent of the families reported adequate intakes of ascorbic acid. The outstanding weakness of diets, of 1800 individuals surveyed in six states of the Northeast Central Region was the inadequacy of ascorbic acid (8).

Experimental Method

In 1957, the Carman Farm District Business Association was organized for the purpose of co-operating in farm management research with the Department of Agricultural Economics and Farm Management at the University of Manitoba. The membership included farmers from the areas of Carman, Elm Creek, Homewood, Sperling, Myrtle, Roland, Miami, and Graysville. Appendix A). The farms were mainly of the mixed farming type. Carman is approximately 60 miles south of Winnipeg.

In January 1960, a letter was sent to the members of the group who had been visited during earlier surveys. (Appendix B). Preliminary findings of the family food consumption survey conducted in the fall of 1957 were reported and their co-operation was enlisted in an assessment of the nutritional status of the individual by dietary record and biochemical test.

Laboratory facilities at the Carman Memorial Hospital were placed at the disposal of the University for use as a collection centre for blood specimens. Initially, arrangements were made to have the volunteers visit the hospital. Later, visits to the farm homes were found to be more convenient for the subjects. Most of the specimens were collected in this manner. All subjects appeared to be in good health except for one with asthma and two with slight head colds.

Blood Collection and Analyses

The meal preceding the collection of the blood sample was free of foods high in ascorbic acid. The subjects were asked to omit fruit and vegetables from this meal. Blood samples were obtained from a finger prick (Appendix C).

Capillary tubes were found to be the best receptacle for the collection of the micro blood sample. According to Davies (11), collection tubes should meet the following requirements:

- a. be convenient to the clinician.
- b. provide freedom from hemolysis.
contamination.
evaporation.
breakage.
leakage.
- c. yield a maximum amount of serum.
- d. be easily transported.
- e. be economical.

The capillary tube of the size stated in the method met all these requirements under the situation tested.

The average time lapse between the time of collection and centrifuging was 4 hours.

The method of analysis of blood serum for total ascorbic acid is outlined in Appendix D. The Beckman DU spectrophotometer was adapted for micro samples by the method suggested by Lowry and Bessey (27). Before each series of readings, the correction factor of the cell was determined. The cells were cleaned between individual determinations with distilled water, ethyl alcohol and ethyl ether in that order. The amount of ascorbic acid in the sample was read directly from a standard curve. Samples were read in trip-

licate. In some cases the amount of serum was limited and it was not possible to obtain enough liquid for triplicate readings.

Food Records

Individual food records were kept for a 3 day period either prior to or following the sample collection depending on individual circumstances.

The number of days and which days the records are kept depends on the precision required (4). Previous food consumption studies with this group revealed that their food habits did not vary greatly from day to day. A shorter interval is less time-consuming for the homemaker and was thought to provide sufficient precision in this situation. Tables of average values (44) were used to calculate the intake of ascorbic acid. Cooking losses were deducted as an average value of 35 per cent where there were no analytical values for cooked foods. The homemaker was asked to record the food eaten in terms of household measures. The accuracy of the records varied from families who measured the food in a measuring cup to those who estimated the size of the servings. The importance of stating whether the food was raw, frozen, or canned was emphasized.

Experimental Results

The results reported here are the analysis of 45 food records and the serum of 45 micro blood samples for total ascorbic acid. Eight serum values were discarded; 4 because of inadequate amounts of serum and hemolysis; the dilution of 1 was questionable; 1 serum sample appeared to be turbid; 1 sample evaporated during centrifuging and a corresponding food record was not obtained for 1 blood sample. One individual intake was recorded but it was not possible to obtain a blood sample. The final group consisted of 7 adult males, 24 adult females, 3 males and 1 female over 12 years of age and 10 children under 12 years.

Ascorbic Acid Intake

The average daily ascorbic acid intake as estimated from the food records was 91.0 ± 41.6 mg. with a range of 15.2-214.4 mg. Only 2 persons consumed less than the recommended allowance of 30 mg. per day. This represents 4.4 per cent of the group. Twenty individuals or 44.4 per cent of the group recorded intakes ranging from 30-75 mg. daily. Twenty-three persons recorded intakes over 76 mg. per day. This represents 51.0 per cent of the group. Of these, 4 persons consumed amounts over 150 mg. daily.

A survey of family food consumption conducted in the fall, 1958, and spring, 1959, with 49 families and 32 families respectively in the same rural area revealed that the consumption of citrus fruits and tomatoes and other fruits, leafy

green and yellow vegetables and milk were below the intake recommended by Canada's Food Rules in a considerable number of families. Potato consumption was below the recommended intake in a minority of families (Table I).

The percentage of ascorbic acid contributed by food groups was compared with a similar analysis of the Canadian dietary during 1956 (44). The percentage contribution of ascorbic acid by tomatoes and citrus fruits and other fruits was above the Canadian average. The contribution by vegetables other than potatoes to the total ascorbic acid intake was similar to the Canadian average. However, in this group, the amount of ascorbic acid ingested from potatoes and dairy products was lower than the reported Canadian average (Figure I).

Blood Serum Levels

The total ascorbic acid values for serum ranged from a trace to 3.36 mg. per cent. The average value was 1.25 ± 0.74 mg. per cent. The correlation coefficient was $-.051$ for dietary intake and serum levels. However, there appeared to be a trend for high intakes to be associated with higher serum levels (Table II, Table III). In the group consuming over 30 mg. per day of ascorbic acid, 12 individuals or 27.9 per cent had serum levels below 0.8 mg. per cent.

There was a difference of 0.02 mg. per cent between the mean values of the ascorbic acid serum levels for males and females. The mean value of the male group was 1.24 ± 0.20 mg. per cent while the mean value of the female group was $1.26 \pm .28$ mg. per cent.

Weekly food consumption in pounds (edible portions) per person during August-September, 1958 (Total of 49 families) and April-May, 1959 (Total of 32 families).

Food Group	Recommended Weekly Intake Per Person	Fall Survey			Spring Survey		
		Average Intake	Range	Below Recommended Intake (%)	Average Intake	Range	Below Recommended Intake (%)
Tomatoes and Citrus Fruits	1.54	1.77	0.04-5.02	44	1.90	0.0-5.40	31
Other Fruits	1.54	1.92	0.17-6.13	37	1.62	0.0-3.85	41
Leafy, green and yellow vegetables	1.54	2.04	0.11-4.93	42	1.63	0.63-3.78	50
Potatoes	1.54	2.70	1.11-5.68	19	2.75	0.57-9.51	19
Milk	adult 4.41 adol. 13.30 child 9.10	7.21	2.03-15.58	54	7.41	0.97-15.83	50

FIGURE 1

Percentage Contribution of Food Groups
to Ascorbic Acid Intake

Canadian Dietary 1956

This Study

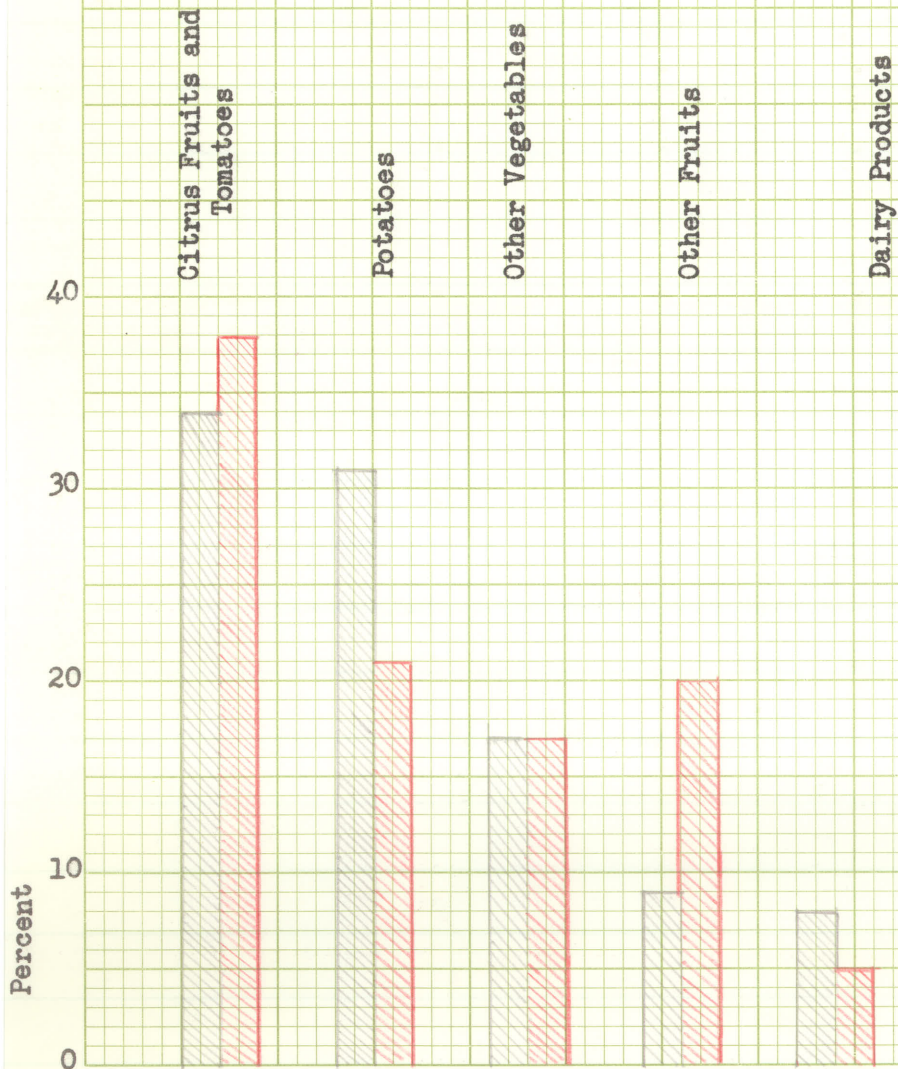


Table II

Blood Serum Levels as Related to Dietary Intake

Dietary Intake (mg)	Serum Concentration of Ascorbic Acid mg/100 ml						
	Below 0.4	0.4 - 0.8	0.9 - 1.2	1.3 - 1.6	1.7 - 2.0	2.1 - 2.4	Over 2.4
Below 30		1	1				1
31 - 75	2	4	1	6		1	
76 - 150	2	5	8	2	4	2	1
Over 150	1	1					

Table III

Serum concentration of ascorbic acid and dietary intake of ascorbic acid
of 45 subjects

Number of Subjects	Intake Mg/Day				Blood Concentration Mg/100 ml		
		Mean	St. dev.	Range	Mean	St. dev.	Range
2	Below 30	18.92	-	15.17-22.68	1.77	-	0.73-2.88
16	31-75	56.94	14.6	30.73-75.59	1.19	0.68	0.32-2.88
24	76 - 150	108.58	11.1	76.38-150.85	1.29	0.74	0.10-3.36
3	151 and over	177.67	-	153.40-214.40	.90	-	trace-1.83
Total		90.5	12.8	15.17-214.40	1.29	0.71	trace-3.36

Discussion of Results

Correlation between dietary intake of ascorbic acid and blood serum levels has not been reported by all investigators. The negative correlation coefficient obtained here indicates that there is not, in this group, a direct relationship between these two factors. Although adult females were in the majority in the total group of 45, a wide range of ages in both sexes was included. Although it would have been desirable to increase the number of subjects in the study, the availability of subjects was a limiting factor. Children, under the age of 6 years, were not encouraged to participate in the survey because of the difficulty of keeping food records and of obtaining blood samples. As the final group was somewhat smaller than originally anticipated, there may be some question as to whether the 3 day measured food record provided adequate information about food intake. It is of interest to note that the Michigan group (49) increased the number of subjects 3 times (i.e. from 17 to 56 adult women) when the measured record was used instead of the weighed record. However, the limited time of the rural homemaker to devote to record keeping of this type makes a longer term record less practical. The weighing of individual diets seems to be impractical in the rural meal setting. Since this group had had previous experience keeping weighed records for family intakes, there was a good possibility that the measured food record was a close estimate of the actual intake.

The average serum levels reported here appear to be higher than those reported in similar studies. In view of this, an assessment of ascorbic acid excretion would have been of interest, but collection of these data would have been very difficult with the group under study.

The findings revealed a wide range of serum levels at a given intake although there was a general trend for the majority of intakes over 30 mg. to correspond to serum concentrations over 0.8 mg. per cent. The variability of biological materials would tend to be reflected in the range of serum values. Low serum values corresponding to high intakes may indicate increased physiological requirement or loss during analysis. The methods used for the collection of blood and for the chemical analysis were thought to prevent significant losses of ascorbic acid. High serum concentrations associated with low intakes may have been the result of the consumption of ascorbic acid rich foods immediately prior to the collection of the blood samples. Although the subjects were asked to omit fruits and vegetables from the meal immediately preceding the collection of the blood sample, it is possible that potatoes were consumed in amounts sufficient to cause a rise in serum levels.

High serum levels should correspond with high intakes of ascorbic acid (40). The average intake of the group, 91.0 mg. per day, was well above the Canadian Dietary Standard Allowance of 30 mg. daily, and the recommended allowance of the U.S. National Research Council of 70 and 75 mg. per day.



The Canadian Dietary Standard was established for 3 purposes (34); (1) as a basis for planning food supplies for persons or groups, (2) to assess the adequacy of observed intakes, (3) to indicate a "nutritional floor" beneath which the maintenance of health cannot be assumed. The basis of the recommendations of the National Research Council is the "standard" man or woman, 25 years of age, living in a temperate climate, weighing 65 kg. and 55 kg. respectively and involved in moderate physical activity.

McHenry (34) points out that the Canadian Standard is not minimal but a conservative estimate of the optimal requirement. In many areas the Canadian and American Allowances are identical, e.g. calcium. The adequacy of the diet should not be judged by these allowances as there is evidence that nutritional deficiencies do not occur below the recommended amounts. The U.S. Recommended Allowance includes a margin of safety above the minimal requirement to allow for differences in individual requirements.

Previous unpublished data (13) indicated that the ascorbic acid intake on a family basis was above the Canadian Dietary Standard for all 49 families, but the majority of the families were below intakes assessed to be adequate on the basis of Canada's Food Rules (Table I). One can conclude that the consumption of foods rich in ascorbic acid recommended by Canada's Food Rules supplies more than adequate amounts of the vitamin. The percentage contribution of ascorbic acid from fruits, citrus fruits and tomatoes was found

to be higher than the Canadian average for 1956 (Figure 1). Due to favorable economic conditions in the area and the general interest in good nutrition among the group, consumption of adequate nutrients might be expected.

It is of interest that significant correlations between the dietary intake and blood concentration of ascorbic acid were reported by Bryan et al (1) and Morgan et al (39) in two markedly different studies. Bryan et al, in the analysis of 65 weighed dietary intakes over a 10-14 day period and plasma for total ascorbic acid, using subjects 12-35 years of age, observed that plasma concentrations above 1.0 mg. per cent did not vary with dietary intake. Morgan analysed blood serum for total ascorbic acid and assessed dietary intake using the 7 day measured records of 577 men and women over 50 years of age and found a positive correlation, significant at the 1 per cent level. Roderuck et al (49) demonstrated a curvilinear relationship between blood concentrations of ascorbic acid in 569 women, aged 20-99 years, and dietary ascorbic acid, expressed in terms of body weight, and recorded by several types of food records.

Serum concentrations reported by Nutrition Division, Ottawa (33), are low compared to those of the Manitoba group. It should be noted that the Ottawa Laboratory evaluates groups where deficiencies are expected to occur. A study by Miller (37) on University girls living in the University residence revealed a majority of low serum levels, although the dietary

intakes were high. The serum samples were frozen and shipped to Ottawa without the addition of trichloroacetic acid. It would seem that these values might represent a loss of serum ascorbic acid.

There appears to be other factors which affect the direct relationship between blood concentrations of ascorbic acid and intake which has been noted particularly at high levels of intake (41) and blood concentration (49). The proposed physiological function of ascorbic acid indicates that the requirements could vary with body weight and perhaps metabolic activity. A followup study of pregnant women over a 9 month period with blood and urinary analysis would be of interest.

While we cannot demonstrate that blood serum levels are directly related to dietary intake, the Manitoba group follows a trend which has been reported by other investigators, that is, that the serum levels tend to rise with increased ascorbic acid intake. The two extreme groups, intakes below 30 mg. per day and above 151 mg. per day, do not show the same trend. However, the number of subjects in each group in this study is too small for conclusions to be drawn. Previous studies by Kyhos et al (23), Mayer et al (32), Merrow et al (35) and Roderuck et al (49) have shown that there is an association of adequate serum ascorbic acid levels with adequate intakes of ascorbic acid, that ascorbic acid deficiencies responded to increased intakes of the vitamin, and that adequate serum levels were found more frequently in subjects who were judged

to be "better nourished". In general, adequate levels of blood ascorbic acid indicate adequate ascorbic acid nutrition of groups. Evidence obtained from this study indicates that the group was adequately nourished in respect to ascorbic acid nutrition.

SUMMARY AND CONCLUSIONS

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.

General conclusions about the ascorbic acid nutrition of rural Manitobans should not be made because of the smallness of the group and the superior economic conditions prevailing in the area.

The average intake of ascorbic acid was 91.0 ± 41.6 mg. per day. This figure is well above both the Canadian Dietary Standard and the U.S. National Research Council's Recommended Allowance. Two persons consumed amounts below the Canadian Dietary Standard of 30 mg. per day.

Although this group consumed less than the amounts of ascorbic acid rich foods recommended for intake by Canada's Food Rules, the individual intakes were above the Canadian Dietary Standard. The former standard of dietary adequacy appears to offer a liberal daily intake of ascorbic acid.

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

The average serum level of ascorbic acid was 1.25 ± 0.74 mg. per cent. In general, the serum concentrations rose with increased intakes. A majority of the 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 serum concentration. The relationship of ascorbic acid intake and blood concentration appears to be affected by other factors.

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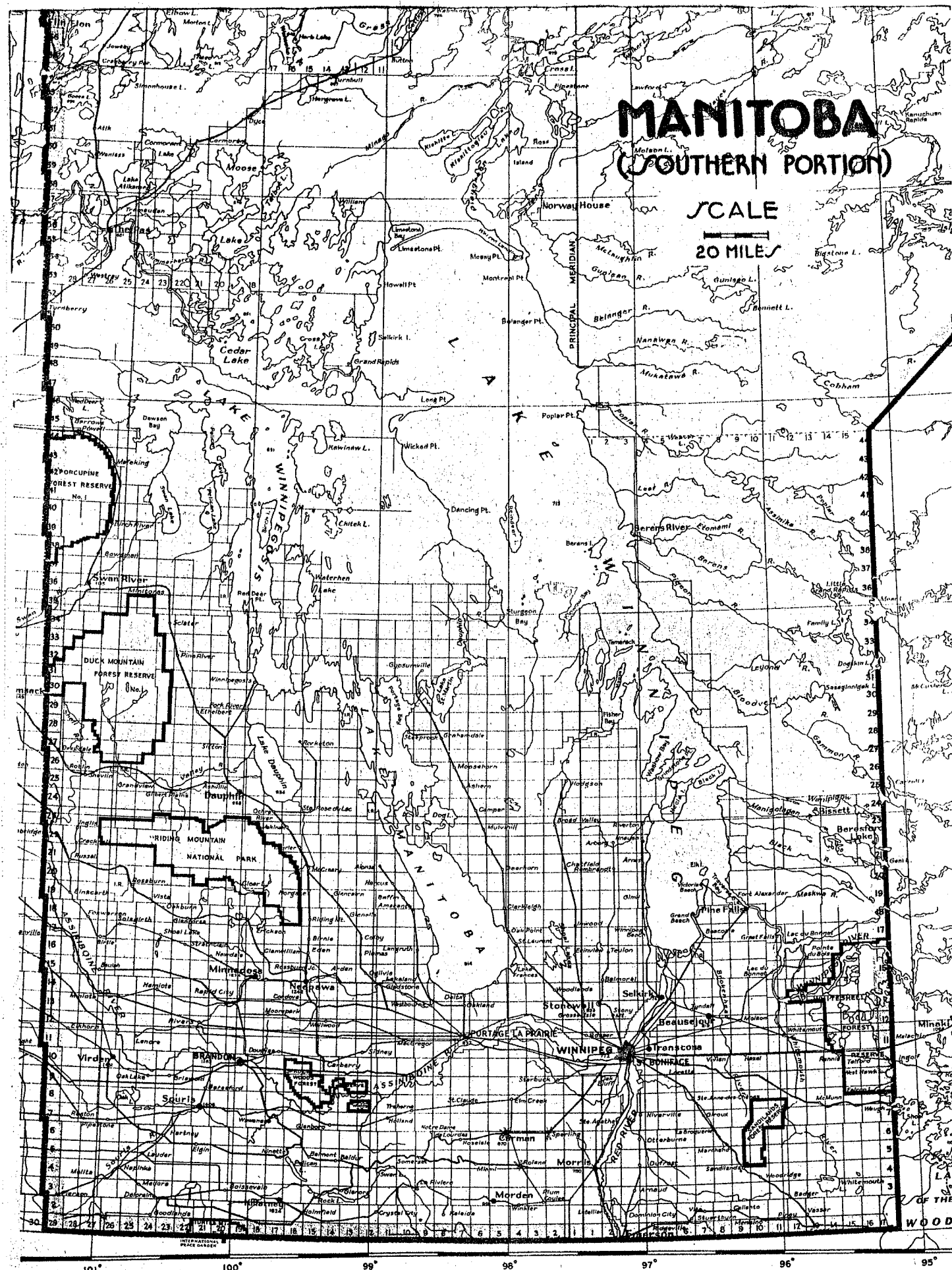
APPENDIX A

MANITOBA

(SOUTHERN PORTION)

SCALE

20 MILES

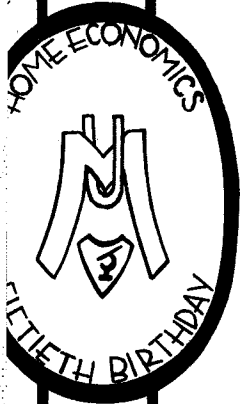


APPENDIX B

THE UNIVERSITY OF MANITOBA

SCHOOL OF HOME ECONOMICS

WINNIPEG, CANADA



Dear Madam:

We hope that you have been interested in the summary of the food consumption survey in the Carman district. We feel that the information we have gained about food consumption levels and nutrient intake of Manitobans has been very worthwhile. The summary of the nutrient content of your family's food intake and comparison with average value is almost ready to send to you. The overall findings from the Fall survey were as follows:

"Twenty-nine (60%) out of the 48 families met the requirements of the Canadian Dietary Standard for the nine nutrients considered, namely: calorie, protein, calcium and iron, vitamin A, thiamine, riboflavin, niacin, and ascorbic acid. One family fell slightly below the requirement for calories. Thirty-nine percent of the families did not meet the requirement for calcium. No families fell below the standard for protein, iron or the five vitamins."

As in all food consumption studies it becomes important to evaluate the individual. We are interested in knowing how the nutrient intake of your teenage son compares with your own, for example. Such a study would involve a record of individual food intake kept for a 3-day period, the food being recorded in household amounts. At the same time, blood and urine analysis are usually done to correlate the dietary record with the nutrient status of the individual. We are interested in obtaining a sample of finger prick blood only which in your case would involve a trip to Carman at a predetermined time.

1960

- 2 -

Would you like to continue with this further aspect of our study? It would not be necessary for the entire family to participate in the survey, although we would like to have as complete a group as possible.

If you are willing to participate in this final stage of the foods and nutrition survey, will you indicate on the checklist below, and mail back to us as soon as possible? We tentatively plan to get the records and blood samples toward the end of February.

Sincerely yours,

(Mrs.) Elizabeth Feniak,
Associate Professor,
Foods and Nutrition

_____ yes Our family would be interested in participating
 in the project on individual nutrition.

_____ no

_____ The number of food records and blood samples that
 might be obtained from our family.

Name: _____

Address: _____

School of Home Economics
University of Manitoba

Individual Food Record

Name

Date

Address

Age

(If adult, indicate by "adult")

Please keep a record of the food consumed for 3 consecutive days. Three days are taken as being typical of your food intake for this season of the year. On the day the blood sample is taken, it is important that you do not consume any fruits or vegetables. The blood level of Vitamin C is elevated considerably immediately after the intake of foods high in Vitamin C.

Example:

Food	Household Measure	Description
Apple	1 medium	raw
Butter	1 teaspoon	
Salmon	$\frac{1}{2}$ cup	creamed
Beef	4 ounces	in stew

Name _____

Day _____

MORNING

HOUSEHOLD MEASURE

DESCRIPTION

NOON

NIGHT

BETWEEN MEAL LUNCHES

APPENDIX C

Method of Blood Collection

1. Wash the hand in warm water. Swab the finger with rubbing alcohol and prick the fleshy part of the finger using a disposable lancet.
2. Collect blood in a capillary tube 2.25x10 mm. Seal the ends with modelling clay.
3. Allow the blood to clot.
4. Wrap tubes in pieces of paper. Fold the ends over to prevent breakage and centrifuge in regular centrifuge tubes at 2400 rpm for 10 minutes.
5. Break the capillary tube above the red blood cells.
6. Remove the serum with 10 mm³ Canlab self-filling pipettes.
7. Transfer the serum to a 10x75 mm. tube using a 3 mm Pasteur pipette.
8. Add a known volume of 5% trichloroacetic acid.
9. Seal with Saran and masking tape.
10. Freeze.

APPENDIX D

DETERMINATION OF ASCORBIC ACID IN SMALL AMOUNTS OF BLOOD

METHOD OF LOWRY, LOPEZ, AND BESSEY

A.. REAGENTS AND EQUIPMENT

Dinitrophenylhydrazine-Thiourea-Copper Sulfate Reagent. Dissolve 2 g. of 2,4 dinitrophenylhydrazine, 0.25 g. of thiourea, and 0.03 g. of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 100 ml. of 9 N H_2SO_4 (3 volumes of distilled water to 1 volume of concentrated H_2SO_4). If a precipitate develops, centrifuge or filter through sintered glass. Store in a refrigerator and make up weekly.

65% Sulfuric Acid. Add 70 ml. of concentrated H_2SO_4 to 30 ml. of distilled water. Keep in a refrigerator and use while cold.

5% Trichloroacetic Acid.

Micropipets. Levy-Lang constriction micropipets to measure 10, 30, and 40 mm.³ are used.

Beckman Spectrophotometer. This is fitted with a special adapter and cuvettes to permit the use of 0.05 ml. fluid volumes (obtainable from Pyrocell Mfg. Co., 207 East 84 Street, New York).

6 x 50 mm. Tubes. Kimble No. 45060, for example.

B. Procedure

Into a 6 x 50 mm.³ tube pipet 10 mm of whole blood or serum and 40 mm.³ of 5% trichloroacetic acid. Mix the contents of the tube by tapping with the finger. Cap the tube with Parafilm and centrifuge for 10 minutes at 3000 r.p.m. Transfer 30 mm.³ of the supernatant fluid into another 6 x 50 mm. tube.

Prepare a standard as follows: Pipet 10 ml. of a 1 milligram per cent standard ascorbic acid solution into a 50 ml. volumetric flask. Make up to volume with 5% trichloroacetic acid. Mix thoroughly. Pipet 30 mm.³ of this solution into a 6 x 50 mm. tube.

Now add 10 mm.³ of dinitrophenylhydrazine-thiourea-copper sulfate reagent to the unknown and the standard tubes. Mix the contents of the tubes thoroughly by tapping, cap them with Parafilm, and place them in a water bath at 37°C. for 4 hours. Remove the tubes and chill them in ice water. Add to each tube 50 mm.³ of ice-cold 65% H_2SO_4 and mix very

thoroughly. Allow to stand at room temperature for 30 minutes and read the tubes in the spectrophotometer at a wavelength of 520 *mμ*. For a blank to set the spectrophotometer, pipet into a 6 x 50 mm. tube, 30 mm.³ of 5% trichloroacetic acid. Add 50 mm.³ of ice-cold 65% H₂SO₄ and 10 mm.³ of dinitrophenylhydrazine-thiourea-copper sulfate reagent.

C. CALCULATION

$$\frac{DU}{DS} \times 1 = \text{mg. ascorbic acid per 100 ml. of whole blood}$$
or serum where DU = optical density of the unknown, and DS = optical density of the standard.

The authors state that values average 0.1 milligram per cent higher by this simplified procedure than by the original method of Roe and Kuether. This probably results from omission of the treatment with charcoal, which apparently removes some non-ascorbic acid chromogenic material from the filtrate.

D. THE FOLLOWING ADAPTATIONS WERE MADE TO THE ABOVE METHODS.

1. Canlab self-filling micropipets to measure 10, 20, and 50 mm.³ were used.
2. Large volumes of serum were obtained. Instead of 6 x 50 mm. tubes, 10 x 75 mm. tubes were found to be more convenient to transport. Serum volumes of 30 mm.³ were used. All reagents were increased proportionately.
3. A standard ascorbic acid curve was prepared and readings were determined from it. Concentrations of optical density were read directly from the curve.
4. All samples were centrifuged at 2400 rpm for 10 minutes.
5. Pasteur pipets were made from 3 mm. glass tubing, and were used for transferring unmeasured volumes.

APPENDIX E

Procedure for Preparing a Standard Curve

1. Dissolve 40 mgm. L-ascorbic acid in 5% trichloroacetic acid and make up to volume in a 200 cc. flask.
2. Take aliquots of 3ml., 4ml., 5ml., 8ml., 10ml., and make to volume in 100 ml. flasks. Store in refrigerator.
3. To prepare a microcurve take 10 mm³ aliquots of the above standard solutions and 10 mm³ 2,4 dinitrophenylhydrazine reagent.
4. Incubate for 3 hours at 37°C.
5. Chill in ice water. Add 50 mm³ chilled 65% H₂SO₄.
6. Let stand at room temperature for 30 minutes and read in a spectrophotometer at 520 mμ.
7. Prepare a blank using 10 mm³ 5% trichloroacetic acid and treat as the standard solutions.

Average Optical Density of Standard Solutions of 2,4 Dinitrophenylhydrozine Derivatives of L-Ascorbic Acid.

L-Ascorbic acid concentration (mg. per cent)	N	Optical Density	
		Range	Mean
.6	22	.020-.132	.077
.8	6	.041-.091	.064
1.0	22	.057-.157	.096
1.4	14	.084-.179	.127
1.6	9	.083-.157	.123
1.8	14	.112-.212	.156
2.0	9	.103-.202	.148
2.2	9	.152-.214	.188
2.4	9	.127-.215	.161
2.6	5	.176-.227	.206

NOTE - Several readings were made of some standards because of the wide range of readings obtained.

Standard Curve for Spectrophotometric Determination of
Total Ascorbic Acid Using Light of 520 mμ Wave Length

