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CHANGES IN THE APPARENT ASCORBIC ACID OF STRAWBERRIES
DURING FROZEN STORAGE

by

Chuan-huan Wu

AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan
State University of Agriculture and Applied Science
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Hilma D. Brewer

Chuan-huan Wu

CHANGES IN THE APPARENT ASCORBIC ACID OF STRAWBERRIES
DURING FROZEN STORAGE

Changes in the apparent ascorbic acid of strawberries of the Catskill and Robinson varieties during frozen storage were studied.

Concentrations of total apparent ascorbic acid, reduced ascorbic acid, dehydroascorbic acid, 2,3-diketogulonic acid, reductones, reductic acid, total ascorbic acid, reducing sugar and total solids were determined in strawberries which had been frozen for 30 hours and in strawberries during frozen storage at -27 to -31 degrees Centigrade. Analyses were made at monthly intervals for a period of ten months in strawberries of the Catskill variety and six months in strawberries of the Robinson variety. The concentrations of the various components in the strawberries which had been frozen for 30 hours were employed as base line to study the changes of these components during frozen storage.

There was little change in the total apparent ascorbic acid of the strawberries during the first month of frozen storage; however, there was a marked decrease in reduced ascorbic acid and an increase in the concentrations of dehydroascorbic acid and 2,3-diketogulonic acid of the frozen strawberries during this period. Fluctuations in concentrations of the components of the strawberries of both varieties oc-

Changes in the Apparent Ascorbic Acid of Strawberries
During Frozen Storage

curred throughout the entire period of frozen storage; there was a significantly inverse relationship between the concentrations of 2,3-diketogulonic acid and total ascorbic acid of the frozen strawberries.

The influence of the changes of apparent ascorbic acid in strawberries during frozen storage on the utilization of ascorbic acid in strawberries was studied with six healthy young women as subjects. Subjects were given 75 milligrams crystalline reduced ascorbic acid as a supplement to their customary diet for seven days before each test period. Test doses given at four test periods were (a) 200 grams strawberries of Catskill variety, frozen for 30 hours; (b) an amount of crystalline reduced ascorbic acid equivalent to the amount of total apparent ascorbic acid provided in the test dose of 200 grams of strawberries; (c) an equivalent amount of dehydroascorbic acid in the form of orange juice treated with activated charcoal, and (d) 250 grams of strawberries, frozen and stored for four months. Blood samples were taken before and at hourly intervals after the test doses for a five-hour period. Urine was collected for a one-hour period preceding and for a five-hour period following each test dose. The blood serum was analyzed for reduced and total apparent ascorbic acid; urine was analyzed for reduced ascorbic acid.

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Changes in the Apparent Ascorbic Acid of Strawberries
During Frozen Storage

There was not a statistically significant difference among the maximum concentrations of ascorbic acid in the blood serum following the test doses, nor in the urinary excretions of ascorbic acid after the test doses. When the total apparent ascorbic acid of the strawberries which had been held in frozen storage was corrected for the amount of 2,3-diketogulonic acid present in the berries, the total available ascorbic acid appeared to be as well utilized as that of the strawberries which had been frozen for only 30 hours.

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TABLE OF CONTENTS

	PAGE
INTRODUCTION	1
REVIEW OF LITERATURE	5
Chemical methods for the determination of ascorbic acid in foods	5
Changes in the total ascorbic acid content of foods during storage	15
Possible role of biosynthesis in the ascorbic acid content of frozen foods	23
Studies of human utilization of ascorbic acid in foods	27
Ascorbic acid in the blood and urine	38
EXPERIMENTAL PROCEDURE	45
Determination of the apparent ascorbic acid content of frozen strawberries	45
Plan of experiment	45
Freezing and sampling of strawberries	46
Chemical methods	47
Reduced ascorbic acid	48
Total apparent ascorbic acid	49
Dehydroascorbic acid and 2,3-diketogulonic acid	51
Reductones and reductic acid	52
Reducing sugar	53
Total solids	54

	PAGE
Studies of the physiological utilization of the	
apparent ascorbic acid of frozen strawberries . .	54
Plan of experiment	54
Subjects	56
Preparation of test doses	56
Strawberries	56
Crystalline ascorbic acid	57
Dehydroascorbic acid	57
Collection and treatment of blood and urine	
samples	58
Chemical analysis	60
RESULTS AND DISCUSSION	61
Changes of the total apparent ascorbic acid in	
frozen strawberries	61
Reduced ascorbic acid	66
Total apparent ascorbic acid	70
Dehydroascorbic acid	75a
2,3-diketogulonic acid	76
Total ascorbic acid	78
Reducing sugar	79
Total solids	80



	PAGE
Concomitant changes in ascorbic acid and related compounds in frozen strawberries	80
Physiological utilization of the total apparent ascorbic acid of frozen strawberries	89
Description of the subjects	89
Serum concentration and urinary excretion of ascorbic acid before test dose	91
Ascorbic acid supplied in test doses	99
The utilization of ascorbic acid of the various test doses	106
Test dose of crystalline reduced ascorbic acid .	112
Test dose of dehydroascorbic acid	118
Test dose of strawberries, frozen for 30 hours .	120
Test dose of strawberries, frozen and stored for four months	123
Comparison of the various test doses	126
SUMMARY AND CONCLUSION	140
LITERATURE CITED	145

LIST OF TABLES

TABLE	PAGE
I. The Average Concentration of Ascorbic Acid and Related Compounds in Strawberries (Catskill Variety) at Monthly Periods of Storage	62
II. The Average Concentration of Ascorbic Acid and Related Compounds in Strawberries (Robinson Variety) at Monthly Periods of Storage	64
III. Analysis of Variance of the Concentration of Reduced Ascorbic Acid in Strawberries (Catskill Variety) Frozen for 30 Hours, Four Months and Eight Months	71
IV. Significance of the Difference of the Means of Reduced Ascorbic Acid in Strawberries (Catskill Variety) Frozen for 30 Hours, Four Months and Eight Months	71
V. Analysis of Variance of the Concentration of Total Apparent Ascorbic Acid in Strawberries (Catskill Variety) Frozen for 30 Hours, Four Months and Eight Months	75
VI. Significance of the Difference of the Means of Total Apparent Ascorbic Acid in Strawberries (Catskill Variety) Frozen for 30 Hours, Four Months and Eight Months	75

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TABLE	PAGE
VII. Correlation Coefficients of the Various Components of Strawberries (Catskill Variety) During Frozen Storage	84
VIII. Correlation Coefficients of the Various Components of Strawberries (Robinson Variety) During Frozen Storage	85
IX. Description of Subjects	90
X. The Reduced and Total Apparent Ascorbic Acid of the Blood Serum and the Urinary Excretion of Ascorbic Acid of the Subjects Preceding the Administration of the Test Doses for the Successive Experimental Periods	92
XI. Analysis of Variance of Concentrations of Serum Ascorbic Acid Preceding the Administration of Various Test Doses	94
XII. Analysis of Variance of the Concentration of Reduced Ascorbic Acid in the Urine of Six Subjects Preceding the Administration of Various Test Doses	97
XIII. Ascorbic Acid Content of Individual Test Doses of 200 Grams of Strawberries, Frozen for 30 Hours	100
XIV. Ascorbic Acid Content of Individual Test Doses of 250 Grams of Strawberries, Frozen for Four Months	102

TABLE	PAGE
XV. The Concentration of Ascorbic Acid and Related Compounds in the Various Test Doses	105
XVI. Reduced Ascorbic Acid Concentrations of Blood Serum of Six Subjects Preceding and at Periodic Intervals after the Administration of Various Test Doses	108
XVII. Total Apparent Ascorbic Acid Concentrations of Blood Serum of Six Subjects Preceding and at Periodic Intervals after the Administration of Various Test Doses	110
XVIII. Calculated Increment of Reduced Ascorbic Acid of Blood Serum of Six Subjects after Administration of Various Test Doses	113
XIX. Calculated Increment of Total Apparent Ascorbic Acid of Blood Serum of Six Subjects after Administration of Various Test Doses	114
XX. Average Urinary Excretions of Reduced Ascorbic Acid before and after Administration of the Various Test Doses	115
XXI. Analysis of Variance of the Maximum Concentrations of Ascorbic Acid in the Serum of Six Subjects after Various Test Doses: F-Values Adjusted by Analysis of Co-variance to Remove the Influence of Basal Blood Values on Maximum Concentrations	127

TABLE

XVII. Analyses

Chemical

Physical

.....

.....

.....

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.....

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.....

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TABLE	PAGE
XXII. Analysis of Variance of Concentration of Reduced Ascorbic Acid Excreted per Hour after Different Test Doses	133

LIST OF FIGURES

FIGURE		PAGE
1.	Graph Showing the Changes in Concentration of Ascorbic Acid and Related Compounds, Total Solids and Reducing Sugar in Strawberries of the Catskill Variety during Frozen Storage	63
2.	Graph Showing the Changes in Concentration of Ascorbic Acid and Related Compounds, Total Solids and Reducing Sugar in Strawberries of the Robinson Variety during Frozen Storage	65
3.	Concentrations of Blood Ascorbic Acid of Six Subjects before and after Various Test Doses	93
4.	Urinary Excretions of Reduced Ascorbic Acid by Six Subjects Preceding and Following the Administration of Various Test Doses.	96
5.	Concentrations of Reduced Ascorbic Acid of Blood Serum of Six Subjects before and at Periodic Intervals after the Administration of Various Test Doses	109
6.	Concentrations of Total Apparent Ascorbic Acid of Blood Serum of Six Subjects before and at Periodic Intervals after the Administration of Various Test Doses	111

FIGURE	PAGE
7. Mean Concentrations of Reduced Ascorbic Acid and Total Apparent Ascorbic Acid of Six Subjects before and at Periodic Intervals after Various Test Doses	129
8. Mean Successive Changes in Concentrations of Re- duced Ascorbic Acid and Total Apparent Ascorbic Acid of Blood Serum of Six Subjects after Vari- ous Test Doses	131

INTRODUCTION

Retention of ascorbic acid in stored or processed foods is important in providing foods which are of high nutritive value. Freezing is the most satisfactory method of preservation of ascorbic acid in foods for relatively long periods of storage, since chemical changes which affect ascorbic acid take place more slowly in foods held at lower temperatures than at higher temperatures.

It is generally accepted that the concentration of reduced ascorbic acid decreases in foods during frozen storage. However, contradictory statements have been found in the literature relative to the effect of frozen storage on the total ascorbic acid (reduced ascorbic acid and dehydroascorbic acid) content of foods. Bedford and McGregor (1948), Hewston, Fisher, and Orent-Keiles (1951), and Lee (1951) found that the total ascorbic acid concentrations of fruits and vegetables were decreased during frozen storage, although there were fluctuations in values at different time intervals. On the other hand, Paul, Wiant, and Robertson (1949) reported that increases in the concentration of total ascorbic acid occurred during frozen storage in a variety of fruits and vegetables. The highest concentration of total ascorbic acid was after a three months storage period. An

increase in the total ascorbic acid content of strawberries held in frozen storage for three months as compared with the total ascorbic acid content of fresh berries also was found in this laboratory by Einbecker and her co-workers (1947; 1950).

Apparent differences in the effect of frozen storage on the total ascorbic acid content of foods as reported from various laboratories may be due to (a) the possible presence of substances, related to ascorbic acid, which interfere with the chemical determination of total ascorbic acid in certain foods (Wokes, Organ, and Jacoby, 1943; Snow and Zilva, 1944; Purinton, 1947; and Somers, Kelly, Thacker and Redder, 1951); (b) the conversion at different rates under various conditions of the reduced form of the vitamin to its biologically inactive oxidation product which also is determined in the analysis of total ascorbic acid (Pi Joan and Gerjovich, 1946; Hartzler, 1948); (c) the possible biosynthesis of this vitamin from its precursors during frozen storage (Isherwood, 1953).

Biologically inactive reductones and reductic acid which are derivatives of carbohydrates and which have been reported to be present in foods (Wokes et al., 1943; Roe, Mills, Oestering and Damron, 1948; and Goldblith and Harris, 1948), react with 2,4-dinitrophenylhydrazine (Penney and Zilva, 1945), which is used as a reagent for the analysis of

total ascorbic acid. Changes in the relative concentrations of these substances during the storage of foods may therefore be a possible cause of variations in determined total ascorbic acid values.

The reduced form of the vitamin reacts rapidly with various oxygen carriers present in plant tissue to form the reversible oxidation product, dehydroascorbic acid. Above pH five dehydroascorbic acid readily undergoes a rearrangement in which the lactone ring is split. The product, 2,3-diketogulonic acid, is not reducible by sulfhydryl compounds in cells and is no longer biologically active (Borsook, Davenport, Jeffreys and Warner, 1937; Penney and Zilva, 1943a). This substance combines with 2,4-dinitrophenylhydrazine more rapidly than does total ascorbic acid to give the characteristic color of the osazone (Mills, Damron, and Roe, 1949). Thus, in a determination of total ascorbic acid content of foods based upon this color reaction, 2,3-diketogulonic acid also would be measured if present, and the apparent ascorbic acid values would exceed the true ascorbic acid concentration of the material.

Synthesis of l-ascorbic acid from reducing sugars in plants has been reported by Ray (1934) and Isherwood (1953) and in animals by Jackel, Mosbach, Burns and King (1950) and Horowitz, Doerschuk and King (1952). The synthesis of ascorbic acid in foods from reducing sugar during frozen stor-

age has not been demonstrated, but it is possible that biosynthesis of the vitamin also may occur under this condition in fruits which are not blanched before freezing.

This study was planned to investigate the relative concentrations of reduced ascorbic acid and total ascorbic acid of fresh strawberries and of strawberries stored in the frozen state and further to investigate the various substances which contribute to the apparent ascorbic acid values of frozen strawberries. The utilization of the total ascorbic acid of frozen strawberries by humans also was studied to investigate whether the changes in apparent ascorbic acid in strawberries during frozen storage represented true changes in the vitamin content of the berries.

Strawberries were selected as the food for the investigation of the changes in apparent ascorbic acid during frozen storage, since strawberries are a relatively rich source of ascorbic acid and since a marked increase of total ascorbic acid of strawberries during frozen storage was reported previously from this laboratory (Paul et al., 1949; Einbecker et al., 1947; 1950).

REVIEW OF LITERATURE

Chemical Methods for the Determination of Ascorbic Acid in Foods

Ascorbic acid values of food are reported usually as reduced ascorbic acid or as total ascorbic acid. The latter term has been used to designate both the reduced ascorbic acid and the oxidized form of ascorbic acid, dehydroascorbic acid. Both forms of ascorbic acid are biologically available, although some workers have reported that dehydroascorbic acid was not as well utilized as reduced ascorbic acid (Gould and Schwachman, 1943; Roe and Baunum, 1936). Early studies of the vitamin C content of foods by chemical analysis were based upon oxidation-reduction reactions of reduced ascorbic acid with an oxidizing substance as iodine (Stevens, 1938), methylene blue (Martini, 1934), or 2,6-dichlorophenolindophenol (Bessey and King, 1933). Results from these analyses corresponded closely with the antiscorbutic potency of foods as measured by the protective action of the food against the development of scurvy in guinea pigs (Penney and Zilva, 1943a). This relationship existed more closely, however, with the amount of reduced ascorbic acid in fresh foods than with the amount of reduced ascorbic acid in processed foods (Strobecker and Vaubel, 1936; Mack and

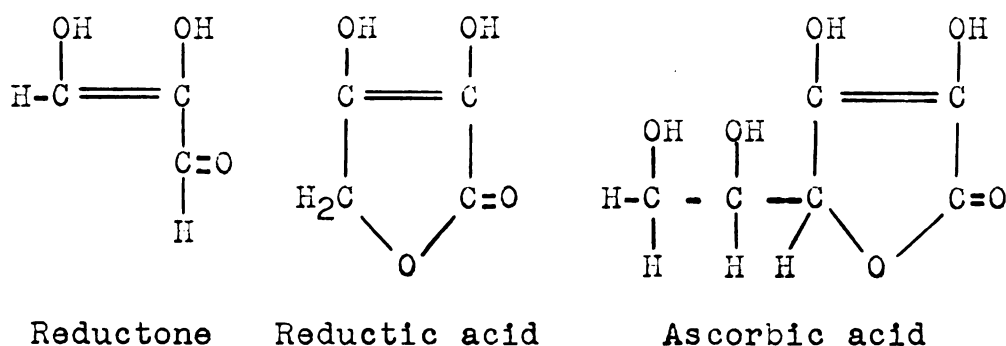
Tressler, 1937; Hou, 1937). This observation stimulated interest in the development of a method of analysis of ascorbic acid in foods which would measure both the reduced ascorbic acid and the dehydroascorbic acid.

Roe and Keuther (1942; 1943) reported a method for the determination of ascorbic acid. In the method, reduced ascorbic acid was converted to dehydroascorbic acid by treatment with an activated charcoal. The dehydroascorbic acid thus formed was coupled with 2,4-dinitrophenylhydrazine to form an osazone. When this osazone was treated with 85 percent sulfuric acid, a reddish colored product was formed which absorbed maximally at 500 to 550 millimicrons and 350 to 380 millimicrons.

A year later Roe and Oesterling (1944) modified the dinitrophenylhydrazine method to differentiate dehydroascorbic acid from the reduced form of ascorbic acid in plant tissue. In the modified procedure, tissue extract was treated with activated charcoal as in the Roe and Keuther method (1943). However, when thiourea was added to a second aliquot of tissue extract in an amount to give a one percent solution, the reduced ascorbic acid was protected by the thiourea in its reduced state and only the dehydroascorbic acid in the tissue extract reacted with dinitrophenylhydrazine. By a combination of the two procedures, then, the authors obtained a

value for the concentration of combined reduced ascorbic acid and dehydroascorbic acid in the plant extract and a value also for dehydroascorbic acid alone.

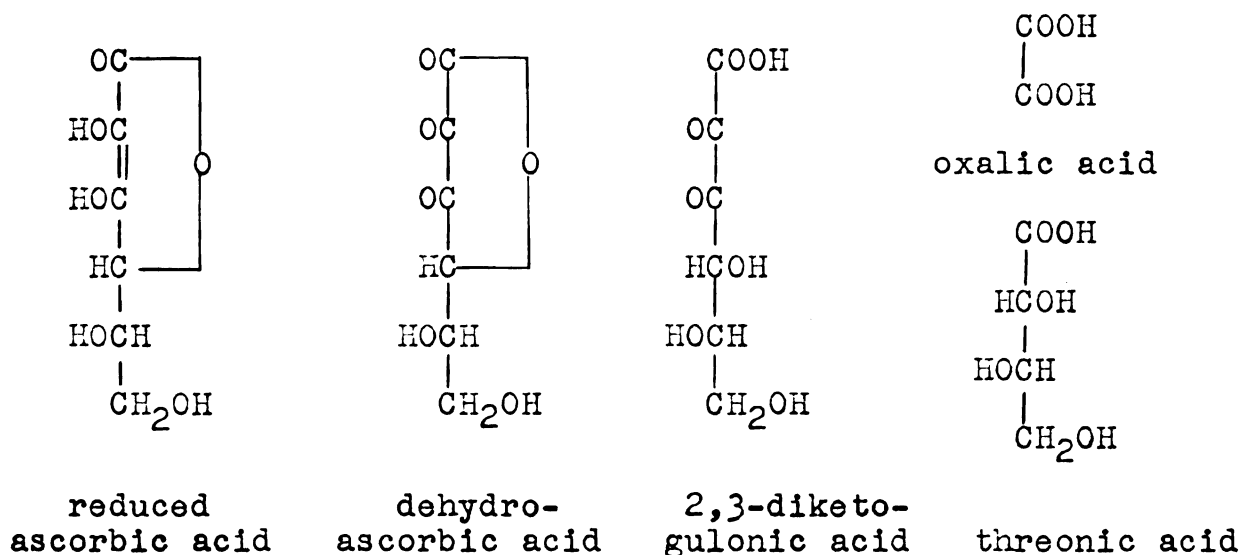
Reductones and reductic acid are related closely in chemical structure to ascorbic acid.



The enediol group is common to the three compounds. Carpeni (1938) has reported that the ultra-violet absorption spectra of the compounds were similar with respect to three absorption maxima. Penney and Zilva (1945) reported that l-ascorbic acid, reductone and reductic acid solutions incubated at 25 degrees Centigrade for fifteen minutes with dinitrophenylhydrazine produced 2.5, 48, and 92 percent respectively of the color given by ascorbic acid and dinitrophenylhydrazine after three hours at 37 degrees Centigrade. These authors suggested that interferences by the reductones and reductic acid in the determination of ascorbic acid might be controlled by regulating the conditions of incubation. However, this procedure failed to differentiate the reductones and reductic acid from ascorbic acid when all were present in the same extract. Later, Schocken and Roe (1952) devised a

method for the determination of reductones and reductic acid based upon the fact that the reductones and reductic couple with the 2,4-dinitrophenylhydrazine in 18 normal sulfuric acid at room temperature, while ascorbic acid, dehydroascorbic acid, and diketogulonic acid do not form derivatives with 2,4-dinitrophenylhydrazine under these conditions. This procedure made it possible to determine reductones and reductic acid simultaneously with the determination of ascorbic acid and to correct ascorbic acid values for interference by these compounds.

Penney and Zilva (1943) found that 2,3-diketogulonic acid interfered more markedly than reductones and reductic acid in the determination of total ascorbic acid with 2,4-dinitrophenylhydrazine. Two, three-diketogulonic acid is the irreversible oxidation product of dehydroascorbic acid. According to Borsook and his co-workers (1937) further oxidation of 2,3-diketogulonic acid yielded oxalic acid and threonic acid as metabolic products:



Both Borsook et al. (1937) and Penney and Zilva (1943) found that 2,3-diketogulonic acid does not possess the biological activity of ascorbic acid. When the 2,3-diketogulonic acid is coupled with the dinitrophenylhydrazine reagent, however, the osazone formed cannot be distinguished from that formed by dehydroascorbic acid. Thus, if diketogulonic acid is present in food, determination of total ascorbic acid by coupling with dinitrophenylhydrazine may give a higher value for the food than is represented by the two biologically active forms of the vitamin. Roe, Mills, Oesterling and Damron (1948) published details of a procedure which was developed to correct for the presence of diketogulonic acid in the tissue extract. In this procedure, tissues were extracted with a mixture of 0.5 percent solution of stannous chloride in five percent metaphosphoric acid. Diketogulonic acid was determined by reducing dehydroascorbic acid to reduced ascorbic acid with hydrogen sulfide and

then only diketogulonic acid was coupled with dinitrophenylhydrazine. When the same procedure was applied to the tissue extract with the omission of the reduction reaction, values were obtained for both dehydroascorbic acid and diketogulonic acid. Therefore the difference of the values obtained by the two processes represented the concentration of dehydroascorbic acid in the tissue extract. The determination of reduced ascorbic acid was performed on a second aliquot of the acid extractant by passing hydrogen sulfide through the aliquot to remove the stannous ions by the formation of precipitated stannous sulfide. The precipitated stannous sulfide was separated by filtration and the filtrate was treated with bromine to oxidize the reduced ascorbic acid before coupling with the 2,4-dinitrophenylhydrazine reagent. This gave the total concentration of reduced ascorbic acid, dehydroascorbic acid, and diketogulonic acid. By subtracting the concentration of dehydroascorbic acid and diketogulonic acid, the reduced ascorbic acid concentration of the extract was obtained.

As indicated above, the reduced ascorbic acid fraction of the total ascorbic acid of foods can be determined by difference by the method of Roe and Oesterling (1944) and the method of Roe, Mills, Oesterling and Damron (1948). Chemical analyses of reduced ascorbic acid in foods based upon the oxidation-reduction reaction of reduced ascorbic

acid with 2,6-dichlorophenolindophenol have been reported from many laboratories (Menaker and Guerrant, 1938; Harris and Olliver, 1942; Daniel and Munsell, 1937). This method was originally developed by Bessey and King (1933) for visual titration of ascorbic acid of the plant tissue with a solution of an alkaline salt of 2,6-dichlorophenolindophenol. In the oxidized state 2,6-dichlorophenolindophenol is blue in an alkaline medium and red in an acid medium; in the reduced state the 2,6-dichlorophenolindophenol is colorless. The method of Bessey and King (1933) was developed to apply these characteristics of the dye, i.e. the change in color when the solution was changed from an alkaline to an acid state and the change from a colored to a colorless state when the dye was reduced.

Since the method was a colorimetric procedure, there was interference in the determination of ascorbic acid in plant extracts by water soluble plant pigments. Various adaptations of the method were developed to eliminate the effect of the plant pigments on the color developed by the reaction of ascorbic acid and 2,6-dichlorophenolindophenol. These included the use of water immiscible solvents such as amyl acetate (Fujita and Numata, 1941) and xylene (Nelson and Somers, 1945) to extract the excess 2,6-dichlorophenolindophenol reagent from the aqueous mixture of 2,6-dichlorophenolindophenol and the plant extract. The intensity of the color

of the organic solvent then was proportional to the amount of dye which had not been reduced by the ascorbic acid of the plant extract.

Since the reaction of reduced ascorbic acid and 2,6-dichlorophenolindophenol was also an oxidation-reduction reaction, there was interference with the procedure by various metal ions and reducing substances present in biological extractants. The interference of ferrous iron with the determination of ascorbic acid by the 2,6-dichlorophenolindophenol method has been reported by several workers (Eekelen and Emmerie, 1936; Basu and Nath, 1938). It was found, however, that in a sufficiently acid medium small quantities of ferrous salts do not interfere with the indophenol titration (Eekelen and Emmerie, 1936). Ott (1941) reported that ferric salts interfered in the determination of reduced ascorbic acid by increasing the rate of oxidation of ascorbic acid and that metaphosphoric acid was effective in arresting the catalytic action of traces of ferric ions. Barron, Guzman, and Klemperer (1936) and Dekker and Dickinson (1940) reported that interference might result from the reducing action of cuprous salts upon the indophenol dye or through the catalytic oxidative action of the cupric ion upon ascorbic acid. The latter effect was thought to be due to enzyme systems which contain copper in combination with various protein groups. Both the reducing property of the cuprous

ion and the catalytic effect of cupric ion can be controlled by compounds such as 8-hydroxyquinoline (Sendroy, 1937), acetic acid, and metaphosphoric acid (Ott, 1941).

Reducing substances like hydrogen sulfide, reductones, reductic acid, glutathione, cysteine, which are present in biological extracts, have been found to reduce the 2,6-dichlorophenolindophenol reagent similarly to ascorbic acid. (King, 1936; Mack and Tressler, 1937). Therefore it was realized that the presence of these substances in foods might result in the determination of falsely high values for reduced ascorbic acid in foods. The effects of these extraneous reducing substances upon the determination of ascorbic acid were controlled, in part at least, by regulating the time of the determination, since the rates of reaction were found to differ for the various reducing substances with 2,6-dichlorophenolindophenol, and the rate of reaction of ascorbic acid with 2,6-dichlorophenolindophenol was more rapid than the reaction rate of many other reducing compounds with the dye. Bessey (1938) described a photocolometric method for the determination of small quantities of ascorbic acid and dehydroascorbic acid in turbid and colored solutions in the presence of other reducing substances. In principle, this method involved the addition of an excess of an aqueous 2,6-dichlorophenolindophenol solution to a buffered metaphosphoric acid extract of the unknown and meas-

urements of the percent transmission of light with a photoelectric colorimeter at 15 and 30 second intervals after the addition of the dye. Correction for other interfering substances which reduced the dichlorophenolindophenol at a slower rate than ascorbic acid was made by subtracting the drift of the galvanometer needle between the 15 and 30 second period from the transmission reading obtained after the first 15 seconds. Later, Loeffler and Ponting (1942) reported that when plant tissue was macerated in a one percent metaphosphoric acid solution, ascorbic acid could be determined in fruits and vegetables, whether fresh, frozen, or dehydrated, by taking the transmission reading on a photoelectric colorimeter at 15 seconds after the start of the addition of the dichlorophenolindophenol reagent to the metaphosphoric acid tissue extract. The authors considered that a single measurement of percent transmission was adequate since the amounts of interfering substances in plant tissues were relatively small.

Recognition of the probable interference by various related substances in the determination of ascorbic acid has led to the use of the term "apparent" by some investigators in reports of ascorbic acid values. The term "apparent ascorbic acid" was used originally by Butler and Cushman (1941) to describe ascorbic-acid-like reducing substances in the white layers of centrifuged blood from leukemic patients

and later applied to foods and food products by Wokes, Organ, Duncan and Jacoby (1943). These authors referred to a group of substances which resembled ascorbic acid in chemical and physical properties but did not possess the anti-scorbutic properties. Einbecker, Jackson, Paul and Ohlson (1947) used the term "apparent total ascorbic acid" for the amount of ascorbic acid determined by the method of Roe and Oesterling (1944) with 2,4-dinitrophenylhydrazine. Somers, Kelly, Thacker and Redder (1951) defined "total apparent ascorbic acid" as a term which referred to the amount of ascorbic acid equivalent to the total amount of 2,6-dichlorophenolindophenol reduced by the buffered aliquot of the tissue extract, whether the dye was reduced by ascorbic acid or by some other substances. Stewart, Horn and Robson (1952) referred to the substances in blood which reacted with 2,4-dinitrophenylhydrazine to form osazones by the method of Roe and Kuether (1943) as "apparent ascorbic acid."

Changes in the Total Ascorbic Acid Content of Foods During Storage

The development of methods for the analysis of both dehydroascorbic acid and reduced ascorbic acid in foods provided data from various laboratories which demonstrated that a relatively larger amount of ascorbic acid may be present in the form of dehydroascorbic acid in processed foods than in

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fresh foods (Mack and Tressler, 1937; Hou, 1937; Woessner, Elvehjem, and Schuette, 1940; Wokes, 1943). This observation stimulated interest in the investigation of the influence of various factors upon the relative concentrations of reduced ascorbic acid and dehydroascorbic acid in foods.

Changes which occurred in the concentrations of reduced ascorbic acid, dehydroascorbic acid and total ascorbic acid of cabbage after cutting and shredding were studied by McMillan and Todhunter (1946). There was a reduction in the amount of reduced ascorbic acid of the cabbage immediately after cutting and shredding; however, there was an increase in the concentration of dehydroascorbic acid of the cabbage after cutting and shredding. The increased concentration of dehydroascorbic acid was maintained for 30 minutes, after which the concentration of dehydroascorbic acid also was reduced. At the end of 120 minutes after the cutting and shredding of the cabbage, values for reduced ascorbic acid, dehydroascorbic acid and total ascorbic acid were less than for the fresh cabbage.

Dodds, Price and Moore (1948) reported studies of the concentrations of reduced ascorbic acid and dehydroascorbic acid of sweet potatoes which were cured by storage at a temperature of 80 to 85 degrees Fahrenheit and high humidity for seven to ten days and then stored at a temperature of 50

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to 55 degrees Fahrenheit and low humidity. The concentration of dehydroascorbic acid of the sweet potatoes increased after six weeks of storage and then was reduced gradually throughout the experimental period of 24 weeks. The concentration of reduced ascorbic acid decreased gradually throughout the entire storage period.

Changes in the concentration of reduced ascorbic acid and dehydroascorbic acid of vegetables during frozen storage were reported by Bedford and McGregor (1948). These authors found that whereas four to thirteen percent of the ascorbic acid was present as dehydroascorbic acid in the fresh, green vegetables, from 22 to 94 percent of the ascorbic acid was in the form of dehydroascorbic acid in frozen vegetables which had been held in frozen storage for six months. The total amount of ascorbic acid of the vegetables was less after six months of frozen storage than in the fresh vegetables.

Wolfe et al. (1949) reported that fresh samples of muskmelon contained an average of 0.40 milligrams of reduced ascorbic acid and 0.42 milligrams of total ascorbic acid per gram; after the muskmelon was frozen and stored, the retention of ascorbic acid in the frozen melon at successive time intervals was 36, 41, 39, and 37 percent of the reduced ascorbic acid and 60, 59, 48 and 54 percent of the total ascorbic acid at periods of one, three, six and nine months

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respectively. Thus, although there was a marked reduction in the concentration of both reduced and total ascorbic acid after one month of frozen storage, the ascorbic acid content of the frozen muskmelon remained relatively constant during the remainder of frozen storage. The average retention of total ascorbic acid was about 18 percent higher than that of the reduced ascorbic acid.

Hewston, Fisher, and Orent-Keiles (1951) studied the influence of time and temperature on changes of both reduced and total ascorbic acid in several canned fruits, fruit juices and one vegetable during storage. The temperatures ranged from 45 to 73 degrees Centigrade and the length of storage period varied from three to 42 days. Canned grapefruit, orange juice, orange-grapefruit juice, tomato juice, and spinach, were studied. The total ascorbic acid content of the grapefruit was reduced approximately 80 percent after five days of storage at 73 degrees Centigrade. There was a reduction of 58 percent in the total ascorbic acid of canned orange juice under the same storage conditions. Considerable loss of total ascorbic acid also occurred in the canned orange-grapefruit juice which was stored for seven days at 73 degrees Centigrade. However, there was a loss of only 18 percent of the total ascorbic acid of tomato juice which was stored at a temperature of 45 degrees Centigrade for 14 days. Among the foods stored, only spinach showed an increase in

the concentration of total ascorbic acid during storage. The canned spinach was stored for 42 days at 45 degrees Centigrade. At the end of this storage period the total ascorbic acid concentration had increased from 15.1 milligrams per 100 grams, which was the value before storage, to 20.6 milligrams per 100 grams.

Paul, Wiant and Robertson (1949) found that after three months of frozen storage there was an increase in the total ascorbic acid concentration of strawberries. The fresh berries contained 47.5 milligrams of total ascorbic acid per 100 grams; after three months of frozen storage, the berries contained 83.3 milligrams per 100 grams. The concentration of total ascorbic acid was lowered to 66.5 milligrams per 100 grams at the end of six months of frozen storage and 50.8 milligrams per 100 grams at the end of nine months of frozen storage. A similar increase in the total ascorbic acid content of red cherries, red and black raspberries, and rhubarb also was observed after three months of frozen storage, although the increase was of less magnitude than that for the frozen strawberries. These workers did not report separate values of reduced ascorbic acid and dehydroascorbic acid of the frozen fruits.

Following the work of Paul et al. (1949), Einbecker and co-workers (1947; 1950) studied the relative concentration of reduced ascorbic acid and total ascorbic acid of fresh strawberries and strawberries which had been frozen and held in frozen storage over a period of time. Berries of the

Robinson variety were studied. The fresh strawberries contained 65.6 milligrams reduced ascorbic acid and 65.4 milligrams of total ascorbic acid per 100 grams of strawberries. After three months of frozen storage, the strawberries contained 45.5 milligrams of reduced ascorbic acid and 78.1 milligrams of total ascorbic acid per 100 grams. The concentration of reduced ascorbic acid in the frozen strawberries was 40.2 milligrams per 100 grams after six months of storage and 32.3 milligrams per 100 grams after nine months of storage. Some reduction in the concentration of total ascorbic acid was found in the frozen strawberries after six months of storage; the value was 50.7 milligrams per 100 grams. The concentration of total ascorbic acid in the stored frozen strawberries after nine months of storage was similar to that after six months of storage.

Since 1948, when Roe and his co-workers modified the method for the determination of total ascorbic acid to differentiate diketogulonic acid from reduced ascorbic acid and dehydroascorbic acid, some studies have been reported of processed foods which have included measurements of diketogulonic acid as well as reduced ascorbic acid and dehydroascorbic acid. Mills, Damron and Roe (1949) investigated the ascorbic acid content of 27 vegetables and fruits and reported that fresh foods as purchased at the market contained some dehydroascorbic acid and diketogulonic acid but that the

amounts were small unless the foods had been allowed to deteriorate considerably. Quality of the product also appeared to affect the amount of diketogulonic acid in frozen foods since frozen strawberries in poor condition contained no reduced ascorbic acid, 12.7 milligrams dehydroascorbic acid and 37.5 milligrams of diketogulonic acid per 100 grams, whereas frozen strawberries of good quality contained 80.0 milligrams of reduced ascorbic acid, 12.0 milligrams of dehydroascorbic acid and 3.0 milligrams of diketogulonic acid per 100 grams.

Fresh plant tissues were reported by Stokstad and Jukes (1949) to contain small amounts of dehydroascorbic acid and diketogulonic acid. These authors found that frozen foodstuffs contained from 0 to 75 percent of the total ascorbic acid derivatives in the form of diketogulonic acid.

Changes in the quantitative relationship of reduced ascorbic acid, dehydroascorbic acid and diketogulonic acid during storage were investigated by Mills and her co-workers (1949) in orange juice and in a slurry prepared from potatoes. Orange juice and a slurry of white potatoes were stored in the refrigerator at two degrees Centigrade. Originally all the ascorbic acid of the orange juice was present in the reduced form. Analysis on the second day of storage showed that five percent of the reduced ascorbic acid had been changed to dehydroascorbic acid. The amount of dehy-

dehydroascorbic acid increased gradually throughout the storage period. On the thirteenth day of the study, diketogulonic acid was found to be present for the first time. The amount of diketogulonic acid increased slowly until at the last analysis on the thirty-eighth day, it comprised the largest percentage of the substances measured as total ascorbic acid. The total concentration of the three compounds remained constant for the first 30 days, then decreased slowly so that by the thirty-eighth day the total ascorbic acid content was equivalent to approximately 83 percent of the concentration of ascorbic acid in the fresh orange juice.

A potato slurry was prepared by Mills and her co-workers by blending potatoes with distilled water (ratio: one to 50) in the container of an electric blender. The original slurry contained almost no reduced ascorbic acid, but about 90 percent of the substances measured as total ascorbic acid was dehydroascorbic acid and 10 percent was diketogulonic acid. Within six hours, however, diketogulonic acid comprised over half of the total of the substances measured as total ascorbic acid. By the nineteenth day, 90 percent of the total amount of the three compounds was in the form of diketogulonic acid. The values for the total concentration of dehydroascorbic acid and diketogulonic acid did not change appreciably during the first week, but later decreased rapidly until at the last analysis only five per-

cent of the concentration of total ascorbic acid of the freshly prepared slurry was present.

The interference of reductones with the determination of total ascorbic acid was reported by Hewston et al. (1948) who found that as much as 45 percent of the apparent ascorbic acid in french-fried potatoes was reductones.

The studies which have been reported here indicate that the oxidation of reduced ascorbic acid to dehydroascorbic acid and possibly to diketogulonic acid may occur during the storage of foods. The oxidative process apparently can take place in foods stored in cold storage, in canned foods, and in frozen foods. In those studies in which an actual increase in the total ascorbic acid concentration of processed foods was observed, it would appear that other chemical changes occurred in the foods during storage, perhaps of an oxidative nature, which yielded compounds capable of reacting with the 2,4-dinitrophenylhydrazine. An actual increase in the total ascorbic acid content of the food would result, it would seem, only from the possible synthesis of ascorbic acid during storage.

Possible Role of Biosynthesis in the Ascorbic Acid Content of Frozen Foods

Storage of foods in the frozen state does not preclude the possibility of enzymatic reactions occurring in the food.

Inactivation of the enzymes of vegetables is achieved before freezing by blanching of the vegetables. Many fruits, however, are frozen in the raw state, without blanching. Sizer and Josephson (1942) reported that the storage of lipase, trypsin and invertase in aqueous solution with their respective substrates for 27 days at -70 degrees Centigrade did not affect their activity. Ball and Lineweaver (1938) in studying the action of enzymes at low temperature concluded that enzyme activity may continue at low temperatures and may be an important factor in problems of food preservation. These studies suggest that certain enzymatic changes may occur in fruits in the frozen state, and provided a basis for the hypothesis that biosynthesis of ascorbic acid from its precursors might occur by enzymatic action in frozen fruit.

The conversion of sugars, i. e., d-glucose, d-fructose, and d-mannose into l-ascorbic acid, was demonstrated to occur in cotyledonless pea seedlings by Ray (1934). Ghosh (1946) suggested that pyruvic acid is a precursor of ascorbic acid in the rat, since the incubation of liver tissue with sodium pyruvate under suitable conditions produced an increase of from 15 to 20 percent ascorbic acid above the amount originally in the liver tissue. In the presence of vitamin B₁ the increase was more than 30 percent. Horowitz and his co-workers (1952) have reported that experiments with C¹⁴-d-glucose indicated that there was a direct conver-

sion of glucose to l-ascorbic acid. This observation was based on the fact that administration of C¹⁴-d-glucose to rats resulted in the excretion in the urine of labelled l-ascorbic acid. A year later Horowitz and King (1953) also found that glucuronic acid might serve as a precursor of ascorbic acid in the albino rat. Nath, Belavady, Sahu and Chitale (1953) found that intramuscular injection of glucose, sodium acetate alone, sodium acetate followed immediately by glucose, or the condensation product of glucose and ethyl acetoacetate, resulted in a temporary rise in the plasma concentration of ascorbic acid within an hour following the injection. Isherwood (1953) attempted to establish the nature of the hexose precursor and elucidated the mechanism by which hexose is transformed into l-ascorbic acid by adding certain compounds postulated as intermediates in the reaction to the solution in which cress seedlings were grown. The amount of l-ascorbic acid present in the treated seedlings was compared with that in seedlings grown in water. He found that the γ -lactones of d-glucuronic and l-gulonic acids, when added to cress seedlings, were transformed into l-ascorbic acid. d-galacturonic acid methyl ester and l-galactono- γ -lactone behaved similarly. Mapson (1953) was interested to find whether an enzyme extract could be obtained to catalyze the conversion of either l-gulono- γ -lactone or l-galactono- γ -lactone to l-ascorbic acid. He

found that mitochondria prepared from partially germinated pea seedlings were able to convert l-galactono- γ -lactone rapidly to l-ascorbic acid, but could not effect the corresponding conversion of l-gulonolactone to l-ascorbic acid. This reaction was catalyzed most efficiently by oxygen; however, cytochrome oxidase also was involved. The reaction proceeded smoothly until approximately 40 percent of the lactone had been transformed into l-ascorbic acid and then ceased, but began again if an additional quantity of the lactone was added. The disappearance of the lactone, apart from that portion converted to l-ascorbic acid, was shown to be due to its conversion into the free galactonic acid, a reaction which proceeded simultaneously with the enzymically catalyzed formation of l-ascorbic acid. Since galactonic acid was not itself converted to l-ascorbic acid, this reaction decreased the yield of l-ascorbic acid obtained from the γ -lactone. This side reaction indicated that biosynthesis of ascorbic acid from its precursors was not a simple process.

Thus, Isherwood (1953) and Mapson (1953) have demonstrated that biosynthesis of ascorbic acid from hexose derivatives may occur in living plant tissues. There is no evidence at present that similar synthesis of ascorbic acid from reducing sugars may occur in processed foods, but it is possible that enzymatic changes occurring in food, even at

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Studies of Human Utilization of Ascorbic Acid in Foods

Reduced ascorbic acid is a water-soluble vitamin. The absorption of the vitamin from the intestinal tract has been found to occur quite rapidly and blood plasma or serum concentrations of the vitamin have been found to reflect changes in the intake of the vitamin in relatively short periods of time (Todhunter, Robins, and McIntosh, 1942; Clayton and Folsom, 1940; Linkswiler, 1954). Changes in the concentration of ascorbic acid in the urine also have been found to reflect changes in vitamin intake in short periods of time (Belser, Hauck, and Storvick, 1939; Berryman, French, Harper and Pollack, 1944). Therefore changes in the blood and urine concentrations of ascorbic acid corresponding to changes in the intake of the vitamin have been used as a basis for the study of the human utilization of ascorbic acid in foods.

Early in 1936, Hawley and her co-workers (1936) studied the urinary excretion of ascorbic acid following the administration of comparable quantities of the vitamin in orange juice and in pure crystalline form and found that the vitamin C of orange juice was as well utilized as the pure substance.

Clayton and Folsom (1940) compared the blood concentrations and urinary excretions of ascorbic acid of subjects following the administration of a dietary supplement of potatoes which supplied 50 milligrams of ascorbic acid and 25 milligrams of the pure vitamin with the blood concentrations and urinary excretions of the subjects following the administration of a dietary supplement of 75 milligrams of the pure vitamin. Each period was continued for four days. Blood values for two of the four subjects were slightly higher after the potatoes were given than when the test dose was crystalline ascorbic acid. Blood values for one subject were approximately the same for the two experimental periods. One subject had a slightly lower concentration of vitamin C in the blood when potato was used as a partial source of the dietary ascorbic acid than when the ascorbic acid was supplied as the pure vitamin, although the blood concentration of ascorbic acid was greater than one milligram per 100 milliliters. The authors concluded therefore that the vitamin from the potato was well utilized. Urinary excretions of vitamin C also were determined and were found to be comparable in the two test periods. This indicated that there was good absorption of the ascorbic acid of the potato from the intestinal tract and that the retentions of the vitamin were comparable for the two test periods.

Todhunter and Fatzer (1940) studied the utilization of ascorbic acid by seven college women. The blood ascorbic acid values and the urinary excretions of ascorbic acid were reported to be comparable for each subject after a diet which contained 20 milligrams of ascorbic acid and a dietary supplement of raspberries containing 40 milligrams of ascorbic acid was fed for six days and after the same diet supplemented with 40 milligrams of crystalline ascorbic acid was administered for a comparable period of time. The subjects were saturated with orange juice containing 200 milligrams of ascorbic acid for three days before each experimental period. The authors estimated the so-called "utilization index," $\frac{\text{Intake} - \text{Output}}{\text{Body weight}} \times \sqrt{\text{Age}}$, for each subject; the utilization index indicated that the ascorbic acid of the red raspberries was as well utilized as crystalline ascorbic acid.

The ability of the test dose supplement to maintain tissue saturation and to support plasma ascorbic acid concentrations was the criterion which was used by Clayton and Borden (1943) in studies of the availability of ascorbic acid in raw cabbage and canned tomato juice. Four college students on a basal diet containing ten milligrams ascorbic acid were given 150 milligrams ascorbic acid daily for eight days before each experimental period of nine days. During the experimental periods, subjects were on the basal diet supplemented with (a) 75 milligrams of ascorbic acid, (b) 25

milligrams of ascorbic acid plus an amount of raw cabbage which provided 50 milligrams of ascorbic acid, and (c) 25 milligrams of ascorbic acid plus an amount of tomato juice which provided 50 milligrams of ascorbic acid. Ascorbic acid was determined in a sample of blood taken when the subject was in a fasting state and on a 24-hour urine composite. Both blood and urinary ascorbic acid were determined on the last day of each experimental period. The concentrations of ascorbic acid in the blood of the subjects following the experimental periods when cabbage and tomato juice were given as dietary sources of the vitamin were comparable to the concentration of ascorbic acid in the blood following the period when the ascorbic acid was supplied in the pure form. The urinary excretions of ascorbic acid were somewhat lower when the natural foods were a source of dietary ascorbic acid than when the dietary supplement was crystalline ascorbic acid. Therefore, the authors concluded that the ascorbic acid contained in the raw cabbage and in the canned tomato juice was as well utilized or even better than ascorbic acid in the pure form.

Hartzler (1945) studied the availability of ascorbic acid in papayas and in guava juice. On the first day, nine subjects, four women and five men, were given 300 milligrams of ascorbic acid. Following this, there was a control period of six days in which subjects were given a basal diet

plus 75 milligrams of pure ascorbic acid. Each experimental period was six days in length. The administration of the massive dose of 300 milligrams of ascorbic acid and the control period were repeated preceding each experimental period. During one experimental period, the subject received 75 milligrams of crystalline ascorbic acid daily. The test foods were given as the source of ascorbic acid in the experimental periods; each test food was given in an amount which supplied 75 milligrams of ascorbic acid. Urinary excretions of ascorbic acid were determined daily and blood plasma ascorbic acid was determined once each week. Application of analysis of variance to the experimental data indicated that the ascorbic acid of the papayas and of the guava juice was as well utilized as the pure ascorbic acid.

Elliot and Schuck (1947) reported that the ascorbic acid of grapefruit was as well utilized as the crystalline vitamin. This observation was based upon studies of the urinary excretion of ascorbic acid by nine subjects during two three-day test periods and studies of the concentration of ascorbic acid in whole blood of three subjects at the end of each test period.

Goldsmith and Ellenger (1939) were among the first to study the changes of ascorbic acid content in blood at periodic intervals after the administration of a test dose. The subjects were 22 persons; three were considered to be normal

and nineteen were hospital patients who had no fever, acute infectious disease or vitamin C deficiency. The blood plasma concentration of reduced ascorbic acid was determined at intervals of one, three and six hours after the oral administration of a test dose of 600 milligrams of ascorbic acid. At the end of one hour, there was a rise in the concentrations of blood plasma ascorbic acid. The highest concentrations ranged from 1.7 to 2.9 milligrams per 100 milliliters and occurred at the end of three hours after the administration of the test dose. After six hours had elapsed, there appeared to be a reduction of the amount of vitamin C in the blood plasma. The curves of plasma ascorbic acid following the test dose resembled those for dextrose tolerance tests as described by Greenberg, Rinehart and Phatak (1936).

In 1942, Todhunter, Robbins and McIntosh (1942) reported a study of the rate of increase of blood plasma ascorbic acid after ingestion of 50 milligrams of ascorbic acid in the crystalline form, and after consumption of equivalent amounts of ascorbic acid in cauliflower, orange sections, orange juice or strawberries. The ascorbic acid content of blood plasma began to increase within 30 to 60 minutes after the test dose was given and returned to the fasting concentration in three or four hours. The maximum increase in plasma concentration of ascorbic acid was reached within one and one-half hours after ingestion of 50 milligrams of as-

corbic acid either as crystalline ascorbic acid, as orange juice, or as orange sections. The maximum increase in plasma concentration of ascorbic acid occurred in two hours when strawberries were the source of ascorbic acid, and in two and one-half hours when cauliflower was eaten.

Investigation of the rate of increase of plasma reduced ascorbic acid after ingestion of strawberries and crystalline ascorbic acid was reported also by Einbecker, Jackson, Paul, and Ohlson (1947). The plasma ascorbic acid concentrations of five young women were measured in the fasting state and one-half, one, one and one-half, two and one-half, and three and one-half hours after an ascorbic acid-free breakfast which was supplemented with (a) no supplement, (b) 150 grams unsweetened frozen strawberries, (c) crystalline ascorbic acid equivalent to the amount of reduced ascorbic acid provided by the strawberries and (d) crystalline ascorbic acid equivalent to the amount of apparent total ascorbic acid provided by the strawberries. The increases in plasma ascorbic acid obtained after ingestion of the strawberries were comparable to those obtained when crystalline ascorbic acid was taken in amounts equivalent to the reduced ascorbic acid in the strawberries. The maximum concentration occurred one and one-half hours after ingestion of the berries, while two and one-half hours passed after the administration of crystalline ascorbic acid before the maximum concentration was

reached. Increases in blood ascorbic acid concentration obtained when crystalline ascorbic acid was taken in amounts equivalent to the apparent total ascorbic acid were greater than those obtained after the ingestion of strawberries. In this study, blood plasma ascorbic acid was measured by oxidation of reduced ascorbic acid with 2,6-dichlorophenolindophenol according to the procedure of Farmer and Abt (1936). Any ascorbic acid which was present in the blood as dehydroascorbic acid would not have been measured by this procedure.

Studies of human utilization of ascorbic acid also have been concerned with the relative utilization of dehydroascorbic acid in comparison with crystalline reduced ascorbic acid. Earlier reports of studies of the utilization of dehydroascorbic acid by guinea pigs indicated that dehydroascorbic acid had 25 percent (Roe and Barnum, 1936) and 80 percent (Gould and Schwachman, 1943) of the potency of reduced ascorbic acid. However, Penney and Zilva (1943) reported that studies with guinea pigs indicated that dehydroascorbic acid was as well utilized as reduced ascorbic acid.

Borsook, Davenport, Jeffreys, and Warner (1937) studied the effect with human subjects when orange juice as a source of reduced ascorbic acid was replaced by charcoal-treated orange juice as a source of dehydroascorbic acid. These authors found that the concentrations of reduced and total ascorbic acid in the blood plasma of subjects were comparable

after a test dose of fresh orange juice and after a test dose of orange juice which had been treated with charcoal to oxidize the ascorbic acid. A similar study was conducted by Todhunter and her co-workers (1950) on young women. These authors also used activated charcoal to oxidize the reduced ascorbic acid in orange juice to dehydroascorbic acid. It was concluded that when dehydroascorbic acid was fed, the concentrations of reduced ascorbic acid in blood serum and in the urine of young women were comparable to the concentrations of reduced ascorbic acid in the blood serum and urine when the subjects received reduced ascorbic acid; this was considered to be indicative of satisfactory utilization of the dehydroascorbic acid. Ritter and Cohen (1951) found, too, that differences between the average urinary excretions of ascorbic acid after oral doses of reduced ascorbic acid and after oral doses of dehydroascorbic acid were not statistically significant.

Linkswiler (1954) also studied the blood ascorbic acid of subjects following the ingestion of crystalline dehydroascorbic acid (method of preparation not given) and reduced ascorbic acid. Reduced and total ascorbic acid determinations were made on the blood serum of five women, 30-40 years of age, before and after the oral administration of 50, 150, or 300 milligrams crystalline dehydroascorbic acid or 150 milligrams reduced ascorbic acid. The concentration of ascor-

bic acid in the serum was maximum at a period of one and one-half hours after the oral dose. The maximum increment in the reduced ascorbic acid values in blood averaged 0.18, 0.34, and 0.79 milligrams per 100 milliliters respectively following the ingestions of 50, 150, and 300 milligrams dehydroascorbic acid; the corresponding total ascorbic acid values in blood averaged 0.21, 0.54, and 0.91 milligrams per 100 milliliters. The maximum increment following ingestion of 150 milligrams reduced ascorbic acid was 0.45 milligrams of reduced ascorbic acid and 0.53 milligrams of total ascorbic acid per 100 milliliters of blood plasma. Thus, although the increment in reduced ascorbic acid in the blood was higher after the test dose of reduced ascorbic acid than after the test dose of dehydroascorbic acid, the increments in total ascorbic acid in the blood were comparable after test doses of reduced ascorbic acid and dehydroascorbic acid.

The availability of ascorbic acid in canteloupe and grapefruit was studied by Chen and Schuck (1951) by comparison of the urinary excretions of dehydroascorbic acid, reduced ascorbic acid and diketogulonic acid by five healthy women on a basal diet supplemented with the test food and on a basal diet supplemented with crystalline ascorbic acid. The basal diet supplemented with 40 milligrams crystalline ascorbic acid provided 8.42 milligrams diketogulonic acid, 6.61 milligrams dehydroascorbic acid, and 69.67 milligrams

reduced ascorbic acid per day. The average daily excretions in urine for the last four days of the six-day experimental period were 4.88 milligrams diketogulonic acid, 6.73 milligrams dehydroascorbic acid and 38.91 milligrams reduced ascorbic acid. When the basal diet was supplemented with cantaloupe, the daily diet provided 19.78 milligrams diketogulonic acid, 14.88 dehydroascorbic acid, and 56.24 milligrams reduced ascorbic acid. The average urinary excretions were 4.72 milligrams diketogulonic acid, 6.00 dehydroascorbic acid, and 42.21 milligrams reduced ascorbic acid. The basal diet, supplemented with grapefruit, supplied 9.67 milligrams diketogulonic acid, 7.15 dehydroascorbic acid, and 73.61 milligrams reduced ascorbic acid per day. The corresponding urinary excretions were 4.86 milligrams diketogulonic acid, 5.39 milligrams dehydroascorbic acid, and 36.87 milligrams reduced ascorbic acid. The data indicated that the intake of the basal diet plus the cantaloupe supplement, which contained higher intakes of dehydroascorbic acid and diketogulonic acid, did not increase the excretion of these two substances above that observed on the basal diet supplemented with crystalline ascorbic acid or grapefruit. The authors postulated that the occurrence of dehydroascorbic acid and diketogulonic acid in urine might be due in part to oxidation of reduced ascorbic acid during filtration and storage in the kidneys and bladder.

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The studies reported above indicate that, although various procedures have been used in the study of the utilization of ascorbic acid by humans, these procedures have been based essentially upon the influence of the test food on the blood and/or urinary concentration of ascorbic acid. The procedures which have been used may be grouped as follows:

(a) Blood and/or urinary concentrations of ascorbic acid of subjects maintained on a basal diet, low in ascorbic acid, and supplemented by pure ascorbic acid or an equivalent amount of ascorbic acid from the test food.

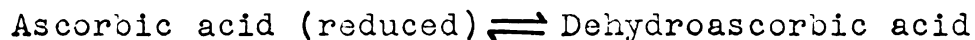
(b) Blood and/or urinary concentrations of ascorbic acid of subject at periodic intervals following a test dose of pure ascorbic acid or an equivalent amount of ascorbic acid from the test food. This pattern of study has been used both when subjects were maintained on a diet low in ascorbic acid and when subjects were on self-selected diets.

Ascorbic Acid in the Blood and Urine

Blood concentrations of ascorbic acid have been used widely as an index of nutritional status with respect to ascorbic acid. According to Youmans (1941), a concentration of 0.7 milligrams of ascorbic acid per 100 milliliters of blood plasma or serum is indicative of good ascorbic acid nutrition and concentrations of 1.2 milligrams per 100 milliliters of serum or above have been observed frequently among

well-nourished individuals. Youmans considered that blood plasma or serum ascorbic acid values within the range of 0.4 to 0.7 milligrams of ascorbic acid per 100 milliliters represented a borderline state of ascorbic acid nutrition, and that values less than 0.4 or 0.5 milligrams of ascorbic acid per 100 milliliters were indicative of a deficiency state with respect to ascorbic acid. Similar interpretations of blood ascorbic acid values have been reported by Farmer (1944), Adamson and co-workers (1945), Borsook et al. (1946) and King (1951).

Borsook and his co-workers in 1937 suggested that an equilibrium may be maintained between reduced ascorbic acid and dehydroascorbic acid in the blood:



and that the glutathione of the cells may be a factor in maintaining this balance. Penney and Zilva (1943), however, were unable to demonstrate the presence of dehydroascorbic acid in guinea pig blood after the administration of test doses, either of 120 milligrams of dehydroascorbic acid or 120 milligrams of 2,3-diketogulonic acid. Todhunter and co-workers (1950) reported also that human blood did not contain dehydroascorbic acid. In contrast, however, Stewart, Horn and Robson (1952; 1953) reported that the average vitamin C concentration of fasting plasma of ten healthy subjects

was 0.56 milligrams of reduced ascorbic acid per 100 milliliters of plasma as determined by the method of Farmer and Abt (1936) using 2,6-dichlorophenolindophenol as an oxidizing agent, and 0.77 milligrams of total ascorbic acid per 100 milliliters of plasma as determined by the method of Roe and Kuether (1943) based on the reaction of oxidized ascorbic acid with 2,4-dinitrophenylhydrazine. When the plasma extract was treated with hydrogen sulfide and the ascorbic acid then determined by titration with 2,6-dichlorophenolindophenol, the values were more nearly comparable. The authors interpreted this to indicate the presence of dehydroascorbic acid in the blood plasma in amounts which averaged about 20 percent of the total ascorbic acid. Davey, Wu, and Storvick (1952) also found that dehydroascorbic acid was present in blood.

Relatively little attention has been given to the possible occurrence of diketogulonic acid in the blood. Penney and Zilva (1943) found that the blood plasma of guinea pigs, after five days on scorbutic diet, contained five milligrams of diketogulonic acid per 100 milliliters of plasma. Fifteen minutes after the oral administration of 120 milligrams of dehydroascorbic acid to guinea pigs weighing 300-350 grams, the average diketogulonic acid content in the plasma was five milligrams per 100 milliliters of plasma and remained at this concentration for one hour. When 120 milli-

grams dehydroascorbic acid were given intramuscularly, the plasma concentration of diketogulonic acid of the guinea pigs reached a maximum concentration of 36 milligrams per 100 milliliters within fifteen minutes, then decreased gradually to 17 milligrams per 100 milliliters by one hour after injection. When 120 milligrams of diketogulonic acid were administered orally, the plasma diketogulonic acid was from four to seven milligrams per 100 milliliters. However, when the same amount of diketogulonic acid was given intramuscularly, the plasma diketogulonic acid increased to 55 milligrams per 100 milliliters after 15 minutes and then decreased gradually to 23 milligrams per 100 milliliters at the end of one hour. In contrast with the study of Penney and Zilva (1943), Damron, Monier and Roe (1952) did not find diketogulonic acid in the blood of full grown guinea pigs, either on a control diet or after five days on a scorbutic diet. In human subjects, Chen and Schuck (1951) reported that both dehydroascorbic acid and diketogulonic acid were found in fasting blood samples and also in the blood at intervals after the ingestion of crystalline ascorbic acid. In the experiment reported by Stewart, Horn, and Robson (1953), the ascorbic acid in the blood plasma was converted to the reduced form after treatment with hydrogen sulfide and the values for total ascorbic acid by the Roe and Kuether (1943) method and reduced ascorbic acid by the Farmer and Abt

(1936) method were similar. This indicated that the substances in the blood which had reacted with the 2,4-dinitrophenylhydrazine were reduced ascorbic acid and dehydroascorbic acid rather than 2,3-diketogulonic acid, since the 2,3-diketogulonic acid, if present, would not have been reduced to ascorbic acid. As reported previously, Chen and Schuck (1951) found that the average daily urinary concentrations of diketogulonic acid, dehydroascorbic acid and reduced ascorbic acid of subjects on a basal diet supplemented with pure ascorbic acid were 4.88, 6.73 and 38.91 milligrams respectively. The concentrations of diketogulonic acid and dehydroascorbic acid were relatively constant when canteloupe was substituted for pure ascorbic acid as the dietary supplement, although the canteloupes supplied approximately 13 milligrams of diketogulonic acid and eight milligrams of dehydroascorbic acid. The total ascorbic acid concentration in urine has been found to be highly correlated with the reduced ascorbic acid in the urine ($r = 0.914$, $P \leq 0.01$) (Berryman, French, Harper, and Pollack, 1944).

It was recognized early that the amount of vitamin C in the urine was closely related to the vitamin C intake (Harris and Ray, 1935; Abbasy, et al., 1937) and that ascorbic acid has the characteristics of a renal threshold substance (Faulkner and Taylor, 1938; Lewis, et al., 1943). Thus the urinary excretion of ascorbic acid is related to the blood

concentration of ascorbic acid, which in turn is dependent upon the dietary intake of ascorbic acid. Levcowich and Batchelder (1942) reported that ascorbic acid excretions on a vitamin C free diet ranged from seven to 16 milligrams per day. According to Haines and co-workers (1947), the mean daily urinary excretions of ascorbic acid ranged from 15 to 30 milligrams for subjects on intakes from 33 to 70 milligrams of ascorbic acid per day. When test doses of 400 milligrams of ascorbic acid were administered, the urinary excretion of ascorbic acid ranged from 15 to 125 milligrams on the first day and from 152 to 302 milligrams on the third day that the test dose was given. The authors suggested that for two subjects who had previously been on a diet supplying 33 milligrams ascorbic acid per day, the tissues were taking up the available ascorbic acid, so that although the renal threshold was exceeded for these subjects in the third day of administration of the 400 milligrams test dose, little urinary excretion of ascorbic acid occurred. Melnick et al. (1945) studied the physiological availability of water soluble vitamins using five male subjects on ordinary adequate diet. During the experimental days, subjects were put on a basal diet which supplied 115 milligrams ascorbic acid for one day. The average 24-hour urine excretion was 37 milligrams ascorbic acid. After a test dose of 200 milligrams ascorbic acid was given in addition to the basal diet, the

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Todhunter, Robbins, and McIntosh (1942) found that ascorbic acid intakes from 60 to 90 milligrams per day were required, in addition to a basal diet which supplied 20 milligrams of ascorbic acid, to saturate the tissues of three ~~women~~ subjects. Tissue saturation was judged on the basis of urinary excretions of ascorbic acid following test doses of 400 milligrams ascorbic acid which were administered at the end of each period of controlled ascorbic acid intake.

Urinary excretion of ascorbic acid following a test dose has been found to occur in a relatively short period after the administration of the test dose. The urinary excretion of ascorbic acid during a four-hour period after the test dose has been found to give as satisfactory an estimate of the state of the tissues with respect to ascorbic acid as in the urinary excretion of ascorbic acid in a 24-hour period after the test dose (Melnick, Hochberg and Oser, 1945; Engelfried, 1944; Sigurjonsson, 1951).

EXPERIMENTAL PROCEDURE

Determination of the Apparent Ascorbic Acid Content of Frozen Strawberries

Plan of Experiment

Fresh strawberries of the Catskill and Robinson varieties were sampled at random for the analysis of reduced and total ascorbic acid. The rest of the berries were packaged in pint containers, frozen at -27 to -31 degrees Centigrade, and kept at this temperature for the entire storage period. It was not possible to analyze all of the components studied here in the fresh strawberries; therefore analyses of reduced ascorbic acid, total apparent ascorbic acid, dehydroascorbic acid, 2,3-diketogulonic acid, reductones and reductic acid, reducing sugar and total solids were carried out on strawberries which had been frozen for 30 hours. These values provided a base line for interpretation of the changes in these components of the strawberries during frozen storage. The frozen berries were sampled every month and analyzed for these components. The period of study was six months for strawberries of the Robinson variety and ten months for strawberries of the Catskill variety.

the same time, the fact that the same person can be both a subject and an object of a relation is not a contradiction. For example, a person can be both a subject and an object of a relation of self-love. In this case, the person is both the one who loves and the one who is loved. This is not a contradiction because the person is acting in two different capacities. Similarly, a person can be both a subject and an object of a relation of self-hatred. In this case, the person is both the one who hates and the one who is hated. This is not a contradiction because the person is acting in two different capacities. In fact, a person can be both a subject and an object of a relation of self-love and self-hatred at the same time. This is not a contradiction because the person is acting in two different capacities. For example, a person can love themselves and hate themselves at the same time. This is not a contradiction because the person is acting in two different capacities. In fact, a person can be both a subject and an object of a relation of self-love and self-hatred at the same time. This is not a contradiction because the person is acting in two different capacities. For example, a person can love themselves and hate themselves at the same time. This is not a contradiction because the person is acting in two different capacities.

Freezing and Sampling of Strawberries

Strawberries of the Catskill and the Robinson varieties were used in this experiment. They were purchased from the local market, Lansing, Michigan, in July, 1952. Immediately after purchase, the strawberries were washed in enamel dish pans by immersing two quarts at a time in about six quarts of tap water. The berries were mixed thoroughly by hand and the water was decanted. This washing was repeated one additional time. The berries were drained in an enamel colander for two minutes and spread on towels for removal of any excess moisture on the surface of the berries. Stems of the berries were removed by hand and the berries were mixed again. Samples were taken for the analysis of the reduced and total ascorbic acid content of fresh strawberries. Since it has been reported that sugar did not aid in the retention of ascorbic acid (Rabak, 1939), the remainder of the berries were packed without sugar or sirup in pint carton boxes.¹ Two hundred grams of berries were weighed into each box. The berries were frozen in a home type deep freeze chest at -27 to -31 degrees Centigrade and stored at this temperature. Four pints of each variety of the strawberries were selected at random from the freezer approximately 30

¹Nestrite carton pint sized box, Lily-Tulip Cup Corp., New York, N. Y.

hours after freezing and the berries were thawed in the cartons overnight (12 hours) in a refrigerator. The contents of the four boxes were mixed thoroughly with a porcelain spatula in a three-liter beaker. Two 200-gram samples of the strawberries of the Catskill variety were weighed for use in the human feeding experiment. The rest of the berries were used for the chemical analyses of reduced ascorbic acid, total apparent ascorbic acid, dehydroascorbic acid, 2,3-diketogulonic acid, reductones and reductic acid, reducing sugar and total solids. Each month following the beginning of the experiment, four pints of each variety of the berries were thawed and sampled for chemical analyses. Strawberries of the Catskill variety were stored in the frozen state for a total period of ten months. There was not an adequate supply of strawberries of the Robinson variety on the local market to permit analyses of the chemical constituents at monthly intervals for ten months; therefore, chemical analyses of strawberries of the Robinson variety were terminated at the end of a six-month storage period.

Chemical Methods

Chemical analyses were carried out on duplicate samples of strawberries. The order of procedure for carrying out the various methods was similar for each series of analyses. On the morning after the frozen strawberries had been thawed, samples were taken for the different procedures and the

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samples added to the acid extractant or the appropriate diluent, and stored in such a manner that no change in the component would be expected to occur which might be attributed to sampling procedure or delay in handling of sample until the analyses could be performed.

Reduced ascorbic acid. The determination of reduced ascorbic acid in strawberries was² by a modification of the procedure reported by Loeffler and Ponting (1942). Fifty grams of strawberries were added to 100 milliliters of five percent metaphosphoric acid in the container of an electric blender.² The mixture was blended for two minutes, transferred to a 500-milliliter volumetric flask and made to volume with distilled water. The contents of the flask were mixed and filtered. Twenty milliliters of the filtrate of the strawberries of the Robinson variety and fifteen milliliters of the filtrate of strawberries of the Catskill variety were diluted respectively to 100 milliliters with one percent metaphosphoric acid. Five milliliters of the diluted extract were added from a rapid delivery pipette to five milliliters of a solution of 2,6-dichlorophenolindophenol in the cuvette of a spectrophotometer.³ The cuvette was placed in position and the percent transmission of the solu-

²Waring Blender, Central Scientific Co., Chicago, Ill.

³Coleman Spectrophotometer, Model 11, Coleman Electric Co., Inc., Maywood, Ill.

tion was read from the galvanometer exactly fifteen seconds after the addition of the fruit extract to the dye reagent. The percent transmission was measured at a wavelength of 540 millimicrons. The instrument was standardized at 100 percent transmission against a decolorized solution containing five milliliters of the dye reagent, five milliliters of the dilute fruit extract and a few crystals of reduced ascorbic acid. A series of standard solutions was prepared of varying concentrations of reduced ascorbic acid in one percent meta-phosphoric acid. Aliquots of the ascorbic acid solutions were combined with the dye reagent and the percent transmission determined as described for the fruit extract. The concentration of the reduced ascorbic acid in the fruit extract was determined by the application of the Beer-Lambert law.

Total apparent ascorbic acid. In this study, the author has used the term "apparent ascorbic acid" to represent the substances in the tissue extract that react similarly to ascorbic acid in a chemical determination of ascorbic acid and therefore are analyzed as ascorbic acid. Particularly, the term "total apparent ascorbic acid" has been used to refer to the substances which form osazones with 2,4-dinitrophenylhydrazine by the Roe and Oesterling procedure (1944). In comparison, "total ascorbic acid" has been used by the author with reference to the results of the study to desig-

nate the ascorbic acid value of foods obtained by subtraction of the diketogulonic acid and reductones and reductic acid from the values obtained by the Roe and Oesterling procedure (1944).

The total apparent ascorbic acid content of the strawberries was determined by the method of Roe and Oesterling (1944). One hundred and twenty-five grams of a solution of five percent metaphosphoric acid in ten percent acetic acid were weighed into 25 grams strawberries in a beaker. This mixture was blended in electric blender for two minutes. Four grams of the slurry of the berries of Catskill variety or five grams of the slurry of the berries of Robinson variety were made to a volume of 100 milliliters with the metaphosphoric-acetic acid solution. One gram of activated charcoal⁴ was introduced into this solution and mixed thoroughly. After standing for five minutes, the mixture was centrifuged at 2500 revolutions per minute for 15 minutes. One milliliter of two percent 2,4-dinitrophenylhydrazine in 10 normal sulfuric acid was added to four milliliters of the supernatant liquid in a test tube and the mixture was incubated for six hours at 37 degrees Centigrade. After incubation, the tube and its contents were cooled in an ice bath and five milliliters of 65 percent sulfuric acid was added

⁴Norit A, Central Scientific Co., Chicago, Ill.

gradually with mixing. The sample then was kept at room temperature for 30 minutes for maximum color development. After this, the percent transmission of the solution was determined with a spectrophotometer⁵ at a wavelength of 540 millimicrons.

Dehydroascorbic acid and 2,3-diketogulonic acid. Dehydroascorbic acid and 2,3-diketogulonic acid were determined by the method of Roe, Mills, Oesterling and Damron (1948). The method was modified by the use of twice as much thiourea as the authors specified and the omission of stannous chloride. Stannous chloride was omitted to avoid the turbidity produced by stannous sulfide which is formed when the procedure of Roe et al. (1948) is followed. The use of stannous chloride was suggested by Roe et al. (1948) to prevent the strong oxidant effect of oxy-hemoglobin when present in tissue. Since oxy-hemoglobin was not present in strawberries, and since thiourea has been reported to be satisfactory for the protection of reduced ascorbic acid in plant tissue by Roe and Oesterling (1944), this modification was adopted.

Twenty-five grams of strawberries were ground in a glass mortar with 50 milliliters of a solution of five percent thiourea in five percent metaphosphoric acid. The slurry was diluted to 250 milliliters with five percent metaphosphoric acid and filtered. Twenty-five milliliters of the

⁵Coleman Spectrophotometer, Model 11, Coleman Electric Co., Inc., Maywood, Ill.

filtrate were diluted to 200 milliliters with five percent metaphosphoric acid. Chemical analysis of the diluted acid extract according to the method for total apparent ascorbic acid, with the exception of the addition of activated charcoal, gave the content of dehydroascorbic acid and diketogulonic acid of the strawberries.

One hundred milliliters of the diluted acid extract were measured into a large test tube; hydrogen sulfide was passed into the test tube from a Kipp generator for 15 minutes and the solution was filtered. Carbon dioxide was bubbled into an aliquot of 40 milliliters of the filtrate in order to displace the excess hydrogen sulfide. Since dehydroascorbic acid had thus been converted to the reduced form of ascorbic acid, chemical analysis of this solution by the procedure for total apparent ascorbic acid, again with the omission of activated charcoal, gave the content of 2,3-diketogulonic acid of the strawberries. The dehydroascorbic acid content of the strawberries was obtained by subtraction of the value for 2,3-diketogulonic acid from the value for 2,3-diketogulonic acid and dehydroascorbic acid.

Reductones and reductic acid. Reductones and reductic acid were determined by the method of Schocken and Roe (1952). The extract which was prepared for the determination of total apparent ascorbic acid was used for the determination of reductones and reductic acid. The procedure was identical to that for the determination of total apparent ascorbic acid

with the exception that five milliliters of 65 percent sulfuric acid were added before the addition of the 2,4-dinitrophenylhydrazine solution; this prevented the coupling of the dehydroascorbic acid and diketogulonic acid with 2,4-dinitrophenylhydrazine to form osazones. The method of Schocken and Roe (1952) was modified to the extent that the incubation period for the tissue extract with 2,4-dinitrophenylhydrazine was six hours rather than three hours. Preliminary experiments indicated that extension of the incubation period did not affect the values for reductones and reductic acid, and the procedures for the determination of total apparent ascorbic acid and for reductones and reductic acid were thus carried out simultaneously in the laboratory.

Reducing sugar. Ten grams of strawberries were blended in 100 milliliters of distilled water for two minutes. The slurry was transferred to a 250 milliliter flask and the contents of the flask were made to a volume of 250 milliliters with distilled water. The diluted slurry was filtered and 100 milliliters of the filtrate was clarified by the addition of 10 milliliters of saturated lead acetate. The excess lead acetate was precipitated by the addition of anhydrous sodium oxalate and the solution was filtered. Fehling's solution was added to the filtrate. The cuprous oxide which was formed was determined gravimetrically. The amount of reducing sugar equivalent to the cuprous oxide was determined from the Munson and Walker table for calculating invert sugar from weights of cuprous oxide (A. O. A. C., 1950).

Total solids. Total solids were determined by the use of a **semi-automatic** moisture tester.⁶

Studies of the Physiological Utilization of the Apparent
Ascorbic Acid of Frozen Strawberries

Plan of Experiment

This phase of the experiment was planned to study the utilization of the apparent ascorbic acid of strawberries frozen for 30 hours and of strawberries frozen and stored for four months, in comparison with the utilization of crystalline ascorbic acid and dehydroascorbic acid. Utilization of the ascorbic acid was studied by determination of the blood concentrations of reduced and total ascorbic acid at periodic intervals after the administration of the test doses. Reduced ascorbic acid of the urine also was determined.

There were four tests. The interval between the tests was about one month. The tests were planned as follows:

(1) The determination of blood and urinary ascorbic acid following the administration of 200 grams of Catskill variety strawberries which had been frozen for 30 hours.

(2) The determination of blood and urinary ascorbic acid following the administration of crystalline reduced as-

⁶Brabender Semi-automatic Moisture Tester, Model DF, Brabender Corporation, Rochelle Park, New Jersey.

corbic acid equivalent to the amount of total apparent ascorbic acid contained in the test dose of strawberries frozen for 30 hours.

(3) The determination of blood and urinary ascorbic acid following administration of orange juice which had been treated with activated charcoal to oxidize the ascorbic acid to dehydroascorbic acid. The amount of orange juice which was given contained an amount of dehydroascorbic acid equivalent to the amount of total apparent ascorbic acid in the test dose of strawberries frozen for 30 hours.

(4) The determination of blood and urinary ascorbic acid following administration of a test dose of strawberries which had been frozen for four months and which was estimated to contain an amount of total ascorbic acid equivalent to the amount of total apparent ascorbic acid in the test dose of strawberries frozen for 30 hours.

The time intervals for the collection of blood following the test doses were one, two, three, four and five hours, when strawberries or dehydroascorbic acid was the test dose. Since other workers have indicated that crystalline ascorbic acid is absorbed quickly into the blood stream (Todhunter and Fatzer, 1940), blood samples were taken at one-half, one, two, three and four hours following the test dose of reduced ascorbic acid. Urine collections were made during a four-hour period after the crystalline ascorbic acid test

dose and a five-hour period after the other test doses were given.

Subjects

The subjects were six healthy adult women who were members of the research staff or graduate students. The range of ages was from 24 to 37 years; body weights were from 47.8 to 61.9 kilograms. Seven-day dietary records were collected from each subject to provide an indication of the usual dietary habits and an estimation of the average daily ascorbic acid intakes of the subjects. The ascorbic acid intakes of the seven-day dietary records were estimated by calculation with the use of the food table of Donelson and Leichseuring (1945). Each subject was given 75 milligrams of crystalline ascorbic acid daily for seven days preceding the administration of each test dose, in order that a comparable state of nutrition with respect to ascorbic acid might be attained for all subjects preceding the various tests.

Preparation of Test Doses

Strawberries. The strawberries were of the Catskill variety; the berries were purchased on the local market and washed, drained and packaged as described previously. Only two subjects were studied at a time; therefore berries used for the test dose were purchased in three separate lots, so that all strawberries given the subjects in the initial test

period were frozen only 30 hours. Four pints of the strawberries of the Catskill variety were selected at random 30 hours after freezing. The berries were thawed in the cartons overnight (12 hours) in a refrigerator. The contents of the four boxes were mixed thoroughly with a porcelain spatula in a three-liter beaker. Two 200-gram samples of the strawberries were weighed as test doses. The rest of the berries were used for the chemical analyses of all the components reported in this study. The test dose of strawberries frozen and stored for four months was 250 grams.

Crystalline ascorbic acid. The test dose of crystalline ascorbic acid was 169 milligrams. This was the quantity of total apparent ascorbic acid supplied the subjects in the test dose of 200 grams of strawberries which had been frozen for 30 hours. The ascorbic acid was dissolved in 50 milliliters distilled water in a 100 milliliter beaker immediately before it was taken by the subject. The beaker was rinsed twice with distilled water and the rinsings were drunk.

Dehydroascorbic acid. At the present time, dehydroascorbic acid is not commercially available. The dehydroascorbic acid used as a test dose was prepared in the laboratory. Fresh oranges⁷ were purchased from the retail market.

⁷Sunkist oranges.

The juice was extracted from the orange by a glass reamer, and filtered with suction through a pad of glass wool in a Büchner funnel. To every 100 milliliters of the filtered juice, four grams of activated charcoal was added. This mixture was shaken for five minutes and then was filtered under suction with a filter paper⁸ and a layer of filter aid⁹ in a Büchner funnel. The filtrate was divided into portions of 1,000 milliliters and 200 milliliters and frozen at -30 degrees Centigrade. The sample of 200 milliliters was analyzed for reduced ascorbic acid and dehydroascorbic acid on the day preceding the administration of the test dose. The juice was given in an amount to supply 169 milligrams of dehydroascorbic acid. The treated, frozen, and thawed orange juice thus prepared from three batches contained 40.4 to 41.7 milligrams dehydroascorbic acid per 100 milliliters; the reduced ascorbic acid content was found to be negligible.

Collection and Treatment of Blood and Urine Samples

All the blood samples were taken by finger tip puncture according to the method of György (1950). The blood was collected in 10 x 50 millimeter vials and allowed to stand for 15 minutes in a refrigerator for formation of the blood clot.

⁸Whatman no. 42, Central Scientific Co., Chicago, Ill.

⁹Hyflo super-cel, Johns-Manville, New York, N. Y.

The clot was loosened with a fine glass rod and the tube and its contents were centrifuged. Two-tenths of a milliliter of the serum was taken for the analysis of reduced ascorbic acid. Titrations were performed about two hours after deproteinization. Ten cubic millimeters of the serum were deproteinized, frozen and held at a temperature of -30 degrees Centigrade for the analysis of total ascorbic acid content.

The subjects were instructed to discard the first voiding of urine after rising in the morning of the test day. The urine, excreted before the test dose was given, was collected in a brown glass, one-liter bottle which contained twenty milliliters of a solution made up of 100 milliliters of five normal sulfuric acid and 100 milliliters of a solution of one percent metaphosphoric acid, containing two milliliters of 1.5 percent 8-hydroxyquinoline in ethyl alcohol. The time at which the subject arose in the morning was noted; the test dose was administered approximately one hour later. The exact time was recorded and the ascorbic acid content of the urine of the subject preceding the test dose was calculated as milligrams per hour.

Urine collections were made for a four-hour period following the test dose of crystalline reduced ascorbic acid and five-hour periods following the other test doses. The urine of each subject was composited for the four- or five-hour pe-

riod. The collection bottles were kept in the refrigerator throughout the test.

Chemical Analysis

The method of Farmer and Abt (1936) was used to determine the reduced ascorbic acid of blood serum.

Total apparent ascorbic acid of blood serum was determined according to the method of Lowry, Lopez, and Bessey (1945). Readings of percent transmission of the solutions after development of color were made in a spectrophotometer¹⁰ at a wavelength of 540 millimicrons.

Urinary reduced ascorbic acid was determined by the method of Evelyn, Malloy, and Rosen (1938). A five-milliliter aliquot of urine in one percent metaphosphoric acid was measured into a cuvette of a spectrophotometer. Five milliliters of a solution of 2,6-dichlorophenolindophenol were added and readings of the percent transmission of the reaction mixture were taken at five, ten, twenty, and thirty seconds after the addition of the dye; the transmission readings were plotted against time on linear graph paper and extrapolated to zero time. The zero time reading was used to determine the ascorbic acid content in urine by the application of the Beer-Lambert law.

¹⁰ Beckman Spectrophotometer, model DU, Beckman Instruments, Inc., South Pasadena, Calif.

RESULTS AND DISCUSSION

Changes of the Total Apparent Ascorbic Acid in Frozen Strawberries

The mean concentrations of reduced ascorbic acid, dehydroascorbic acid, 2,3-diketogulonic acid, total apparent ascorbic acid, total ascorbic acid, reducing sugar and total solids in fresh frozen strawberries of the Catskill variety and in strawberries of the same variety during successive periods of frozen storage are presented in Table I and plotted in Figure 1. Similar data for strawberries of the Robinson variety are given in Table II and Figure 2. Values are expressed in terms of unit weight of wet matter.

The total apparent ascorbic acid was determined by the method of Roe and Oesterling (1944) and included reduced ascorbic acid, dehydroascorbic acid, diketogulonic acid, and any other substances which may have formed osazones with the 2,4-dinitrophenylhydrazine under the conditions of the method. Values for the 2,3-diketogulonic acid were subtracted from the values for total apparent ascorbic acid and the differences are given under the heading of Total Ascorbic Acid. Analyses were made for reductions and reductive acid on all samples of the strawberries. However, this group of compounds was not present in measurable quantities. There-

TABLE I

THE AVERAGE CONCENTRATION OF ASCORBIC ACID AND RELATED
COMPOUNDS IN STRAWBERRIES (CATSKILL VARIETY)
AT MONTHLY PERIODS OF STORAGE

Storage Period	Total Solids	Reduced Ascorbic Acid Col. 1 ^a	Total Apparent Ascorbic Acid Col. 2 ^b
	%	mg./100 gm.	mg./100 gm.
Fresh Berries	-	81.6	83.0
Frozen, 30 Hrs.	11.55	75.0	80.0
Months			
1	10.90	42.3	86.3
2	11.01	40.0	78.0
3	10.51	41.7	77.5
4	10.84	37.0	72.5
5	11.21	42.4	80.0
6	11.41	49.7	74.0
7	11.31	47.2	78.5
8	11.50	50.6	72.5
9	11.45	51.4	68.8
10	11.43	51.0	75.0

^aCol.1-Reduced Ascorbic Acid by Loeffler and Ponting method (1942).

^bCol.2-Total Apparent Ascorbic Acid by Roe and Oesterling method (1944).

^cCol.5 = Col. 2 - Col. 4.

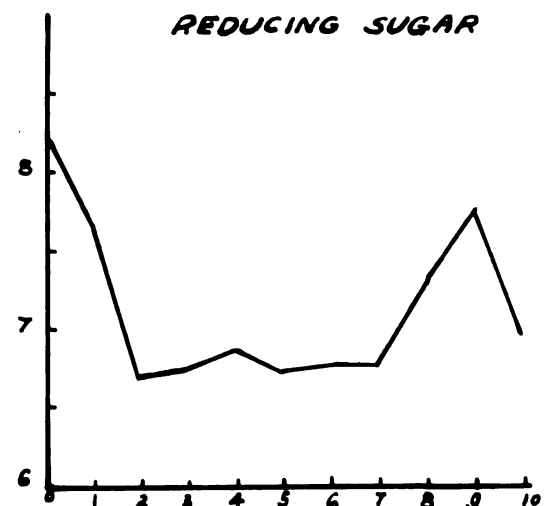
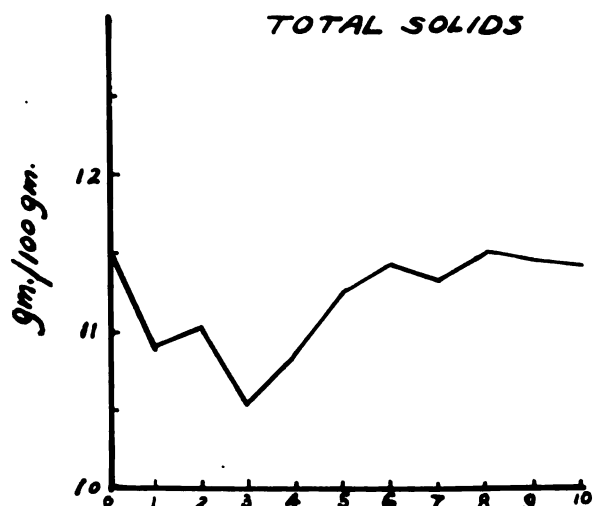
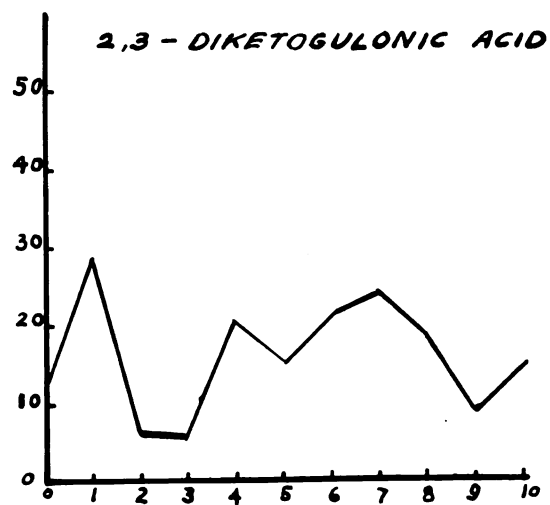
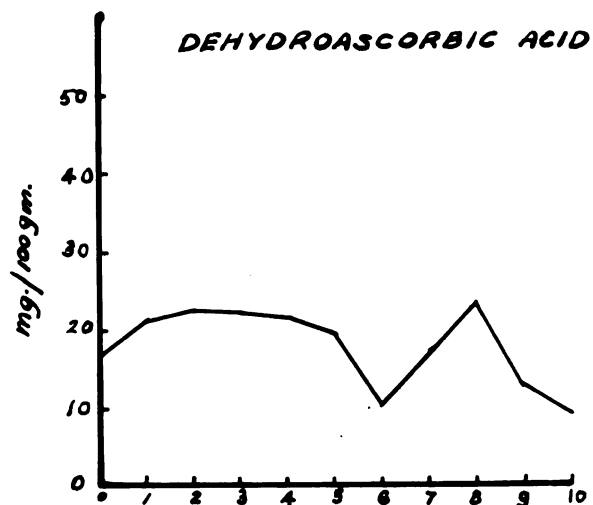
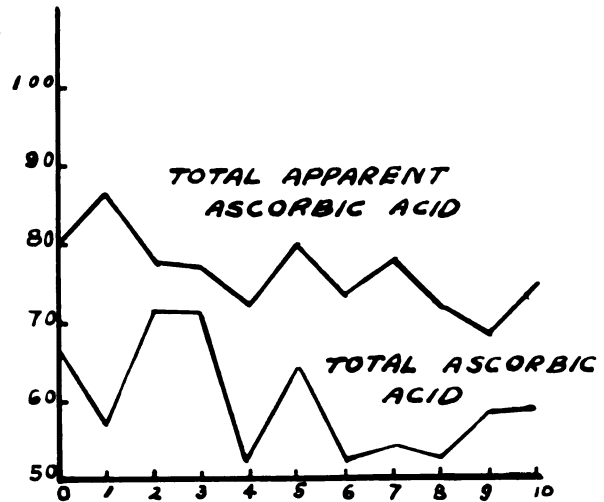
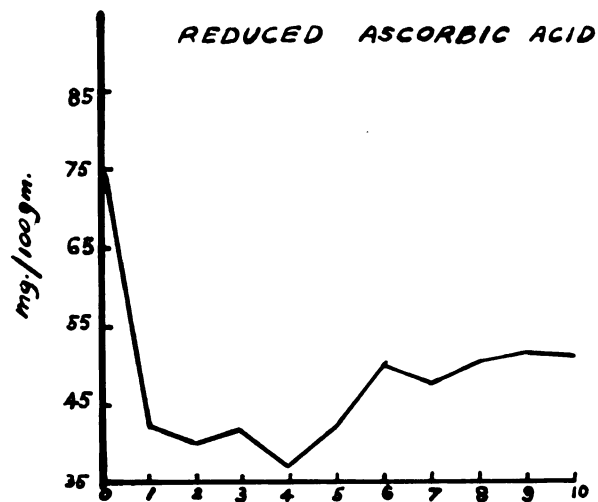
^dCol.6 = Col. 5 - Col. 3.

TABLE I (CONTINUED)

Dehydro- Ascorbic Acid Col. 3	2,3- Diketo- gulonic Acid Col. 4	Total Ascorbic Acid Col. 5 ^c	Reduced Ascorbic Acid Col. 6 ^d	Reducing Sugar
mg./100 gm.	mg./100 gm.	mg./100 gm.	mg./100 gm.	%
-	-	-	-	-
16.3	12.5	67.5	51.2	8.24
21.0	29.0	57.3	36.3	7.65
22.5	6.5	71.5	49.0	6.70
22.0	6.0	71.5	49.5	6.74
21.5	20.5	52.5	31.0	6.87
19.5	15.5	64.5	45.0	6.73
10.0	21.5	52.5	42.5	6.79
16.7	24.3	54.2	37.5	6.79
23.5	19.5	53.0	29.5	7.29
12.7	9.5	59.3	46.6	7.74
9.4	15.6	59.4	50.0	6.97

Figure 1. Graph showing the changes in concentration of ascorbic acid and related compounds, total solids and reducing sugar in strawberries of the Catskill variety during frozen storage.

CATSKILL VARIETY



Storage Time (Month)

TABLE II
THE AVERAGE CONCENTRATION OF ASCORBIC ACID AND RELATED
COMPOUNDS IN STRAWBERRIES (ROBINSON VARIETY)
AT MONTHLY PERIODS OF STORAGE

Storage Period	Total Solids	Reduced Ascorbic Acid Col. 1 ^a	Total Apparent Ascorbic Acid Col. 2 ^b
	%	mg./100 gm.	mg./100 gm.
Fresh Berries	-	47.6	50.0
Frozen, 30 Mns.	8.25	46.6	50.0
Months			
1	8.41	29.7	50.6
2	8.19	24.7	49.0
3	8.06	23.8	43.0
4	8.15	20.5	39.0
5	8.35	22.5	42.0
6	8.30	28.7	39.6

^aCol. 1-Reduced Ascorbic Acid by Loeffler and Ponting method (1942).

^bCol. 2-Total Apparent Ascorbic Acid by Roe and Oesterling method (1944).

^cCol. 5 = Col. 2 - Col. 4.

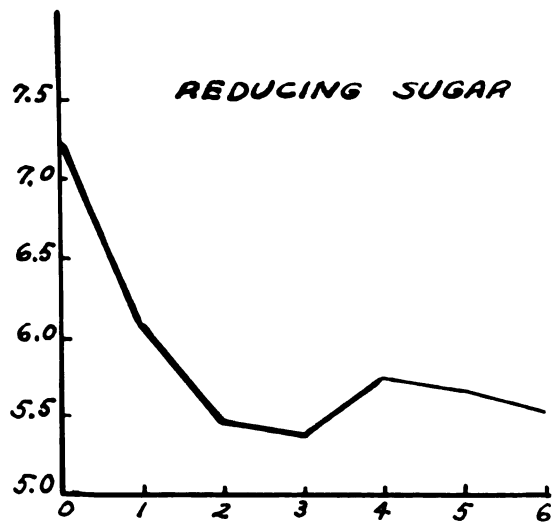
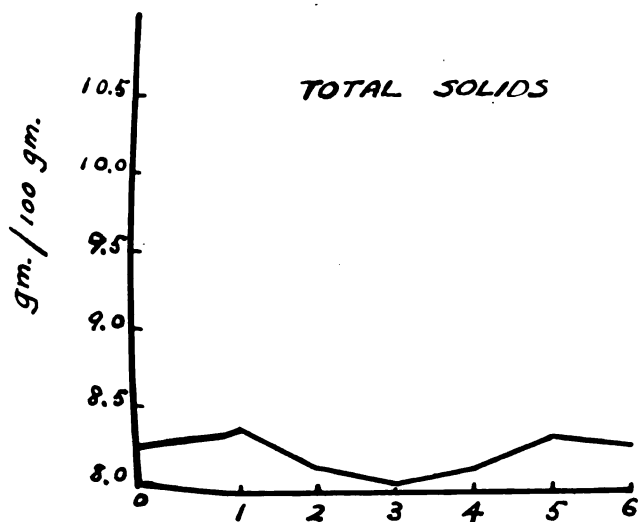
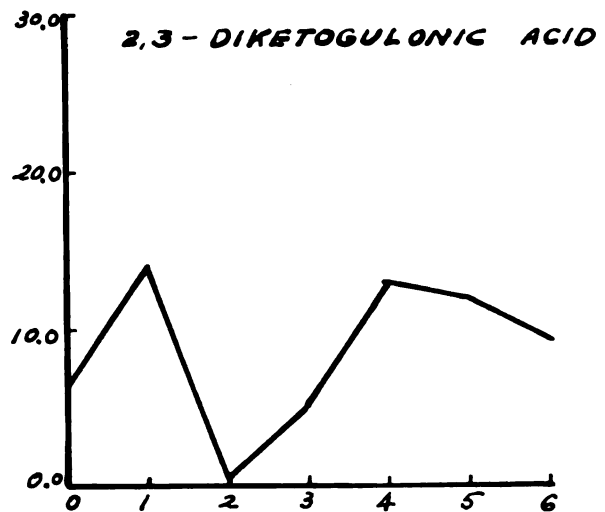
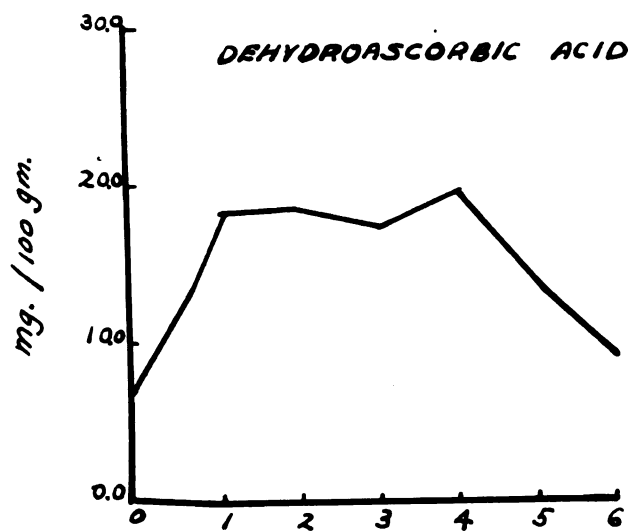
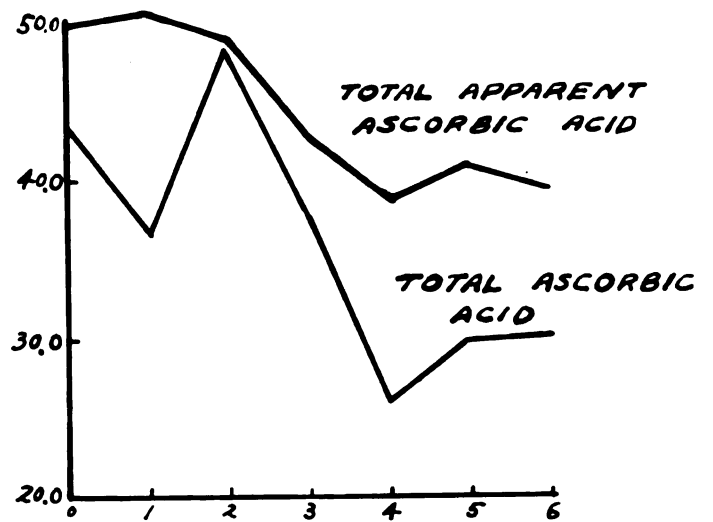
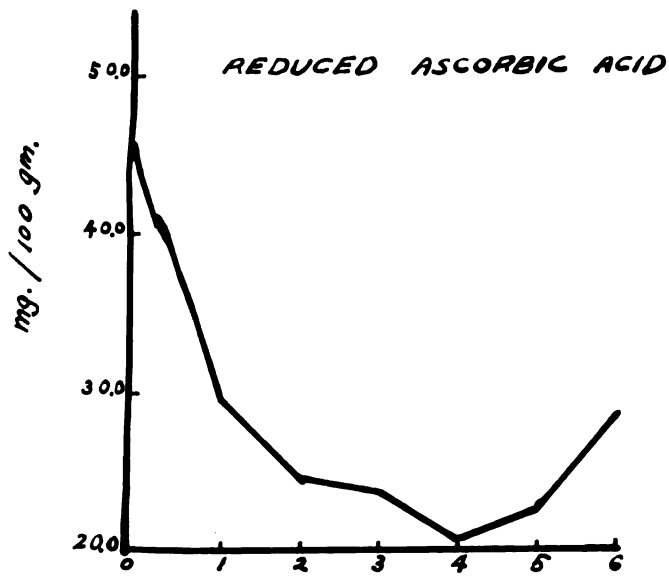
^dCol. 6 = Col. 5 - Col. 3.

TABLE II (CONTINUED)

Dehydro- ascorbic Acid Col. 3	2,3- Diketo- gulonic Acid Col. 4	Total Ascorbic Acid Col. 5 ^c	Reduced Ascorbic Acid Col. 6 ^d	Reducing Sugar
mg./100 gm.	mg./100 gm.	mg./100 gm.	mg./100 gm.	%
-	-	-	-	-
6.5	6.3	43.7	37.2	7.29
18.5	14.0	36.6	18.1	6.09
18.7	0.5	48.5	29.8	5.46
17.5	5.0	38.0	20.5	5.39
19.8	13.0	26.0	6.2	5.74
14.0	12.0	30.0	16.0	5.66
9.2	9.4	30.2	21.0	5.51

Figure 2. Graph showing the changes in concentration of ascorbic acid and related compounds, total solids and reducing sugar in strawberries of the Robinson variety during frozen storage.

ROBINSON VARIETY



Storage Time (month)

fore, no values for reductones and reductic acid were included in Tables I and II and it was assumed that this group of compounds did not contribute to the values for total apparent ascorbic acid.

Reduced ascorbic acid. The reduced ascorbic acid concentrations of the frozen strawberries as determined directly by reaction with 2,6-dichlorophenolindophenol and by subtraction of the dehydroascorbic acid fraction of the strawberries from the total ascorbic acid content both are given in Tables I and II. There was not good agreement between the values for reduced ascorbic acid obtained by the two methods; nor were the differences consistent between the values obtained by the two methods. Although the mean difference in values by the two methods for the strawberries of the Catskill variety was only 5.4 milligrams per 100 grams, the differences in individual values varied from 1.0 to 23.8 milligrams per 100 grams. The mean difference in average values for reduced ascorbic acid at monthly time intervals for strawberries of the Robinson variety was 6.8 milligrams per 100 grams and the range was from 3.3 to 14.3 milligrams per 100 grams.

The determination of reduced ascorbic acid in foods by the two methods used here has been reported by Dodds, Price and Moore (1948). These authors also failed to obtain good agreement between the two methods. However, the **differences**

varied only from 0.9 to 6.3 milligrams ascorbic acid per 100 grams of baked sweet potato and were not as great as the differences of values shown in Tables I and II. In this study, most of the values obtained by the 2,6-dichlorophenol-indophenol procedure exceeded those obtained by the Roe and Oesterling (1944) procedure. However, the reverse was true of the results reported by Dodds and co-workers. Analyses of reduced ascorbic acid of canteloupes reported by Chen, Elliott and Schuck (1948) agreed within 2.0 milligrams per 100 grams for freshly cut canteloupe and for canteloupe halves which were stored in the refrigerator for twenty four hours. Analyses of 2,3-diketogulonic acid were not reported by Dodds and co-workers or by Chen, Elliott and Schuck.

Possible changes in the reduced ascorbic acid concentrations of strawberries during frozen storage have been interpreted on the basis of analysis of reduced ascorbic acid by the 2,6-dichlorophenolindophenol method of Loeffler and Ponting (1942), since this procedure represents a direct measurement of the reduced form of ascorbic acid present in the fruits.

The average reduced ascorbic acid content of the fresh strawberries was 81.6 milligrams per 100 grams for strawberries of the Catskill variety and 47.6 milligrams per 100 grams for berries of the Robinson variety. The values for reduced ascorbic acid for both varieties were within the

range of 25 to 142 milligrams per 100 grams reported for strawberries by various investigators (Illyuvieu and Ulanova, 1939; Chi and Read, 1935; Burrell and Ebright, 1940; Chen and Schuck, 1951). Ezell, Darrow, Wilcox, and Scott (1947) found that Catskill berries contained 81.0 milligrams of reduced ascorbic acid per 100 grams; this was almost identical with the average values found in this study. These authors reported that the reduced ascorbic acid in strawberries of the Robinson variety was 71.1 milligrams per 100 grams and Einbecker and her co-workers (1947; 1950) found the value to be 65.6 milligrams per 100 grams. Both values were much higher than those found in this study. Factors such as degree of ripening (Oliver, 1938) and solar irradiation (McCroy, 1936; Schuphan, 1942; Hansen and Waldo, 1944) have been demonstrated to affect the reduced ascorbic acid content of fruits. Variations of over 100 percent for samples taken from the same plants on different days have been reported by Kirk and Tressler (1941). The procedure of handling the berries from the farm to the market also may affect the ascorbic acid content of the berries. Thus any of various factors might **have** been responsible for the relatively low concentration found in this study for the ascorbic acid in the strawberries of the Robinson variety.

The reduced ascorbic acid concentration of strawberries of the Robinson variety, thirty hours after freezing, was

similar to the concentration of the fresh strawberries. A slightly lower value for reduced ascorbic acid was found for berries of the Catskill variety after 30 hours in the frozen state than for the fresh berries. The strawberries of the Catskill variety contained 81.6 milligrams of reduced ascorbic acid per 100 grams before freezing and 75.0 milligrams per 100 grams after freezing. This represented a loss of about eight percent.

The average concentration of reduced ascorbic acid decreased rapidly during the first month of frozen storage for berries of both varieties. The change in reduced ascorbic acid was from 75.0 to 42.3 milligrams per 100 grams in berries of the Catskill variety and 46.6 to 29.7 milligrams per 100 grams in berries of the Robinson variety. The concentration of reduced ascorbic acid in strawberries of both varieties was relatively constant during the second, third and fourth months of storage. For both varieties an increase in reduced ascorbic acid concentration was observed after five months of storage.

Since strawberries of the Catskill variety were used for the human utilization studies, and since the strawberries were analyzed preliminary to each feeding trial, a greater number of replications was carried out on strawberries of the Catskill variety which had been frozen for 30 hours and which had been held in frozen storage for four

months than at other time intervals of frozen storage or than on strawberries of the Robinson variety. Therefore, six additional replications were made after eight months of frozen storage of the berries of the Catskill variety, so that the data for the ascorbic acid concentrations of the berries frozen 30 hours, four months and eight months could be analyzed statistically by analysis of variance. The analysis of variance presented in Table III indicated that the change in the reduced ascorbic acid of the strawberries during frozen storage of four and eight months was highly significant ($p \leq 0.01$). The significance of the difference of the means was tested by the t-test (Snedecor, 1946). This analysis is presented in Table IV. It is apparent that the loss of reduced ascorbic acid after four and eight months of storage was highly significant; there was not a statistically significant difference between the average reduced ascorbic acid content of frozen strawberries stored four months and that of frozen strawberries stored for eight months.

Total apparent ascorbic acid. The total apparent ascorbic acid contained in the fresh berries of the Robinson variety was 50.0 milligrams per 100 grams which was comparable to that reported by Paul, Wiant and Robertson (1949), who found the average concentration to be 47.5 milligrams per 100 grams. The average total apparent ascorbic acid contained in the fresh berries of Catskill variety was 83.0

TABLE III

ANALYSIS OF VARIANCE OF THE CONCENTRATION OF REDUCED
ASCORBIC ACID IN STRAWBERRIES (CATSKILL VARIETY)
FROZEN FOR 30 HOURS, FOUR MONTHS AND EIGHT MONTHS

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F-value
Total	23	5033.8		
Periods	2	4260.5	2130.3	57.9**
Error	21	773.3	36.8	

**p $\leq$ 0.01; highly significant.

TABLE IV

SIGNIFICANCE OF THE DIFFERENCE OF THE MEANS OF REDUCED
ASCORBIC ACID IN STRAWBERRIES (CATSKILL VARIETY)
FROZEN FOR 30 HOURS, FOUR MONTHS AND EIGHT MONTHS

Comparison	Calculated t-value
Without storage vs. 4 months storage	10.200**
Without storage vs. 8 months storage	8.367**
4 months storage vs. 8 months storage	1.833

**p $\leq$ 0.1; highly significant.

milligrams per 100 grams. No comparison could be made on the total apparent ascorbic acid content of strawberries of the Catskill variety with reported values, since no such values were found in the literature.

The mean concentration of total apparent ascorbic acid of strawberries of the Robinson variety, 30 hours after freezing, was similar to the concentration of the fresh berries. A slightly lower concentration of total apparent ascorbic acid was found in berries of the Catskill variety after 30 hours in the frozen state than in the fresh berries. The value after freezing was four percent less than that of the fresh berries.

There was a slight increase in the concentration of total apparent ascorbic acid in strawberries of the Catskill variety during the first month of frozen storage. The values were 80.0 and 86.3 milligrams per 100 grams for the frozen strawberries without storage and after one month of storage, respectively. There was no change in the total apparent ascorbic acid content of the strawberries of the Robinson variety during the first month of frozen storage (Tables I and II).

The concentration of total apparent ascorbic acid in strawberries of both varieties was lowered gradually through the second, third and fourth months of storage. There was a decrease of nine percent in the total apparent ascorbic acid

content of the berries of the Catskill variety and 22 percent in the total apparent ascorbic acid content of berries of the Robinson variety after four months of frozen storage. A slight increase in total apparent ascorbic acid concentration occurred after the fifth month of storage in comparison with the average concentration of the berries after four months frozen storage; this was followed by a decrease after the sixth month, in berries of both varieties. At the end of six months of frozen storage there was an average loss of 7.5 percent in total apparent ascorbic acid in strawberries of the Catskill variety and 21 percent in strawberries of the Robinson variety in comparison with the values of the berries frozen for 30 hours. In the Catskill variety, there was a slight increase in the concentration of total apparent ascorbic acid from the sixth to the seventh month of storage, then a reduction was observed after eight and nine months. The lowest value for total apparent ascorbic acid of the berries was observed after nine months of frozen storage. This was 68.8 milligrams per 100 grams of strawberries. After ten months of storage, the total apparent ascorbic acid had increased again to a value of 75.0 milligrams per 100 grams of strawberries.

Statistical treatment of the data on the total apparent ascorbic acid content of strawberries of the Catskill variety indicated that there was a highly significant change in

total apparent ascorbic acid concentration of the berries during the storage period (Table V). The 't'-test was applied to test the significance of the differences of the means. The results of this test indicated that there was a highly significant decrease of total apparent ascorbic acid concentration in strawberries after frozen storage for four months and eight months, in comparison with frozen strawberries which had been frozen only for 30 hours (Table VI). There was not a statistically significant difference between the total apparent ascorbic acid content of strawberries frozen and held for four months and that of strawberries frozen and held for eight months.

The changes in total apparent ascorbic acid content of strawberries in this study were in accord with the results of Bedford and McGregor (1948), Dodds, Price, and Moore (1948), Hartzler (1948), Wolfe (1949), and Mills, Damron and Roe (1949). These authors found that the total ascorbic acid content of foods decreased during storage and that the total ascorbic acid of the food varied from time to time. The change in total apparent ascorbic acid of strawberries observed in this study did not follow the pattern of the study reported by Paul and her co-workers (1949), whose data indicated that there was about a 75 percent increase in total ascorbic acid in strawberries of the Robinson variety during the first three months of frozen storage, in comparison with

TABLE V

ANALYSIS OF VARIANCE OF THE CONCENTRATION OF TOTAL APPARENT
ASCORBIC ACID IN STRAWBERRIES (CATSKILL VARIETY) FROZEN
FOR 30 HOURS, FOUR MONTHS AND EIGHT MONTHS

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F-value
Total	23	1,313.4		
Period	2	830.4	415.2	18.1**
Error	21	483.0	23.0	

**p $\leq$ 0.01; highly significant.

TABLE VI

SIGNIFICANCE OF THE DIFFERENCE OF THE MEANS OF TOTAL
APPARENT ASCORBIC ACID IN STRAWBERRIES (CATSKILL
VARIETY) FROZEN FOR 30 HOURS, FOUR MONTHS
AND EIGHT MONTHS

Comparison	Calculated t-value
Without storage vs. 4 months storage	4.167**
Without storage vs. 8 months storage	5.833**
4 months storage vs. 8 months storage	1.667

**p $\leq$ 0.01; highly significant.

the fresh berries and an increase of 40 percent after six months of storage; the total ascorbic acid concentration of the frozen strawberries was greater than that of the fresh strawberries after the end of the experimental period of nine months. The chemical procedure used for the determination of total apparent ascorbic acid in this study was Roe and Oesterling (1944). This was similar to the procedure used by Hartzler (1948), Dodds, Price and Moore (1948), Mills, Damron and Roe (1949), and Paul et al. (1949) for the determination of total ascorbic acid of foods during storage.

Dehydroascorbic acid. The dehydroascorbic acid content of the strawberries frozen for thirty hours was 16.3 milligrams per 100 grams for the Catskill variety (Table I) and 6.5 milligrams per 100 grams for the Robinson variety (Table II). A value of 12.0 milligrams of dehydroascorbic acid per 100 grams of strawberries was reported by Mills, Damron and Roe (1949). The variety of the berries studied by these authors was not given.

The concentrations of dehydroascorbic acid in strawberries increased during the first month of storage from 6.5 to 18.5 milligrams per 100 grams in the strawberries of the Robinson variety and from 16.3 to 21.0 milligrams per 100 grams in strawberries of the Catskill variety. The increase in concentration of dehydroascorbic acid during the first month of storage was in reverse of the change which was observed

for reduced ascorbic acid. The average concentration of dehydroascorbic acid in frozen strawberries of the Robinson variety remained high from the first to the fourth month of storage and then was lowered. The average concentration of dehydroascorbic acid after the fourth month of frozen storage was 19.8 milligrams per 100 grams. At the end of the sixth month the concentration was lowered to 9.2 milligrams per 100 grams. A similar pattern was observed for strawberries of the Catskill variety during the first four months of frozen storage. At the end of four months the dehydroascorbic acid concentration was 21.5 milligrams per 100 grams, which was almost identical with that after the first month. A decrease of 11.5 milligrams per 100 grams of the dehydroascorbic acid in berries of the Catskill variety was observed from the end of the fourth month of frozen storage to the end of the sixth month. A subsequent increase in dehydroascorbic acid occurred, which reached a peak of 23.5 milligrams per 100 grams at the end of the eighth month. The concentration of dehydroascorbic acid was lowered during the ninth and tenth months; at the end of the tenth month of frozen storage, there were 9.4 milligrams of dehydroascorbic acid per 100 grams of strawberries of the Catskill variety.

2,3-diketogulonic acid. After 30 hours of frozen storage, the average concentration of the 2,3-diketogulonic acid was 12.5 milligrams per 100 grams for berries of the Catskill

variety and 6.3 milligrams per 100 grams for berries of the Robinson variety. The maximum increase in the concentration of the diketogulonic acid in the strawberries of the two varieties occurred during the first month of frozen storage. This increase was from 12.5 to 29.0 milligrams of diketogulonic acid per 100 grams in berries of the Catskill variety and from 6.3 to 14.0 milligrams per 100 grams in berries of the Robinson variety. A sudden decrease to less than the original value was observed in both varieties after the second month of frozen storage. In the Robinson berries, the diketogulonic acid concentration was relatively low at the end of the third month, then increased to 13.0 milligrams per 100 grams at the end of the fourth month. Values at the end of five and six months of frozen storage were 12.0 and 9.4 milligrams of diketogulonic acid per 100 grams of strawberries, respectively. In strawberries of the Catskill variety, similar changes in the diketogulonic acid concentration were observed at the end of three, four and five months. The concentrations were 6.0, 20.5 and 15.5 milligrams of diketogulonic acid per 100 grams of strawberries respectively. There was an increase of six milligrams of diketogulonic acid per 100 grams of strawberries during the sixth month of frozen storage and a further increase of three milligrams per 100 grams of berries during the seventh month of frozen storage. A reduction of diketogulonic acid was observed after the eighth month and again after the ninth month of storage. A rise in dike-

togulonic acid concentration to a value of 15.6 milligrams per 100 grams of strawberries had occurred by the end of the tenth month of frozen storage.

The 2,3-diketogulonic acid content of fresh strawberries was reported by Chen and Schuck (1951) as 1.13 to 5.11 milligrams per 100 grams. Mills, Damron, and Roe (1949) found that the diketogulonic acid content in frozen strawberries ranged from 3.0 to 37.0 milligrams per 100 grams depending upon the condition of the berries. All values for diketogulonic acid of the strawberries of this study were within the range reported by Mills and co-workers.

Total ascorbic acid. The total ascorbic acid values which were obtained by subtraction of diketogulonic acid, reductones and reductic acid from the total apparent ascorbic acid values also were presented in Table I for berries of the Catskill variety and in Table II for berries of the Robinson variety.

The total ascorbic acid concentration of berries of the Catskill variety, frozen for 30 hours, was 67.5 milligrams per 100 grams. A marked decrease to 57.3 milligrams per 100 grams was observed after the first month of frozen storage. This value was increased to 71.5 milligrams per 100 grams after the second month of frozen storage and was maintained for another month. A decrease in total ascorbic acid content of berries of the Catskill variety to 52.5 milligrams

per 100 grams after four months storage was followed by an increase to 64.5 milligrams after five months storage. During the last five months of storage, the total ascorbic acid values remained relatively constant, that is, from 52.5 to 59.4 milligrams per 100 grams of berries.

In berries of the Robinson variety, the total ascorbic acid content of strawberries frozen for 30 hours was 43.7 milligrams per 100 grams. This value was decreased to 36.0 milligrams per 100 grams after the first month of frozen storage. An increase to 48.5 milligrams per 100 grams was observed after the second month of storage; this was followed by a decrease of 10.5 milligrams per 100 grams after the third month of storage. By the end of the fourth month of frozen storage, the total ascorbic acid content of berries of the Robinson variety was only 26.0 milligrams per 100 grams. Then a slight increase to 30.0 and 30.2 milligrams per 100 grams after five and six months of storage was found.

Reducing sugar. The concentration of reducing sugar in the berries frozen for 30 hours was 8.24 percent for the Catskill variety and 7.29 percent for the Robinson variety, respectively. These values were within the range reported in the literature for reducing sugar for strawberries (Chatfield and McLaughlin, 1928; Chatfield, 1940). The concentrations of reducing sugar ranged from 6.70 to 8.24 percent in the berries of the Catskill variety during ten months

frozen storage and from 5.39 to 7.29 percent in berries of the Robinson variety during six months frozen storage. The greatest decrease in the concentration of reducing sugar in the berries was observed during the first two months of frozen storage for both varieties. This decrease was 19 percent in the berries of Catskill variety and 25 percent in the berries of the Robinson variety.

Total solids. The percentage of total solids was relatively constant during the frozen storage period. The average range was from 10.51 to 11.55 percent in strawberries of Catskill variety stored for 10 months. The mean percentage of total solids ranged from 8.06 to 8.41 percent in Robinson berries for a period of six months frozen storage.

Concomitant Changes in Ascorbic Acid and Related Compounds in Frozen Strawberries

At the end of the first month of frozen storage, there was a marked decrease in reduced ascorbic acid concentration of strawberries of both varieties in comparison with the concentration of the berries frozen for thirty hours. A definite increase in the concentration of dehydroascorbic acid and 2,3-diketogulonic acid occurred during this period; there was very little change in the total apparent ascorbic acid concentration. This indicated that during the first month of frozen storage some of the reduced ascorbic acid probably was oxidized to dehydroascorbic acid and 2,3-diketogulonic

acid. The reduction in concentration of reduced ascorbic acid during the first month of frozen storage for strawberries of the Catskill variety was greater than the corresponding increase in concentration of dehydroascorbic acid and diketogulonic acid. For strawberries of the Robinson variety, however, the increment in 2,3-diketogulonic acid and dehydroascorbic acid during the first month of frozen storage was greater than the decrement in reduced ascorbic acid.

During the second month of frozen storage there was further reduction of the concentrations of reduced ascorbic acid in the strawberries, but the concentration of dehydroascorbic acid was relatively the same as at the end of the first month. Considerable reduction in the concentration of diketogulonic acid, occurred, perhaps due to further oxidation of this acid to oxalic acid and threonic acid.

Although there was a loss of 22.5 milligrams of 2,3-diketogulonic acid per 100 grams of berries of the Catskill variety during the second month of frozen storage, the total apparent ascorbic acid concentration was reduced only by 7.3 milligrams. Therefore the total ascorbic acid concentration of the strawberries was higher after the second month than after the first month of storage. Similarly, there was a loss of 13.5 milligrams of 2,3-diketogulonic acid per 100 grams of strawberries of the Robinson variety during the second month of storage, but a change only of 0.4 milligrams

of total apparent ascorbic acid per 100 grams of strawberries. Thus, in these berries, too, an increase in total ascorbic acid was observed at the end of the second month of frozen storage. The failure of the marked changes in 2,3-diketogulonic acid during the second month of frozen storage to be reflected in the values for total apparent ascorbic acid suggest that other unidentified substances may have contributed to the determination of total apparent ascorbic acid. If this were true, then the apparent increase in total ascorbic acid during the second month of frozen storage would be an artifact. However, it also is possible that, in the procedure of Roe and Oesterling (1944) for the measurement of the total apparent ascorbic acid, not all of the 2,3-diketogulonic acid of the strawberries formed an osazone with the 2,4-dinitrophenylhydrazine. If this were true, then the values for total ascorbic acid obtained by difference of the 2,3-diketogulonic acid and the total apparent ascorbic acid also would be erroneous. The experimental pattern of this study does not provide adequate basis for evaluating either of these hypotheses. However, values for reduced ascorbic acid and dehydroascorbic acid were relatively constant at the end of the first, second and third months of frozen storage, and, therefore, there appears to be support for the second hypothesis, since if other unidentified substances had contributed to the measurement of to-

tal apparent ascorbic acid, it would seem that these substances too might have affected the determination of dehydroascorbic acid.

The data were analyzed statistically in an attempt to ascertain whether any relationships may have existed among the substances which were determined in this study. Correlation coefficients were calculated for the various components and are given in Tables VII and VIII for strawberries of the Catskill and Robinson varieties, respectively. There was a significant relationship between the total apparent ascorbic acid and total ascorbic acid of the berries of the Robinson variety ($P \leq 0.05$); however, this relationship was not significant for berries of the Catskill variety. A negative relationship existed between the reduced ascorbic acid and dehydroascorbic acid of strawberries of the Robinson variety during the period of frozen storage ($P \leq 0.05$); the relationship between reduced ascorbic acid and dehydroascorbic acid of strawberries of the Catskill variety was not significant, although in these berries, too, the values varied inversely, as indicated by a negative correlation coefficient of 0.408. This supports the hypothesis that some of the reduced ascorbic acid may have been oxidized to dehydroascorbic acid. The reduction in dehydroascorbic acid in the last few months of storage (Figures 1 and 2) suggest that there was further oxidation of the dehydroascorbic acid.

TABLE VII

. CORRELATION COEFFICIENTS OF THE VARIOUS COMPONENTS OF
STRAWBERRIES (CATSKILL VARIETY) DURING FROZEN STORAGE

Components						Correlation Coefficient
Apparent total ascorbic acid & reduced ascorbic acid						-0.005
"	"	"	"	" dehydro	"	0.309
"	"	"	"	" diketogulonic	"	0.314
"	"	"	"	" total ascorbic	"	0.328
"	"	"	"	" reducing sugar		0.133
"	"	"	"	" total solids		-0.183
Reduced		"	"	" dehydroascorbic acid		-0.408
"		"	"	" diketogulonic	"	-0.116
"		"	"	" total ascorbic	"	0.106
"		"	"	" reducing sugar		0.728*
"		"	"	" total solids		0.598
Dehydroascorbic		"	"	" diketogulonic acid		-0.051
"		"	"	" total ascorbic."		0.256
"		"	"	" reducing sugar		-0.105
"		"	"	" total solids		-0.468
Diketogulonic		"	"	" total ascorbic acid		-0.795**
"		"	"	" reducing sugar		0.050
"		"	"	" total solids		0.129
Total ascorbic		"	"	" reducing sugar		0.033
"	"	"	"	" total solids		-0.247
Reducing sugar		"	"	" total solids		0.470

* $p \leq 0.05$; significant.

** $p \leq 0.01$; highly significant.

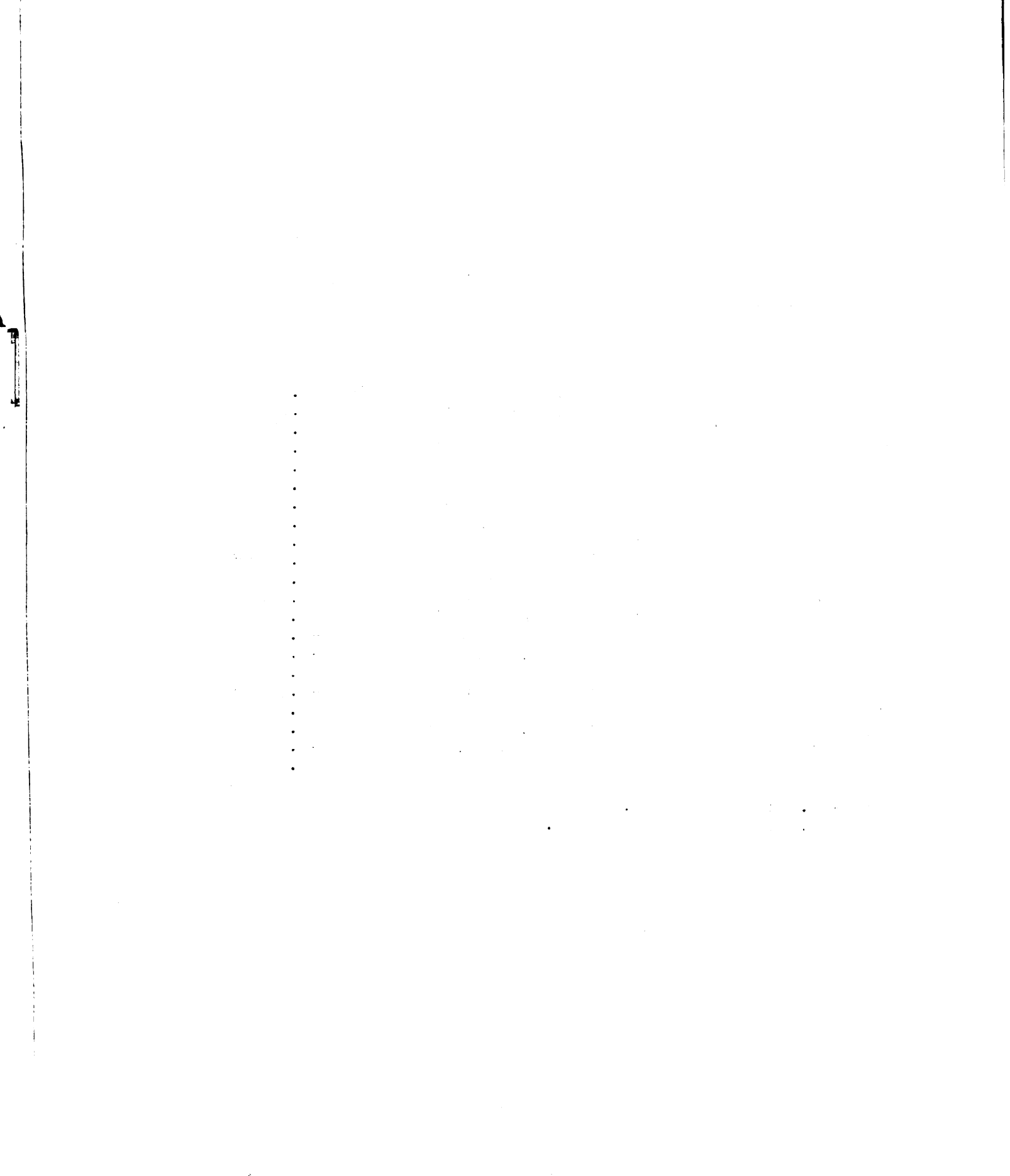
TABLE VIII

CORRELATION COEFFICIENTS OF THE VARIOUS COMPONENTS OF
STRAWBERRIES (ROBINSON VARIETY) DURING FROZEN STORAGE

Components						Correlation Coefficient
Apparent total ascorbic acid & reduced ascorbic acid						0.589
"	"	"	"	" dehydro	"	-0.050
"	"	"	"	" diketogulonic	"	-0.322
"	"	"	"	" total ascorbic	"	0.798*
"	"	"	"	" reducing sugar		0.558
"	"	"	"	" total solids		0.282
Reduced		"	"	" dehydroascorbic acid		-0.763*
"		"	"	" diketogulonic	"	-0.166
"		"	"	" total ascorbic	"	0.465
"		"	"	" reducing sugar		0.920**
"		"	"	" total solids		0.221
Dehydroascorbic		"	"	" diketogulonic acid		0.082
"		"	"	" total ascorbic	"	0.089
"		"	"	" reducing sugar		-0.600
"		"	"	" total solids		-0.275
Diketogulonic		"	"	" total ascorbic acid		-0.822*
"		"	"	" reducing sugar		-0.964**
"		"	"	" total solids		0.526
Total ascorbic		"	"	" reducing sugar		0.302
"	"	"	"	" total solids		-0.161
Reducing sugar		"	"	" total solids		0.200

* $p \leq 0.05$; significant.

** $p \leq 0.01$; highly significant.



There was a significantly inverse relationship between 2,3-diketogulonic acid and total ascorbic acid in strawberries of both varieties. It also is apparent from Figures 1 and 2 that a decrease in total ascorbic acid appeared to occur simultaneously with an increase in 2,3-diketogulonic acid in the strawberries. This supports the hypothesis that ascorbic acid may be oxidized to 2,3-diketogulonic acid during the storage of frozen fruits. However, as indicated earlier, there is not a simple explanation of the observation that a decrease in total ascorbic acid at certain intervals of frozen storage paralleled an increase in 2,3-diketogulonic acid concentration, since it has been reported that 2,3-diketogulonic acid is an irreversible oxidation product of dehydroascorbic acid.

The inverse relationship between diketogulonic acid and reducing sugars was highly significant for strawberries of the Robinson variety ($r = -0.964$). However, this inverse relationship did not exist for strawberries of the Catskill variety during frozen storage. The high values of 2,3-diketogulonic acid during the last three months of frozen storage of the strawberries of the Robinson variety probably accounted for the significantly inverse relationship between 2,3-diketogulonic acid and reducing sugar. Possibly reducing sugar as well as total ascorbic acid may have been oxidized to 2,3-diketogulonic acid during the last three months

of frozen storage of strawberries of the Robinson variety.

Reducing sugar was correlated positively with reduced ascorbic acid during the period of frozen storage for strawberries of both varieties. This does not support the hypothesis that biosynthesis of ascorbic acid may occur from six-carbon sugar molecules during frozen storage. However, the increase in reduced ascorbic acid in the frozen strawberries of the Robinson variety after the fourth month of storage and in the strawberries of the Catskill variety from the fourth to the sixth month of storage and again after the seventh month of storage does indicate that biosynthesis of ascorbic acid may have occurred during frozen storage or that dehydroascorbic acid was reduced to the reduced form of ascorbic acid.

It is of interest that a greater number of relationships existed among the constituents of the frozen strawberries of the Robinson variety than of the strawberries of the Catskill variety. However, the calculations of the correlation coefficients of the constituents of the strawberries of the Robinson variety were based only on six months of frozen storage, whereas the calculations of the correlation coefficients of the strawberries of the Catskill variety were based on ten months of frozen storage. Comparison of the graphs which were presented in Figures 1 and 2 indicates that the changes in the components of strawberries of the

Catskill variety paralleled to a considerable extent the changes in components of the strawberries of the Robinson variety during the first six months of frozen storage, but that further changes during the additional four months of frozen storage were less interdependent among the components of the strawberries than during the first six months of storage.

The data presented here support the reports of Bedford and McGregor (1948), Dodds, Price and Moore (1948), Wolfe, et al. (1949) and Mills, et al. (1949) that the concentration of total ascorbic acid was decreased during frozen storage. The data are not in accord with the findings of Paul et al. (1949) that an increase in total ascorbic acid concentration of strawberries occurred after three months of frozen storage, since the chemical method used by Paul et al. (1949) was the Roe and Oesterling (1944) procedure which measured the ascorbic acid and ascorbic acid-like compounds of strawberries reported in Tables I and II of this study as total apparent ascorbic acid. It was found in this study, however, that the loss of reduced ascorbic acid of the strawberries during frozen storage was much greater than the loss of total apparent ascorbic acid or of total ascorbic acid. Moreover, there did not appear to be a constant relationship between total ascorbic acid and the combined fractions of re-

duced ascorbic acid determined by the 2,6-dichlorophenol-indophenol method and dehydroascorbic acid. It would appear therefore that there is need for further study of the substances which may interfere with the methods for the determination of ascorbic acid and for the development of procedures which may eliminate this interference.

Physiological Utilization of the Total Apparent Ascorbic Acid of Frozen Strawberries

Description of the subjects. A physical description of the six subjects is presented in Table IX. The ages of the subjects ranged from 24 to 37 years and the weights ranged from 47.8 to 61.9 kilograms. The heights of these subjects varied from 152.5 to 173.5 centimeters.

The average daily intake of ascorbic acid for each subject on her customary diet was calculated from a seven-day dietary record kept by the subject between the periods of administration of the test doses of strawberries frozen 30 hours and strawberries frozen for four months. This represented the period between July and November, 1952. The mean range of ascorbic acid intake by the subjects was from 51 to 118 milligrams per day with an average of 96 milligrams, as shown in Table IX. All of the subjects but one, M. M., had mean daily intakes of ascorbic acid which exceeded 70 milligrams per day, the daily allowance recommended for young women by the Food and Nutrition Board of the National Research Coun-

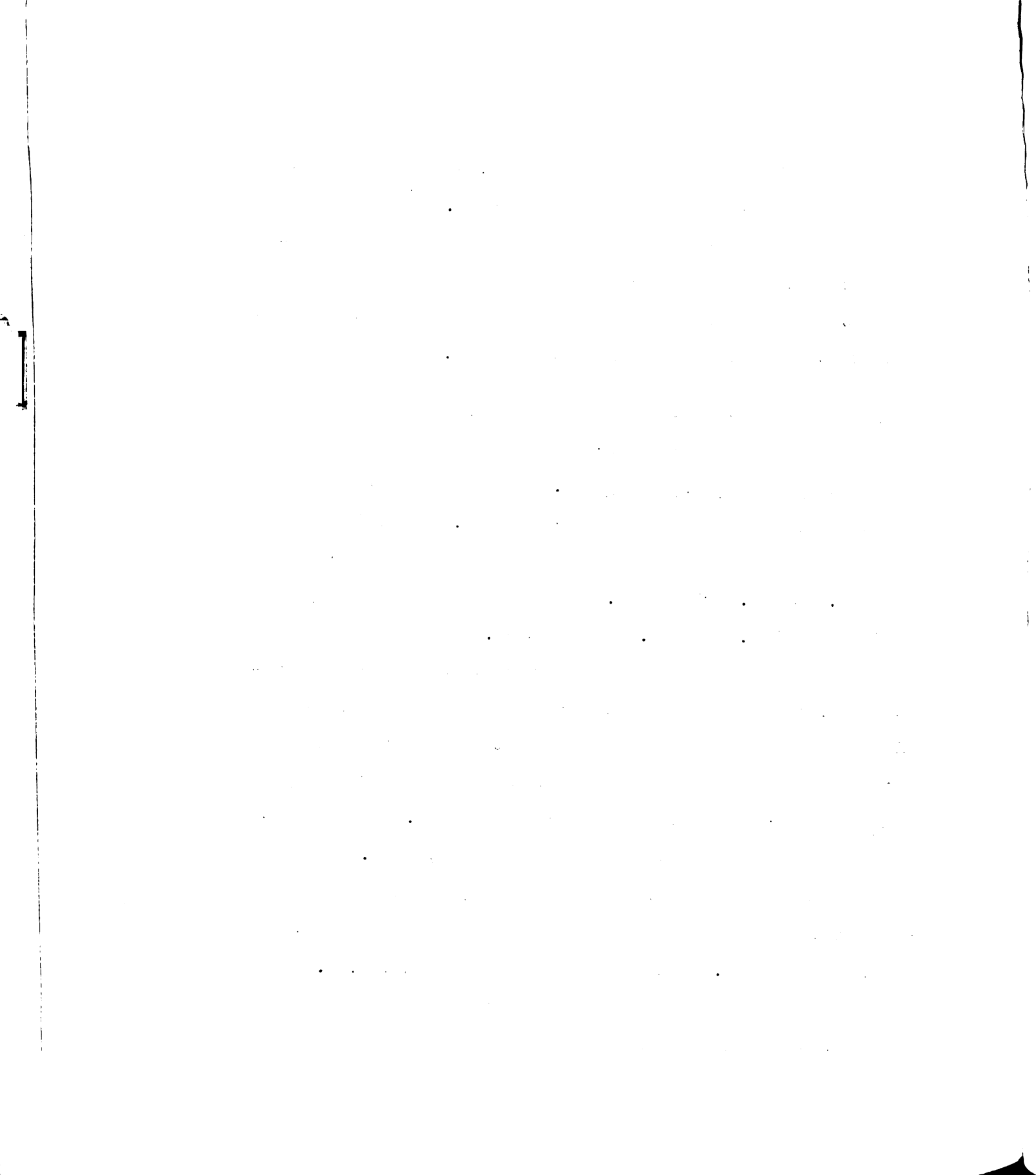


TABLE IX
DESCRIPTION OF SUBJECTS

Subject	Age	Weight	Height	Dietary Intake of Ascorbic Acid*	
				mean mg./day	range mg./day
H. A.	26	47.8	157.5	118	78-140
A. W.	24	57.8	159.5	118	90-125
W. B.	37	59.1	165.2	104	82-112
B. H.	32	61.9	173.5	103	94-120
M. M.	25	53.4	162.5	51	30-85
D. K.	26	49.0	152.5	80	68-105

*Based upon seven-day dietary record.

cil (Shank, 1954). Subject M. M. had an average daily intake of 51 milligrams of ascorbic acid with a range of 30 to 85 milligrams per day.

Serum concentration and urinary excretion of ascorbic acid before test dose. Reduced and total apparent ascorbic acid concentrations of the blood serum of six subjects before the ingestion of each of the test doses are presented in Table X and Figure 3. Each value represents the average of results obtained from tests performed on two consecutive days. The mean serum reduced ascorbic acid concentrations before the respective test doses were 1.21 ± 0.05 , 1.21 ± 0.05 , 0.89 ± 0.03 , and 1.00 ± 0.08 milligrams per 100 milliliters. The corresponding mean concentrations of total apparent ascorbic acid in the serum were 1.38 ± 0.07 , 1.51 ± 0.06 , 1.62 ± 0.07 , and 1.42 ± 0.06 milligrams per 100 milliliters. Statistical treatment of the data by analysis of variance indicated that there was a significant difference among the basal ascorbic acid values before the administration of the different test doses (Table XI). Application of the Fisher's 't'-test indicated that the concentrations of reduced ascorbic acid in the serum before the test dose of 200 grams strawberries, frozen for 30 hours, and before the test dose of crystalline ascorbic acid were significantly higher than before the test doses of charcoal-treated orange juice and of 250 grams strawberries, frozen for four months.

TABLE X

THE REDUCED AND TOTAL APPARENT ASCORBIC ACID OF THE BLOOD
SERUM AND THE URINARY EXCRETION OF ASCORBIC ACID OF THE
SUBJECTS PRECEDING THE ADMINISTRATION OF THE TEST
DOSES* FOR THE SUCCESSIVE EXPERIMENTAL PERIODS

Sub- ject	Test I			Test II		
	Serum		Urine	Serum		Urine
	Reduced ascorbic acid	Total apparent ascorbic acid	Reduced ascorbic acid	Reduced ascorbic acid	Total apparent ascorbic acid	Reduced ascorbic acid
	mg./100 ml.		mg./hr.	mg./100 ml.		mg./hr.
H. A.-	1.28	1.61	0.93	1.10	1.33	0.97
A. W.	1.38	1.44	0.46	1.10	1.50	0.80
W. B.	1.09	1.23	0.80	1.37	1.61	2.22
B. H.	1.29	1.39	1.85	1.28	1.62	7.55
M. M.	1.06	1.14	1.40	1.12	1.33	1.58
D. K.	1.17	1.47	2.94	1.28	1.67	2.43
MEAN	1.21± 0.05	1.38± 0.07	1.40± 0.37	1.21± 0.05	1.51± 0.06	2.59± 1.02

*Test Dose: I--200 grams of strawberries frozen for 30 hours.

II--169 milligrams of crystalline reduced ascorbic acid.

III--169 milligrams of dehydroascorbic acid in the form of charcoal-treated orange juice.

IV--250 grams of strawberries, frozen and stored for four months.

TABLE X (CONT.)

Test III			Test IV		
Serum		Urine	Serum		Urine
Reduced ascorbic acid	Total apparent ascorbic acid	Reduced ascorbic acid	Reduced ascorbic acid	Total apparent ascorbic acid	Reduced ascorbic acid
mg./100 ml.		mg./hr.	mg./100 ml.		mg./hr.
0.87	1.82	1.50	0.96	1.36	0.64
1.02	1.45	1.95	1.00	1.41	1.83
0.93	1.43	1.30	0.98	1.60	1.18
0.82	1.77	2.43	0.95	1.57	0.74
0.83	1.49	2.52	1.00	1.19	1.10
0.85	1.76	1.35	1.11	1.41	0.69
0.89±	1.62±	1.84±	1.00±	1.42±	1.03±
0.03	0.07	0.22	0.08	0.06	0.18

Figure 3. Concentrations of blood ascorbic acid of six subjects before and after various test doses.

Test dose: I--200 grams of strawberries, frozen for 30 hours.

II--169 milligrams of crystalline reduced ascorbic acid.

III--169 milligrams of dehydroascorbic acid in the form of charcoal-treated orange juice.

IV--250 grams of strawberries, frozen and stored for four months.

□ REDUCED ASCORBIC ACID (BASAL) ▨ REDUCED ASCORBIC ACID (INCREMENT)
 ■ TOTAL APPARENT ASCORBIC ACID (BASAL) ▩ TOTAL APPARENT ASCORBIC ACID (INCREMENT)

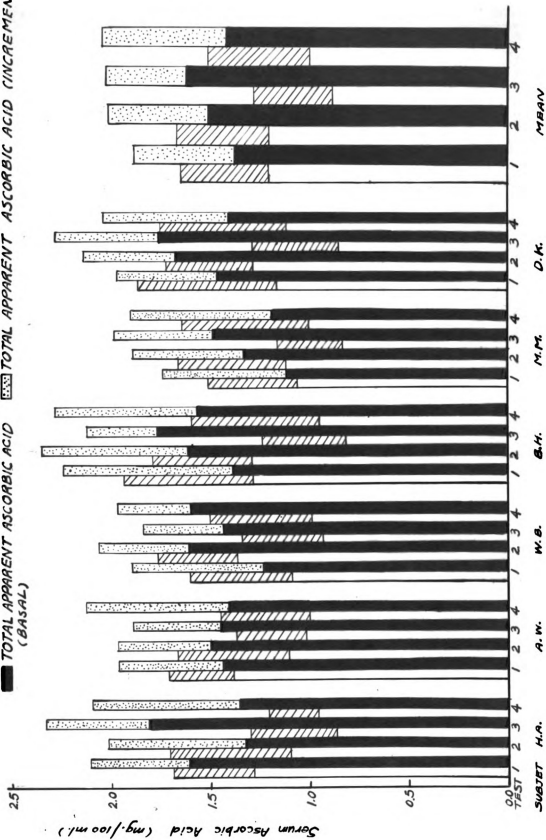


TABLE XI
ANALYSIS OF VARIANCE OF CONCENTRATIONS OF SERUM
ASCORBIC ACID PRECEDING THE ADMINISTRATION
OF VARIOUS TEST DOSES

Source of Variation	Degree of Freedom	F-value
Reduced Ascorbic Acid		
Test Doses	3	21.92**
Subjects	5	2.19
Error	15	
Total Apparent Ascorbic Acid		
Test Doses	3	3.53*
Subjects	5	2.53
Error	15	

* $p \leq 0.05$; significant

** $p \leq 0.01$; highly significant

The concentration of total apparent ascorbic acid in the serum was significantly higher before the test dose of charcoal-treated orange juice than before the test doses of strawberries. However, all individual serum values for reduced and total apparent ascorbic acid preceding the administration of the different test doses exceeded the value of 0.7 milligrams per 100 milliliters, which has been reported as a blood concentration that is indicative of a good state of ascorbic acid nutrition (Moyer, Harrison, Fisher, and Miller, 1948).

The urinary excretions of ascorbic acid before the different test doses were presented in Table X and have been plotted in Figure 4. The ranges of ascorbic acid excreted in the urine before the ingestion of the test doses of strawberries, frozen for 30 hours, crystalline reduced ascorbic acid, charcoal-treated orange juice, and strawberries, frozen for four months, were $0.46 - 2.94$, $0.80 - 7.55$, $1.30 - 2.52$, and $0.64 - 1.83$ milligrams per hour with averages of 1.40 ± 0.37 , 2.59 ± 1.02 , 1.84 ± 0.22 , and 1.03 ± 0.18 milligrams per hour, respectively. Statistical treatment of the data by analysis of variance indicated that there was no significant difference in the amounts of ascorbic acid which were excreted in the urine preceding the various test doses (Table XII).

Figure 4. Urinary excretions of reduced ascorbic acid by six subjects preceding and following the administration of various test doses.

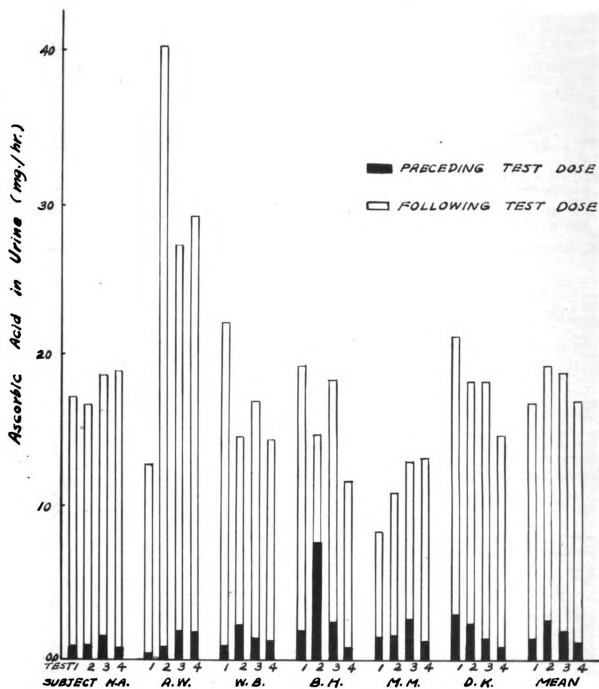


TABLE XII

ANALYSIS OF VARIANCE OF THE CONCENTRATION OF REDUCED
ASCORBIC ACID IN THE URINE OF SIX SUBJECTS PRECEDING
THE ADMINISTRATION OF VARIOUS TEST DOSES

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-value
Total	23	46.27		
Test Doses	3	8.13	2.71	1.53
Subjects	5	11.52	2.30	1.30
Error	15	26.62	1.77	

The concentration of reduced and total apparent ascorbic acid in the serum of the six subjects and the urinary excretions of reduced ascorbic acid by the subjects indicated that the subjects were in a good state of nutrition with respect to ascorbic acid preceding the administration of each of the test doses. It had been anticipated that the administration of 75 milligrams of crystalline ascorbic acid to each subject preceding the various tests might establish a uniform state of ascorbic acid nutrition for the subjects for all of the tests. Statistical differences in the serum concentrations of ascorbic acid preceding the different tests indicated that this was not achieved. Thus it would appear that the variations in dietary ascorbic acid of the subjects were great enough to affect the serum concentrations of ascorbic acid. The differences may have been partly seasonal in nature, since the mean concentrations of reduced ascorbic acid in the serum of the subjects preceding the first two tests were higher than the mean concentrations of reduced ascorbic acid in the serum of the subjects preceding the last two tests. The first two tests were conducted in July and August and the last two tests in September, October and November. Seasonal differences, if they existed, probably reflected the use of a greater amount of fresh fruits and vegetables in the summer months than in the early fall. Un-

fortunately, dietary records were not obtained preceding each test.

Ascorbic acid supplied in test doses. The total apparent ascorbic acid concentration of the test dose of 200 grams of strawberries, frozen for 30 hours, was the basis for selection of the amount of test doses of crystalline ascorbic acid, dehydroascorbic acid and strawberries, frozen for four months. The ascorbic acid content of the individual test doses of strawberries, frozen for 30 hours, is given in Table XIII. The total apparent ascorbic acid content of the test dose of strawberries, frozen for 30 hours, varied from 164.0 to 176.0 milligrams per subject. The mean concentration of total apparent ascorbic acid supplied by the strawberries, frozen for 30 hours, was 169 milligrams in the test dose of 200 grams. This represented the total apparent ascorbic acid of the strawberries, since diketogulonic acid, reductones and reductic acid were not determined separately.

The reduced ascorbic acid of the test dose of strawberries, frozen for 30 hours, ranged from 137.2 to 156.0 milligrams for the individual subjects. The mean ascorbic acid intake supplied by the test dose of strawberries, frozen for 30 hours, was 148 milligrams. This represented the concentration of reduced ascorbic acid measured by the oxidation-reduction method of Loeffler and Ponting (1942), using 2,6-dichlorophenolindophenol as an oxidizing reagent.

TABLE XIII

ASCORBIC ACID CONTENT OF INDIVIDUAL TEST DOSES OF 200 GRAMS
OF STRAWBERRIES, FROZEN FOR 30 HOURS

Nature of Ascorbic Acid	Subject						Mean
	H. A.	A. W.	W. B.	B. H.	M. M.	D. K.	
Reduced a. a., mg.	150.0	150.0	137.2	137.2	156.0	156.0	148
Total apparent a. a., mg.	166.0	166.0	176.0	176.0	164.0	164.0	169

Test doses of crystalline reduced ascorbic acid and dehydroascorbic acid were given in amounts equivalent to the average intake of total apparent ascorbic acid of the strawberries, frozen for 30 hours. The test dose of crystalline reduced ascorbic acid and dehydroascorbic acid each supplied 169 milligrams per subject.

The amount of strawberries, frozen for four months, which was given as a test dose was 250 grams. The increased amount of strawberries after frozen storage for four months in comparison with the amount of freshly frozen strawberries was given to compensate both for the reduction in concentration of biologically active forms of ascorbic acid and the increase in diketogulonic acid concentration in strawberries, frozen and stored for four months. It was considered desirable to adjust the test dose of strawberries for the increase in diketogulonic acid concentration, since it has been reported that diketogulonic acid does not possess the biological activity of reduced ascorbic acid (Borsook et al. 1937). The total apparent ascorbic acid and the reduced ascorbic acid of the individual test doses of strawberries after frozen storage for four months are given in Table XIV. The total apparent ascorbic acid of this test dose of strawberries varied from 173.6 to 192.3 milligrams per subject, with a mean of 183. The reduced ascorbic acid of the test dose of 250 grams of strawberries, frozen and stored for four

TABLE XIV

ASCORBIC ACID CONTENT OF INDIVIDUAL TEST DOSES OF 250 GRAMS
OF STRAWBERRIES, FROZEN FOR FOUR MONTHS

Nature of Ascorbic Acid	Subject						Mean
	H. A.	A. W.	W. B.	B. H.	M. M.	D. K.	
Reduced a. a., mg.	121.9	121.9	123.8	123.8	97.5	97.5	114
Total apparent a. a., mg.	181.9	181.9	192.3	192.3	173.6	173.6	183

months, ranged from 97.5 to 123.8 milligrams for the subjects with a mean concentration of 114 milligrams, as determined by the 2,6-dichlorophenolindophenol method.

Unfortunately, time did not permit analyses of dehydroascorbic acid, diketogulonic acid and reductones and reductive acid in the samples of strawberries fed to the subjects. Therefore these data were obtained from the analyses of the components of the frozen strawberries of the Catskill variety which were sampled monthly during storage and reported in Table I. The total apparent ascorbic acid of the strawberries sampled after 30 hours freezing was 80 milligrams per 100 grams. The average concentration of the strawberries fed in a test dose at this time was 84.5 milligrams of total apparent ascorbic acid per 100 grams. The total apparent ascorbic acid of the strawberries sampled after four months frozen storage was 72.5 milligrams per 100 grams of strawberries. This agreed closely with the average concentration of the total apparent ascorbic acid of the test doses of strawberries fed to the subjects, i. e., 73.2 milligrams per 100 grams. The similarity of values indicated that the sampling procedure was satisfactory and the author was justified in using the data for the various components reported in Table I for calculation of the concentrations of these compounds in the test dose of strawberries.

The concentrations of total apparent ascorbic acid, 2,3-diketogulonic acid, reductones and reductic acid, total ascorbic acid, dehydroascorbic acid, and reduced ascorbic acid of the test doses of strawberries are given in Table XV. The total apparent ascorbic acid contained in the test dose of strawberries, frozen for 30 hours, of crystalline reduced ascorbic acid, of charcoal-treated orange juice, and of strawberries, frozen and stored for four months, were 169, 169, 169, and 183 milligrams, respectively. The corresponding concentrations of total ascorbic acid which were computed by subtraction of the 2,3-diketogulonic acid from the total apparent ascorbic acid were 144, 169, 169, and 132 milligrams, respectively. The 2,3-diketogulonic acid was not present in the test dose of crystalline ascorbic acid and dehydroascorbic acid, but there were 25 milligrams of 2,3-diketogulonic acid in the 200 grams of strawberries which had been frozen for 30 hours and 51 milligrams of 2,3-diketogulonic acid in the test dose of strawberries which had been frozen and stored for four months. Reductones and reductic acid were absent from all of the test doses. No dehydroascorbic acid was present in the test dose of crystalline ascorbic acid, but there were 169 milligrams of dehydroascorbic acid in the charcoal-treated orange juice, 33 milligrams in the test dose of freshly frozen strawberries and 54 milligrams in the berries which were frozen and stored for four months. The

TABLE XV
THE CONCENTRATION OF ASCORBIC ACID AND RELATED COMPOUNDS
IN THE VARIOUS TEST DOSES

Source of Ascorbic Acid	Total Apparent Ascorbic Acid ¹ Col. 1	2,3-Diketo- gulonic Acid Col. 2
	mg.	mg.
Strawberries, Frozen 30 Hours	169	25
Crystalline Ascorbic Acid	169	
Charcoal-treated Orange Juice	169	
Strawberries, Frozen Four Months	183	51

¹Includes reduced ascorbic acid, dehydroascorbic acid, diketogulonic acid, and any other substances which may have formed osazones with 2,4-dinitrophenylhydrazine under the conditions of the method.

²Total apparent ascorbic acid corrected for diketogulonic acid, reductones and reductic acid Col. 1 - (Col. 2 + Col. 3) .

³Reduced ascorbic acid determined by a modification of the procedure of Roe and Oesterling (1944); Col. 4 - Col. 5 .

⁴Reduced ascorbic acid determined by 2,6-dichlorophenol-indophenol method.

TABLE XV (CONTINUED)

Reductones and Reductive Acid Col. 3	Total ² Ascorbic Acid Col. 4	Dehydro- ascorbic Acid Col. 5	Reduced ³ Ascorbic Acid	Reduced ⁴ Ascorbic Acid
mg.	mg.	mg.	mg.	mg.
0	144	33	111	148
	169		169	169
	169	169	0	0
0	132	54	78	114

reduced ascorbic acid values obtained by the subtraction of the dehydroascorbic acid and 2,3-diketogulonic acid from the total apparent ascorbic acid were 111, 169, 0, and 78 milligrams in the test dose of strawberries frozen for 30 hours, crystalline reduced ascorbic acid, charcoal-treated orange juice, and strawberries, frozen and stored for four months. The corresponding values for reduced ascorbic acid determined by the 2,6-dichlorophenolindophenol method were 148, 169, 0, and 114 milligrams, respectively. The reduced ascorbic acid values of the strawberries as determined by the 2,6-dichlorophenolindophenol method were 37 and 36 milligrams higher than the reduced ascorbic acid values of the two test doses of strawberries as determined by the 2,4-dinitrophenylhydrazine method.

The utilization of ascorbic acid of the various test doses. The series of tests in which (a) crystalline reduced ascorbic acid and (b) charcoal-treated orange juice, as a source of dehydroascorbic acid, were the test doses were included in the experiment to provide a basis for the interpretation of the data for blood and urinary ascorbic acid following the administration of the two test doses of strawberries. Therefore the data which were obtained when crystalline ascorbic acid and dehydroascorbic acid were the test doses are presented first, although this was not the order in which the tests were performed.

Table XVI gives the concentrations of reduced ascorbic acid of the blood serum of the six subjects at periodic intervals after the administration of the various test doses. The concentrations of reduced ascorbic acid in the blood serum of the six subjects before the administration of the test dose, although given previously in Table X, are included again in this table so that the increments in serum ascorbic acid resulting from the test doses can be estimated. The concentrations of reduced ascorbic acid of the blood serum of the six subjects preceding and at periodic intervals following the administration of the various test doses also are shown graphically in Figure 5.

Similar data for the concentration of total apparent ascorbic acid of the blood serum of the subjects before and at periodic intervals after the administration of the test doses are given in Table XVII and shown graphically in Figure 6. Each series of tests included the measurement of reduced and total apparent ascorbic acid in the blood serum before and after the administration of the test dose. There was not a statistical difference between the two daily replications of each test; therefore the blood values for ascorbic acid obtained on the two successive days were averaged for each subject and the values given in Tables XVI and XVII are the averages of the two successive daily tests for each subject.

TABLE XVI

REDUCED ASCORBIC ACID CONCENTRATIONS OF BLOOD SERUM OF SIX
SUBJECTS PRECEDING AND AT PERIODIC INTERVALS AFTER
THE ADMINISTRATION OF VARIOUS TEST DOSES

Test Dose	Time After Ingestion of Test Dose		
		H. A.	A. W.
	hrs.		
169 mg. Reduced Ascorbic Acid	0	1.10	1.10
	$\frac{1}{2}$	1.58	1.47
	1	1.69	1.62
	2	1.71	1.66
	3	1.60	1.58
	4	1.59	1.50
169 mg. Dehydroascorbic Acid	0	0.87	1.02
	1	1.12	1.21
	2	1.30	1.37
	3	1.24	1.31
	4	1.16	1.16
	5	1.13	1.11
200 grams Strawberries, Frozen 30 Hours	0	1.28	1.38
	1	1.61	1.60
	2	1.68	1.71
	3	1.69	1.64
	4	1.66	1.57
	5	1.60	1.55
250 grams Strawberries, Frozen for Four Months	0	0.96	1.00
	1	1.24	1.35
	2	1.42	1.45
	3	1.42	1.39
	4	1.34	1.34
	5	1.28	1.32

*Each value represents the average of two consecutive days' tests.

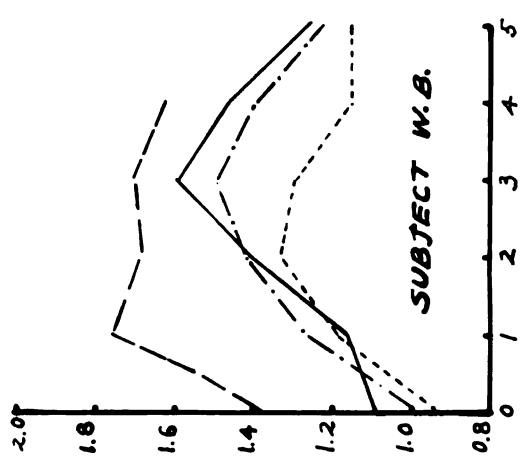
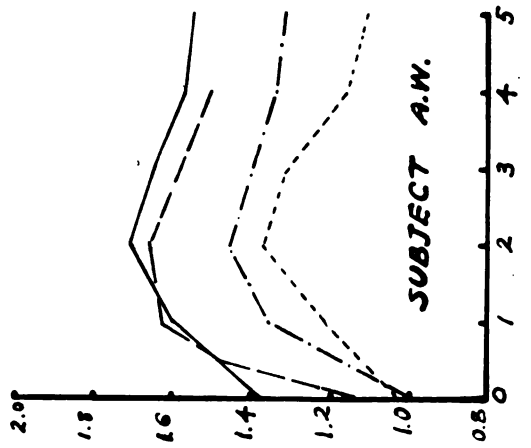
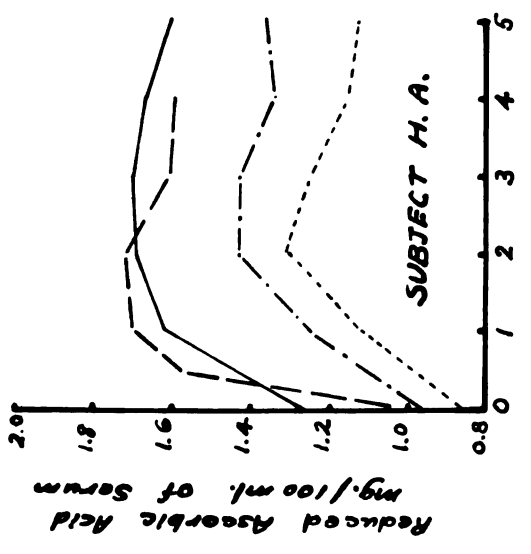
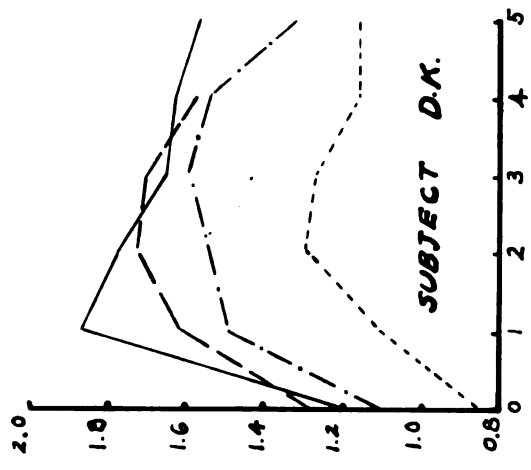
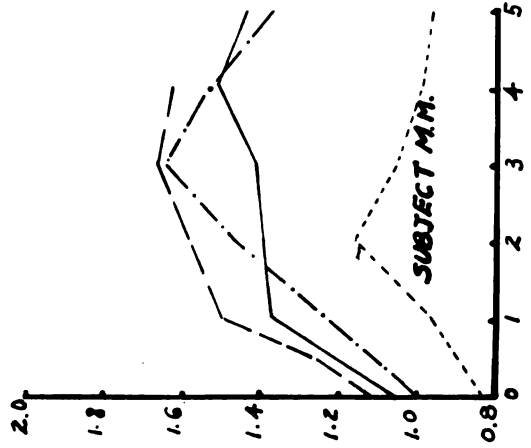
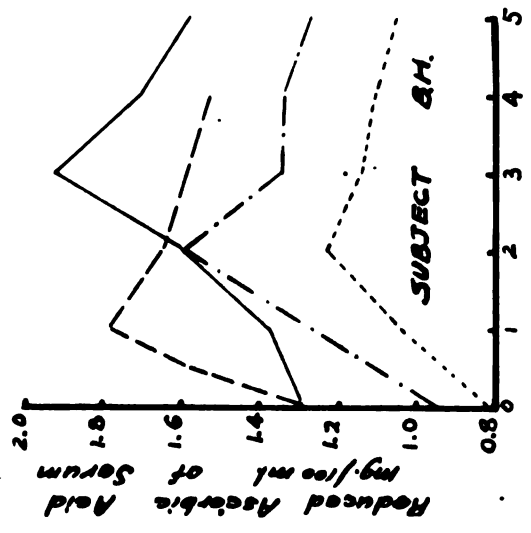
TABLE XVI (CONTINUED)

Reduced Ascorbic Acid* in Blood Serum mg./100 ml.				
Subject				Mean $\pm$ S. E.
W. B.	B. H.	M. M.	D. K.	
1.37	1.28	1.12	1.28	1.21 $\pm$ 0.05
1.54	1.59	1.26	1.44	1.48 $\pm$ 0.05
1.76	1.79	1.49	1.61	1.66 $\pm$ 0.05
1.69	1.65	1.58	1.72	1.67 $\pm$ 0.02
1.71	1.59	1.66	1.70	1.64 $\pm$ 0.02
1.63	1.53	1.62	1.58	1.58 $\pm$ 0.08
0.93	0.82	0.83	0.85	0.89 $\pm$ 0.03
1.19	1.04	0.96	1.11	1.11 $\pm$ 0.04
1.34	1.23	1.16	1.29	1.28 $\pm$ 0.03
1.30	1.14	1.05	1.27	1.22 $\pm$ 0.04
1.16	1.10	0.99	1.16	1.12 $\pm$ 0.03
1.16	1.05	0.96	1.16	1.10 $\pm$ 0.03
1.09	1.29	1.06	1.17	1.21 $\pm$ 0.05
1.17	1.38	1.36	1.86	1.50 $\pm$ 0.10
1.41	1.60	1.39	1.77	1.59 $\pm$ 0.07
1.60	1.93	1.41	1.65	1.65 $\pm$ 0.07
1.47	1.70	1.51	1.63	1.59 $\pm$ 0.04
1.27	1.58	1.43	1.57	1.50 $\pm$ 0.05
0.98	0.95	1.00	1.11	1.00 $\pm$ 0.08
1.27	1.27	1.22	1.49	1.31 $\pm$ 0.04
1.42	1.59	1.46	1.75	1.52 $\pm$ 0.06
1.50	1.34	1.64	1.60	1.48 $\pm$ 0.05
1.40	1.34	1.53	1.54	1.42 $\pm$ 0.04
1.23	1.27	1.37	1.32	1.30 $\pm$ 0.03

Figure 5. Concentrations of reduced ascorbic acid of blood serum of six subjects before and at periodic intervals after the administration of various test doses.

Legend:

- After test dose of crystalline reduced ascorbic acid
- After test dose of dehydroascorbic acid
- After test dose of strawberries, frozen for 30 hours
- - - - - After test dose of strawberries, frozen for four months



Time in Hours After Test Dose

TABLE XVII

TOTAL APPARENT ASCORBIC ACID CONCENTRATIONS OF BLOOD SERUM
OF SIX SUBJECTS PRECEDING AND AT PERIODIC INTERVALS
AFTER THE ADMINISTRATION OF VARIOUS TEST DOSES

Test Dose	Time After Ingestion of Test Dose		
		H. A.	A. W.
	hrs.		
169 mg. Reduced Ascorbic Acid	0	1.33	1.50
	$\frac{1}{2}$	1.73	1.64
	1	1.92	1.80
	2	2.02	1.97
	3	1.82	1.78
	4	1.74	1.75
169 mg. Dehydroascorbic Acid	0	1.82	1.45
	1	2.05	1.79
	2	2.33	1.88
	3	2.25	1.88
	4	2.16	1.65
	5	1.95	1.64
200 grams Strawberries, Frozen 30 Hours	0	1.61	1.44
	1	1.97	1.89
	2	2.11	1.96
	3	1.82	1.78
	4	1.86	1.71
	5	1.81	1.58
250 grams Strawberries, Frozen for Four Months	0	1.36	1.41
	1	1.79	1.85
	2	2.10	2.12
	3	1.74	1.93
	4	1.62	1.70
	5	1.61	1.61

*Each value represents the average of two consecutive days' tests.

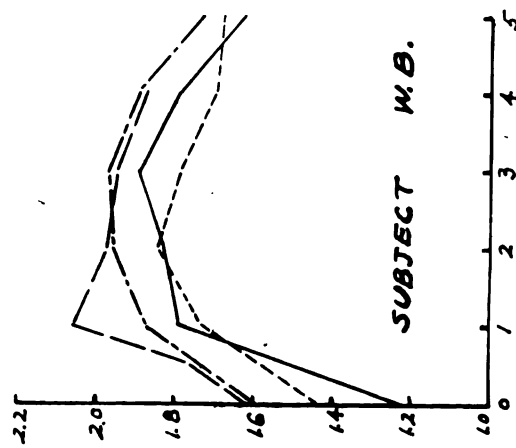
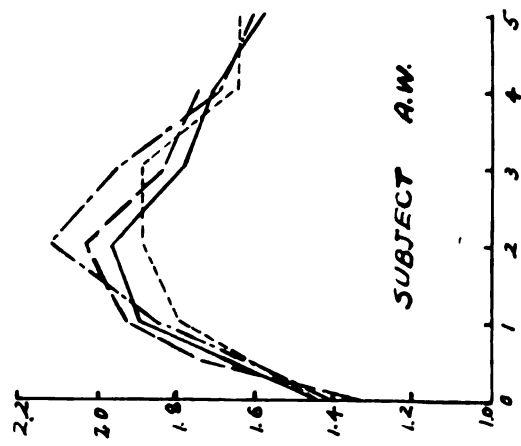
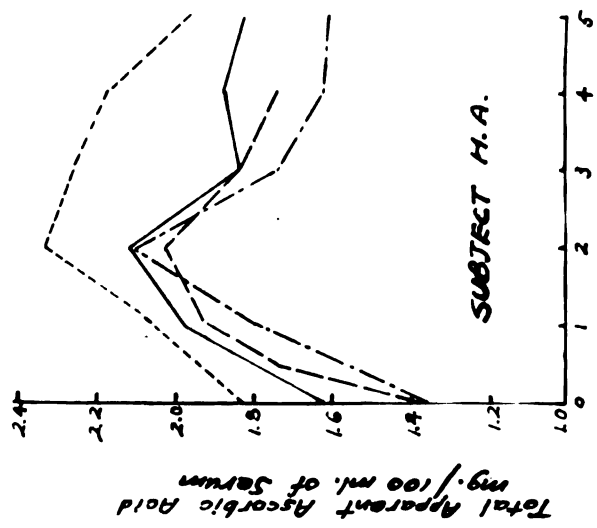
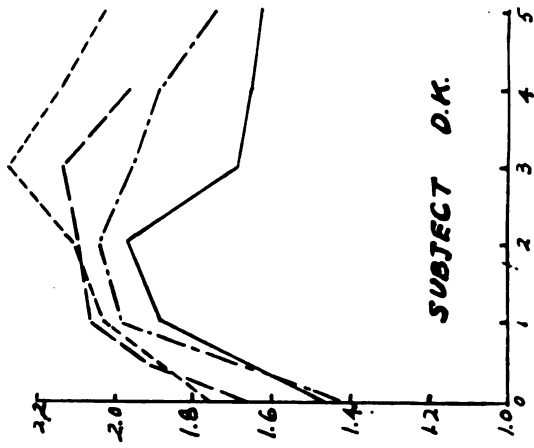
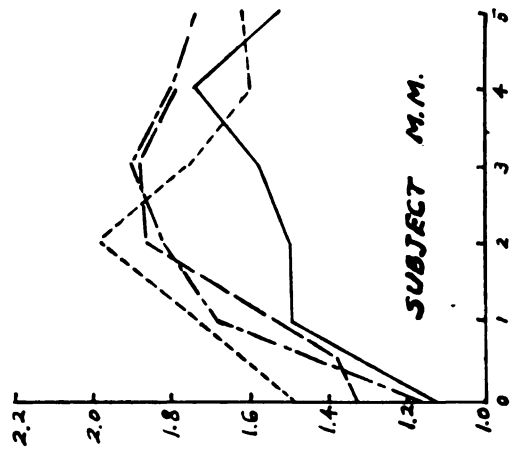
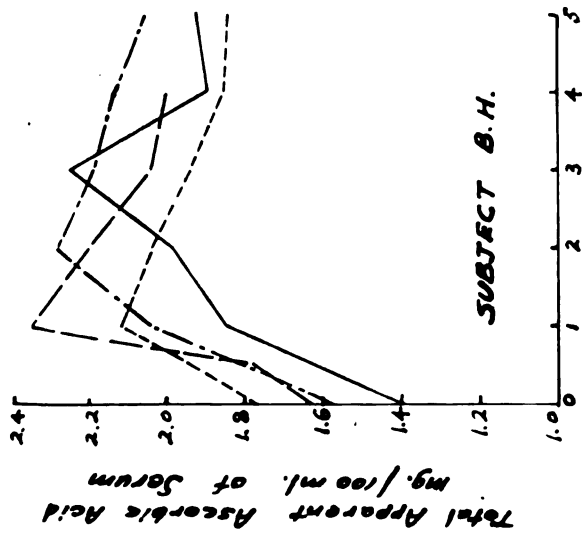
TABLE XVII (CONTINUED)

Total Apparent Ascorbic Acid* in Blood Serum mg./100 ml.				
Subject				Mean $\pm$ S. E.
W. B.	B. H.	M. M.	D. K.	
1.61	1.62	1.33	1.67	1.51 $\pm$ 0.06
1.76	1.77	1.38	1.92	1.70 $\pm$ 0.07
2.06	2.35	1.54	2.06	1.96 $\pm$ 0.11
1.98	2.20	1.86	2.10	2.02 $\pm$ 0.05
1.95	2.04	1.88	2.14	1.94 $\pm$ 0.06
1.88	2.00	1.78	1.97	1.85 $\pm$ 0.05
1.43	1.77	1.49	1.76	1.62 $\pm$ 0.07
1.73	2.12	1.73	2.03	1.91 $\pm$ 0.07
1.84	2.03	1.98	2.11	2.03 $\pm$ 0.07
1.79	1.94	1.76	2.28	1.98 $\pm$ 0.09
1.70	1.85	1.60	2.15	1.85 $\pm$ 0.10
1.68	1.84	1.62	2.03	1.79 $\pm$ 0.07
1.23	1.39	1.14	1.47	1.38 $\pm$ 0.07
1.79	1.84	1.49	1.88	1.81 $\pm$ 0.07
1.83	1.98	1.50	1.97	1.89 $\pm$ 0.08
1.89	2.24	1.58	1.69	1.84 $\pm$ 0.10
1.79	1.89	1.74	1.66	1.77 $\pm$ 0.04
1.62	1.92	1.52	1.63	1.68 $\pm$ 0.06
1.60	1.57	1.19	1.41	1.42 $\pm$ 0.06
1.87	2.03	1.68	1.98	1.87 $\pm$ 0.05
1.96	2.28	1.82	2.04	2.05 $\pm$ 0.06
1.97	2.19	1.90	1.96	1.95 $\pm$ 0.06
1.89	2.13	1.80	1.89	1.84 $\pm$ 0.08
1.73	2.05	1.74	1.75	1.75 $\pm$ 0.07

Figure 6. Concentrations of total apparent ascorbic acid of blood serum of six subjects before and at periodic intervals after the administration of various test doses.

Legend:

- After test dose of crystalline ascorbic acid
- After test dose of dehydroascorbic acid
- After test dose of strawberries, frozen for 30 hours
- .-.-.-. After test dose of strawberries, frozen for four months



Time in Hours After Test Dose

The greatest concentration of reduced and total apparent ascorbic acid in the blood serum of the subjects after the administration of the various test doses was used as a basis for the calculation of the highest increment in blood serum ascorbic acid for each subject following the ingestion of the test doses. The calculated increments of reduced ascorbic acid of the blood serum of the six subjects after the administration of the various test doses are given in Table XVIII and similar data for the total apparent ascorbic acid of the blood serum of the subjects are given in Table XIX.

The excretions of reduced ascorbic acid by the subjects before and after the administration of the test doses are given in Table XX. Values are expressed as milligrams of reduced ascorbic acid excreted in the urine per hour. The values for urinary excretions of ascorbic acid by the subjects also represent averages of two daily replications of each test.

Test Dose of Crystalline Reduced Ascorbic Acid

The test dose of crystalline reduced ascorbic acid was 169 milligrams. This was equivalent to the amount of total apparent ascorbic acid in the 200 grams of the strawberries which were frozen for 30 hours. The average concentrations of reduced ascorbic acid in the blood serum of the subjects at intervals of one-half, one, two, three and four hours after the test dose were 1.48 ± 0.05 , 1.66 ± 0.05 , $1.67 \pm$

TABLE XIX

CALCULATED INCREMENT OF TOTAL APPARENT ASCORBIC ACID OF
BLOOD SERUM OF SIX SUBJECTS AFTER ADMINISTRATION
OF VARIOUS TEST DOSES

Test Dose	Increment* of Serum Total Apparent Ascorbic Acid, mg./100 ml.						Mean $\pm$ S.E.
	Subject						
	H.A.	A.W.	W.B.	B.H.	M.M.	D.K.	
169 mg. Reduced Ascorbic Acid	0.69	0.47	0.45	0.73	0.55	0.47	0.56 $\pm$ 0.05
169 mg. Dehydro- ascorbic acid	0.51	0.43	0.41	0.35	0.49	0.52	0.45 $\pm$ 0.03
200 grams Strawberries, Frozen for 30 Hours	0.50	0.52	0.66	0.85	0.60	0.50	0.61 $\pm$ 0.06
250 grams Strawberries, Frozen for Four Months	0.74	0.71	0.37	0.71	0.71	0.63	0.65 $\pm$ 0.06

*Based on highest concentration of serum ascorbic acid
of the subject after the test dose.

TABLE XX

AVERAGE URINARY EXCRETIONS OF REDUCED ASCORBIC ACID
BEFORE AND AFTER ADMINISTRATION
OF THE VARIOUS TEST DOSES

Test Dose	Urinary Reduced Ascorbic					
	Sub-					
	H. A.		A. W.		W. B.	
	Before	After	Before	After	Before	After
169 mg. Reduced Ascorbic Acid	0.97	16.73	0.80	40.07	2.22	14.56
169 mg. Dehydroascorbic Acid	1.50	18.60	1.95	27.12	1.30	16.95
200 grams Strawberries, Frozen for 30 Hours	0.93	17.20	0.46	12.80	0.80	22.02
250 grams Strawberries, Frozen for Four Months	0.64	18.90	1.83	29.01	1.18	14.48

TABLE XX (CONT.)

Acid, mg./hr., Before and After Test Dose							
ject						Mean $\pm$ S. E.	
B. H.		M. M.		D. K.			
Before	After	Before	After	Before	After	Before	After
7.55	14.59	1.58	10.94	2.43	18.13	2.59 $\pm$ 1.02	19.17 $\pm$ 4.29
2.43	18.38	2.52	13.00	1.35	18.19	1.84 $\pm$ 0.22	18.71 $\pm$ 1.89
1.85	19.22	1.40	8.46	2.94	21.19	1.40 $\pm$ 0.37	16.82 $\pm$ 2.14
0.74	11.74	1.10	13.15	0.69	14.60	1.03 $\pm$ 0.18	16.98 $\pm$ 2.60

0.02, 1.64 ± 0.02 and 1.58 ± 0.08 milligrams per 100 milliliters of serum, respectively (Table XVI). The highest value for the mean concentration of reduced ascorbic acid in the blood serum occurred two hours after the ingestion of the test dose. The highest value for serum reduced ascorbic acid occurred at two hours after the ingestion of the test dose for three of the subjects, at one hour after the test dose was given for one subject, and not until three hours after the ingestion of the test dose for one subject, M. M. The greatest increase in concentration of reduced ascorbic acid in the blood serum ranged from 0.39 to 0.61 milligrams per 100 milliliters for the six subjects; the mean increment was 0.51 ± 0.03 milligrams per 100 milliliters (Table XVIII).

The mean concentrations of total apparent ascorbic acid in the blood serum of the subjects after the test dose of crystalline reduced ascorbic acid were 1.70 ± 0.07 , 1.96 ± 0.11 , 2.02 ± 0.05 , 1.94 ± 0.06 , and 1.85 ± 0.05 milligrams per 100 milliliters at time intervals of one-half, one, two, three and four hours, respectively (Table XVII). The highest value for the average concentration of total apparent ascorbic acid in the blood serum occurred two hours after the administration of the test dose, as was observed for serum reduced ascorbic acid. The highest values of total apparent ascorbic acid in the blood serum after the test doses were given ranged from 1.88 to 2.35 milligrams per 100

milliliters for the six subjects. These values corresponded to increments in serum total apparent ascorbic acid of 0.45 to 0.73 milligrams per 100 milliliters (Table XIX).

The urinary excretions of reduced ascorbic acid of the six subjects before the ingestion of the test dose of 169 milligrams of pure ascorbic acid ranged from 0.80 to 7.55 milligrams per hour. Two of the subjects had excretions of less than one milligram of reduced ascorbic acid per hour; three of the subjects had urinary excretions which ranged from 1.5 to 2.5 milligrams of reduced ascorbic acid per hour. Only one subject, B. H., excreted more than seven milligrams of reduced ascorbic acid per hour before the administration of the test dose. After the test dose of 169 milligrams of pure ascorbic acid was given, the urinary excretions of reduced ascorbic acid ranged from 10.94 to 40.07 milligrams per hour; the average urinary excretion was 19.17 $\pm$ 4.29 milligrams of reduced ascorbic acid per hour for the four-hour period that urinary collections were made. This represented an average increment of 16.58 milligrams per hour in the urinary excretion of ascorbic acid. One subject, A. W., had an unusually high urinary excretion of ascorbic acid during this period; the value for this subject was 40.07 milligrams per hour. This represented a total urinary excretion of 160 milligrams of reduced ascorbic acid during the four-hour period, or an amount almost equivalent

to the amount of test dose administered. Since the blood serum ascorbic acid values of this subject before and after the test dose of reduced ascorbic acid were comparable to those of the other subjects, it would seem that the chemical determination of urinary ascorbic acid may have measured other reducing substances in the urine of this subject, as well as reduced ascorbic acid.

Test Dose of Dehydroascorbic Acid

After a test dose of 169 milligrams dehydroascorbic acid, prepared from fresh orange juice treated with activated charcoal, was given to the subjects; the average values in milligrams per 100 milliliters of serum were 1.11 ± 0.04 , 1.28 ± 0.03 , 1.22 ± 0.04 , 1.12 ± 0.03 , and 1.10 ± 0.03 for reduced ascorbic acid and 1.91 ± 0.07 , 2.03 ± 0.07 , 1.98 ± 0.09 , 1.85 ± 0.10 and 1.79 ± 0.07 for total apparent ascorbic acid at time intervals of one, two, three, four and five hours respectively.

The maximum concentrations of reduced ascorbic acid in the blood serum of the six subjects ranged from 1.16 to 1.37 milligrams per 100 milliliters. Blood serum concentrations of reduced ascorbic acid were lower for subject M. M. than for the other subjects. The highest values of serum reduced ascorbic acid occurred two hours after the ingestion of the test dose.

The highest values of total apparent ascorbic acid in the blood serum of the six subjects ranged from 1.84 to 2.33 milligrams per 100 milliliters after the supplement of 169 milligrams of dehydroascorbic acid. Three subjects had serum values which exceeded two milligrams of total apparent ascorbic acid per 100 milliliters; the average of the highest values of total apparent ascorbic acid in the blood serum of the subjects after the test dose of 169 milligrams of dehydroascorbic acid was 2.03 ± 0.07 milligrams per 100 milliliters. The highest values for total apparent ascorbic acid in the serum of the subjects occurred at two hours after the test dose was given for four of the subjects, at one hour after the test dose for one subject, B. H., and at three hours after the test dose for one subject, D. H.

The urinary excretion of reduced ascorbic acid by the six subjects before the test dose of 169 milligrams of dehydroascorbic acid ranged from 1.30 to 2.52 milligrams per hour. The range of urinary excretions of reduced ascorbic acid after the test dose of 169 milligrams of dehydroascorbic acid was from 13.00 to 27.12 milligrams per hour; the average excretion was 18.71 ± 1.89 milligrams of reduced ascorbic acid per hour. This represented an average increase of 16.87 milligrams per hour above the basal urinary excretion of reduced ascorbic acid, comparable to the average increase in the urinary excretion of reduced ascorbic acid after the test dose of crystalline ascorbic acid. The urinary excre-

tion of reduced ascorbic acid by subject A. W. was 27.12 milligrams per hour, less than that after the test dose of pure ascorbic acid but greater than the urinary excretions of the other subjects.

Test Dose of Strawberries, Frozen for 30 Hours

The test dose of strawberries which had been frozen for 30 hours was two hundred grams. This amount of strawberries supplied 169 milligrams of total apparent ascorbic acid, 25 milligrams of 2,3-diketogulonic acid, 144 milligrams of total ascorbic acid, 33 milligrams of dehydroascorbic acid, and 148 milligrams of reduced ascorbic acid, as determined by the 2,6-dichlorophenolindophenol method (Table XV). Thus the test dose of strawberries, frozen for 30 hours, supplied an amount of total apparent ascorbic acid comparable to that of the test doses of reduced ascorbic acid and dehydroascorbic acid, but the amount of total ascorbic acid and of reduced ascorbic acid present in the test dose of strawberries was less than that of the test dose of pure reduced ascorbic acid or of the total ascorbic acid equivalent to the dehydroascorbic acid in the test dose of charcoal-treated orange juice.

The average concentrations of reduced ascorbic acid in the blood serum of the subjects at intervals of one, two, three, four and five hours after the test dose of strawberries, frozen for 30 hours, were 1.50 ± 0.10 , 1.59 ± 0.07 , 1.65 ± 0.07 , 1.59 ± 0.04 and 1.50 ± 0.05 milligrams per 100

milliliters, respectively. There was considerable variation in the pattern of the response of the subjects after the test dose of strawberries, frozen for 30 hours, as may be seen in the graphs shown in Figure 5. The highest value for serum reduced ascorbic acid after the test dose of strawberries occurred at one hour for subject D. K., and exceeded the peak concentrations of reduced ascorbic acid in the blood serum of this subject after the test doses of reduced ascorbic and of dehydroascorbic acid. The shape of the graph of the blood values of reduced ascorbic acid after the test dose of strawberries for subject A. W. corresponded closely to the shape of the graphs of the blood values of reduced ascorbic acid after the test doses of reduced ascorbic acid and dehydroascorbic acid. The highest values of reduced ascorbic acid in the blood serum of subjects B. H. and W. B. did not occur until the third hour after the test dose of strawberries was given, and the peak of concentration of reduced ascorbic acid in the blood serum of subject M. M. did not occur until the fourth hour after the test dose was given. This indicated that the absorption of the ascorbic acid of the strawberries did not occur as rapidly for these three subjects as the absorption of the ascorbic acid of the test doses of reduced ascorbic acid and dehydroascorbic acid. The increase in serum concentration of reduced ascorbic acid above the basal value after the test dose of strawberries

for subject H. A. was comparable to the increase in serum concentration of reduced ascorbic acid after the test dose of pure ascorbic acid; however, blood values of reduced ascorbic acid declined more rapidly for subject H. A. after the test dose of pure ascorbic acid than after the test dose of strawberries.

The average concentrations of total apparent ascorbic acid in the blood serum at intervals of one, two, three, four and five hours after the supplement of frozen strawberries were 1.81 ± 0.07 , 1.89 ± 0.08 , 1.84 ± 0.10 , 1.77 ± 0.04 , and 1.68 ± 0.06 milligrams per 100 milliliters. The range of the highest values of total apparent ascorbic acid of the blood serum of the six subjects was from 1.74 to 2.24 milligrams per 100 milliliters; this range was greater than that observed after the test doses of reduced ascorbic acid and dehydroascorbic acid. The maximum concentration of total apparent ascorbic acid in the blood serum of the subjects occurred at two hours after the test dose was given for subjects H.A., A. W., and D. K., at three hours after the test dose was given for subjects B. H. and W. B., and not until four hours after the test dose was given for subject M. M. The graphs which were presented in Figure 6 indicated that the pattern of response of blood values of total apparent ascorbic acid after the test dose of strawberries, frozen for 30 hours, was similar to that after the test dose of pure ascorbic acid for subjects H. A. and A. W., but differed both in the rate of ap-

pearance of the total apparent ascorbic acid in the blood serum and in the corresponding values of serum concentrations of total apparent ascorbic acid for the other subjects.

After the test dose of 200 grams of strawberries, frozen for 30 hours, the average urinary excretion of reduced ascorbic acid was 16.82 ± 2.14 milligrams per hour for a five-hour period. Two subjects, W. B. and D. K., excreted quantities greater than 20 milligrams per hour; subject M. M. excreted only 8.46 milligrams per hour. The urinary excretion of subject A. W. was 12.80 milligrams per hour, considerably less than that after the test doses of reduced ascorbic acid and of dehydroascorbic acid. The mean increment in the urinary excretion of ascorbic acid after the test dose of strawberries was 15.42 milligrams per hour above the basal excretion; this was slightly less than the mean increment after the test doses of reduced ascorbic acid and dehydroascorbic acid.

Test Dose of Strawberries Frozen and Stored for Four Months.

The test dose of strawberries, frozen for four months, was 250 grams. As was shown in Table XV, this test dose supplied 183 milligrams of total apparent ascorbic acid, 51 milligrams of 2,3-diketogulonic acid, 132 milligrams of total ascorbic acid, 54 milligrams of dehydroascorbic acid, and 114 milligrams of reduced ascorbic acid, as measured by the 2,6-dichlorophenolindophenol method. Thus the total ap-

parent ascorbic acid of the test dose of strawberries, frozen for four months, exceeded that of the other test doses. Although the amount of total ascorbic acid in the test dose of strawberries, frozen for four months, was 37 milligrams less than that of the test doses of reduced ascorbic acid and of dehydroascorbic acid, the difference between the total ascorbic acid of the test dose of strawberries, frozen for four months and that of the strawberries, frozen for 30 hours, was only 12 milligrams. Thus the test dose of strawberries, frozen for four months, supplied less total ascorbic acid than the other test doses, but a greater quantity of total apparent ascorbic acid. The amount of reduced ascorbic acid was less than in the other test doses also, with the exception of the test dose of dehydroascorbic acid.

The average concentrations of reduced ascorbic acid in the blood serum of the subjects after the test dose of strawberries, frozen for four months, were 1.31 ± 0.04 , 1.52 ± 0.06 , 1.48 ± 0.05 , 1.42 ± 0.04 and 1.30 ± 0.03 milligrams per 100 milliliters at time intervals of one, two, three, four and five hours, respectively. The highest values of reduced ascorbic acid in the blood serum of the subjects after the ingestion of the test dose occurred at two hours for subjects H. A., A. W., B. H. and D. K., and at three hours for subjects W. B. and M. M. The range of the highest values of reduced ascorbic acid in the blood serum of the Subjects was from 1.42 to 1.75 milligrams per 100 milliliters.

The average concentrations of total apparent ascorbic acid in the blood serum of the subjects after the test dose of strawberries, frozen for four months, were 1.87 ± 0.05 , 2.05 ± 0.06 , 1.95 ± 0.06 , 1.84 ± 0.08 , and 1.75 ± 0.07 milligrams per 100 milliliters at intervals of one, two, three, four and five hours after the test dose of strawberries was given. Peak concentrations of total apparent ascorbic acid in the blood serum of the subjects after the test dose of strawberries occurred at two hours for subjects H. A., A. W., B. H. and D. K., and at three hours for subjects W. B. and M. M. The highest values of total apparent ascorbic acid in the blood serum of the subjects after the test dose of strawberries, frozen for four months, ranged from 1.90 to 2.28 milligrams per 100 milliliters.

The average urinary excretion of reduced ascorbic acid after the test dose of strawberries, frozen for four months, was 16.98 ± 2.60 milligrams per hour. This represented an increment of 15.90 milligrams above the basal urinary excretion of reduced ascorbic acid. Again, the urinary excretion of reduced ascorbic acid was high for subject A. W.; the average value for the five-hour period was about 30 milligrams per hour, which represented a total excretion of 150 milligrams of reduced ascorbic acid in the five-hour period.

Comparison of the Various Test Doses

As indicated earlier, there was a significant difference in the concentrations of reduced ascorbic acid and of total apparent ascorbic acid in the blood serum of the six subjects preceding the administration of the various test doses. This difference was apparent in the graphs shown in Figures 5 and 6. There was only one subject, A. W., for whom the basal values of serum ascorbic acid were closely comparable preceding the four test doses, and this was only for the serum concentration of total apparent ascorbic acid. Therefore, the data for serum concentrations of reduced ascorbic acid and total apparent ascorbic acid after the various test doses were analyzed statistically by analysis of covariance in order that the influence of the basal concentration of ascorbic acid on the maximum concentration of ascorbic acid in the blood serum might be eliminated. The adjusted F-values which were obtained after the influence of the basal concentration of ascorbic acid in the blood serum on the maximum concentrations of reduced ascorbic acid and total apparent ascorbic acid had been removed are given in Table XXI. This analysis indicated that there was not a significant difference in the highest values of either reduced ascorbic acid in the blood serum or of total apparent ascorbic acid in the blood serum after the administration of the various test doses.

TABLE XXI

ANALYSIS OF VARIANCE OF THE MAXIMUM CONCENTRATIONS OF
 ASCORBIC ACID IN THE SERUM OF SIX SUBJECTS AFTER VARIOUS
 TEST DOSES: F-VALUES ADJUSTED BY ANALYSIS OF
 CO-VARIANCE TO REMOVE THE INFLUENCE OF BASAL
 BLOOD VALUES ON MAXIMUM CONCENTRATIONS

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F-value
Reduced Ascorbic Acid:				
Test Doses	3	0.08	0.27	1.04
Subjects	5	0.03	0.006	1.22
Error	14	0.36	0.26	
Total Ascorbic Acid:				
Test Doses	3	0.03	0.010	1.11
Subjects	5	0.14	0.028	3.11*
Error	14	0.12	0.009	

* $p \leq 0.05$; significant.

Interpretation of possible differences in the utilization of ascorbic acid after various test doses according to the pattern of this study also is dependent upon consideration of the rate of appearance and disappearance of ascorbic acid in the blood. The average concentrations of reduced ascorbic and of total apparent ascorbic acid in the blood serum of the six subjects at periodic intervals after each of the test doses have been plotted and are shown in Figure 7. Since differences among individuals were relatively great, each point of the graph must be considered to have a high standard error. There was a more rapid increase in the reduced ascorbic acid and total apparent ascorbic acid of the blood serum after the test dose of reduced ascorbic acid than after the other test doses. The pattern of response of total apparent ascorbic acid in the blood serum of the subjects was similar after all of the test doses. There was a greater range between the mean concentrations of total apparent ascorbic acid and reduced ascorbic acid in the blood serum of the subjects after the test dose of dehydroascorbic acid than after the other test doses. This would indicate that the dehydroascorbic acid was absorbed and measured in the blood serum largely as dehydroascorbic acid, although the increase in concentration of serum reduced ascorbic acid after the test dose of dehydroascorbic acid appeared to indicate that some reduction of dehydroascorbic acid to the

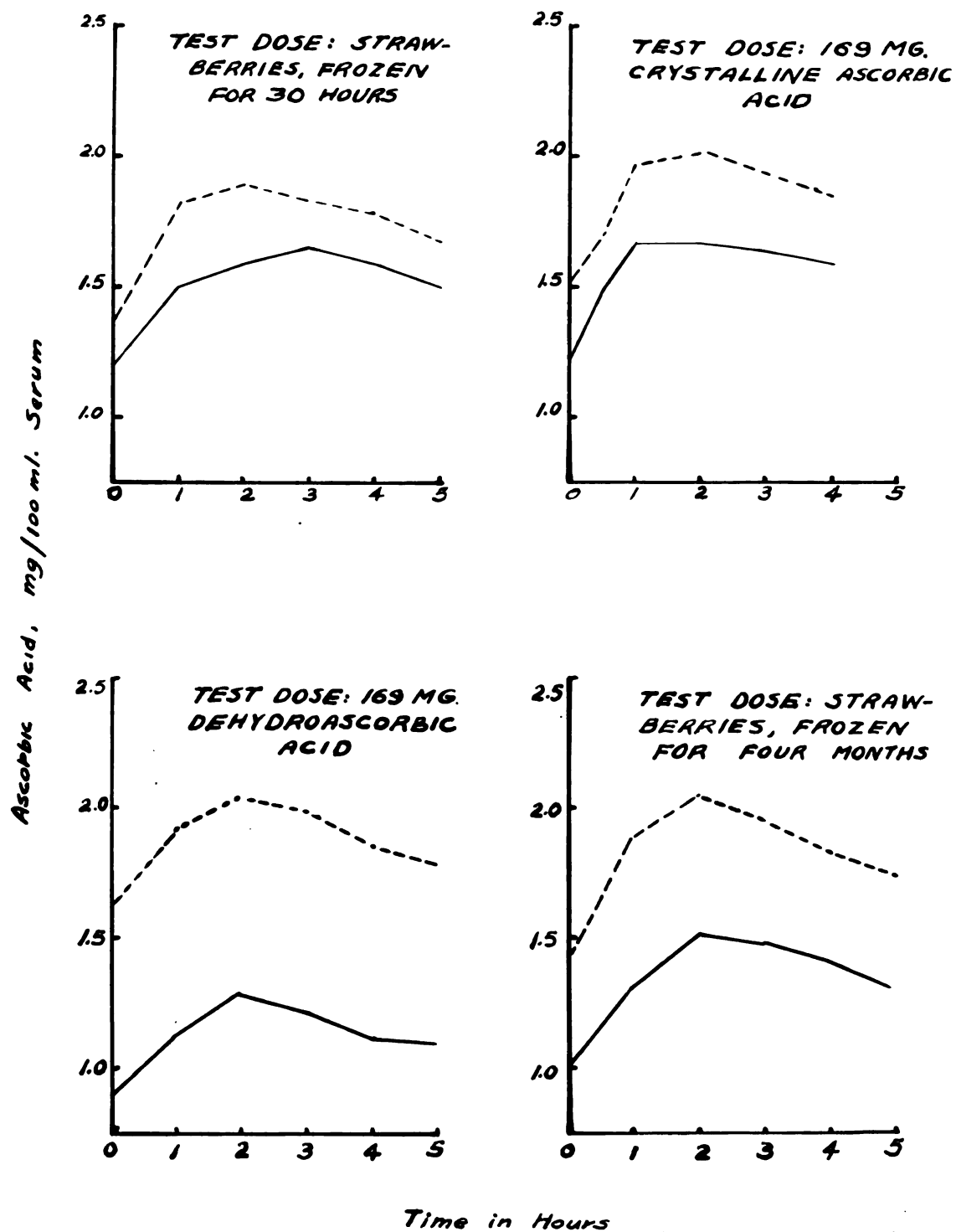
Figure 7. Mean concentrations of reduced ascorbic acid and total apparent ascorbic acid of six subjects before and at periodic intervals after various test doses.

Legend:

.....

Total apparent ascorbic acid

Reduced ascorbic acid



reduced form of ascorbic acid occurred within one hour after the administration of the test dose.

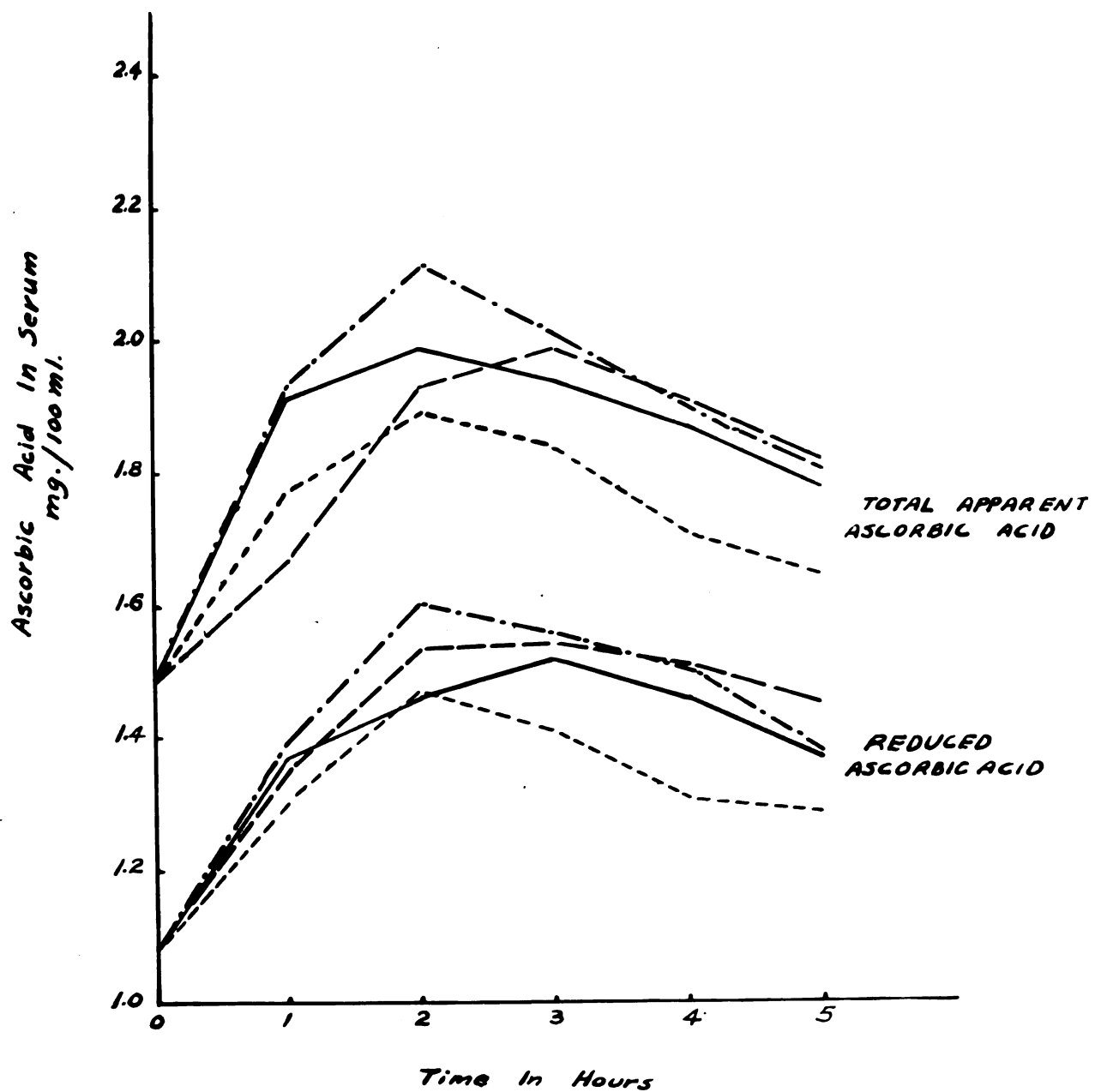
The range between the concentrations of total apparent ascorbic acid and of reduced ascorbic acid in the blood serum was greater after the test dose of strawberries, frozen for four months, than after the test dose of strawberries, frozen 30 hours. This probably reflected the higher concentration of dehydroascorbic acid in the test dose of strawberries, frozen for four months, than in the test dose of strawberries, frozen for 30 hours.

A further attempt was made to compare the blood values of the subjects at periodic intervals after the administration of the test dose by calculation of the average changes in the concentration of reduced ascorbic acid and in the concentration of total apparent ascorbic acid of the blood serum at intervals after the administration of the test doses. The mean increments and/or decrements, based upon the average fasting blood values of the four tests, have been plotted in Figure 8. This graph indicates that the concentrations of total apparent ascorbic acid and of reduced ascorbic acid in the blood serum of the subjects were greater after the test dose of strawberries, frozen for four months, than after the other test doses. The mean increments in total apparent ascorbic acid and reduced ascorbic acid after the test dose of dehydroascorbic acid were less than after the other test doses.

Figure 8. Mean successive changes in concentrations of reduced ascorbic acid and total apparent ascorbic acid of blood serum of six subjects after various test doses.

Legend:

- After test dose of crystalline reduced ascorbic acid
- After test dose of dehydroascorbic acid
- _____ After test dose of strawberries, frozen for 30 hours
- - - - - After test dose of strawberries, frozen for four months



The urinary excretions of reduced ascorbic acid after the four test doses were treated statistically by analysis of variance. Analysis of covariance was not used to remove the influence of the basal urinary excretions of reduced ascorbic acid, since there were not significant differences in the concentrations of reduced ascorbic acid of the urine of the subjects preceding the various test doses. The analysis of variance of the urinary excretions of reduced ascorbic acid after the test doses is given in Table XXII. Although there were significant differences in the urinary excretions of the six subjects, the analysis indicated that there were not significant differences in the mean concentrations of the urinary excretions of reduced ascorbic acid after the different test doses.

The data which have been presented indicate that the ascorbic acid of the test doses of strawberries, frozen 30 hours, and strawberries, frozen for four months was as well utilized as the test doses of crystalline reduced ascorbic acid and of dehydroascorbic acid. There was some indication that the test dose of dehydroascorbic acid was not as well utilized as the ascorbic acid of the other test doses, although there was considerable individual variation in the utilization of the test dose of dehydroascorbic acid.

The observation that the ascorbic acid of the test dose of strawberries which had been frozen and stored for four

TABLE XXII

ANALYSIS OF VARIANCE OF CONCENTRATION OF REDUCED
ASCORBIC ACID EXCRETED PER HOUR AFTER
DIFFERENT TEST DOSES

Source of Variance	Degree of Freedom	Sum of Squares	Mean Squares	F-value
Total	23	1,026.39		
Test doses	3	25.70	8.5	0.21
Subject	5	537.40	107.5	3.48*
Error	15	463.30	30.9	

* $P \leq 0.05$; significant.

months was as well utilized as the ascorbic acid of the other test doses projects the question of which components of the strawberries which had been frozen and stored for four months were the contributing factors to the concentrations of reduced ascorbic acid and total apparent ascorbic acid of the blood serum of the subjects. Although the total apparent ascorbic acid of the strawberries which had been frozen and held in storage was greater than that of the other test doses, this was true only because of the relatively higher concentration of 2,3-diketogulonic acid in the strawberries. Chemical analyses indicated that the reduced ascorbic acid concentration of the strawberries which had been held in frozen storage was 33 milligrams less than in the test dose of the freshly frozen strawberries, as measured by a modification of the Roe and Oesterling method (1944) and 34 milligrams less as measured by the 2,6-dichlorophenolindophenol. The reduced ascorbic acid concentration of the strawberries, which had been held in frozen storage, was 91 milligrams less than the test dose of crystalline reduced ascorbic acid by the method of Roe and Oesterling (1944) or 55 milligrams less than the test dose of crystalline reduced ascorbic acid by the method of Loeffler and Ponting (1942) for determination of reduced ascorbic acid with 2,6-dichlorophenolindophenol. In spite of these differences, the blood values of reduced ascorbic acid of the subjects after the

test dose of strawberries which had been held in frozen storage for four months were comparable to the blood values after test doses of freshly frozen strawberries or of the crystalline reduced ascorbic acid.

The total ascorbic acid of the strawberries which had been held in frozen storage for four months also was less than the total ascorbic acid of the other test doses. There were 12 milligrams less than in strawberries which were freshly frozen and 37 milligrams less than in the test doses of dehydroascorbic acid or of crystalline ascorbic acid. In spite of these differences, the concentrations of total apparent ascorbic acid of the blood serum of the subjects after the test dose of the strawberries which had been in frozen storage were comparable to the concentrations of total apparent ascorbic acid of the blood serum of the subjects after the other test doses.

It might be suggested that the measurement of the total apparent ascorbic acid of the blood serum of the subjects measured 2,3-diketogulonic acid in the blood, as well as reduced ascorbic acid and dehydroascorbic acid. In this case, the higher concentration of 2,3-diketogulonic acid in the test dose of the strawberries, frozen and stored for four months, than in the other test doses might be reflected in the measurement of the total apparent ascorbic acid of the blood serum of the subjects. However, this would not provide an ex-

planation of the fact that the reduced ascorbic acid concentrations of the serum of the subjects after the test dose of strawberries which had been in frozen storage were similar to the reduced ascorbic acid concentrations of the serum of the subjects after the other test doses. A more probable explanation appears to be that the method for study of utilization of ascorbic acid of test doses which was used in this investigation was not precise enough to demonstrate the relatively small differences in total ascorbic acid that existed among the test doses. The variations in patterns of response to the test doses among individuals would support this hypothesis.

The failure of the statistical analysis of the data on the maximum concentrations of ascorbic acid in the blood serum of the individuals after the various test doses to demonstrate significant differences either in the reduced ascorbic acid concentration of the blood serum or the concentration of total apparent ascorbic acid of the blood serum might be interpreted to indicate that the reduced ascorbic acid and the dehydroascorbic acid of the test doses were reversibly oxidized and reduced during the process of absorption and in the blood serum.

Todhunter, McMillan and Ehmke (1950) reported that blood did not contain dehydroascorbic acid. This is not in agreement with the results of this study, since values for

total apparent ascorbic acid of the subjects were uniformly greater than values for reduced ascorbic acid in the serum of the subjects. Moreover, the differences between values of reduced ascorbic acid and total apparent ascorbic acid of the blood serum were greater after the test doses of dehydroascorbic acid and of strawberries, which had been in frozen storage for four months and which contained a larger amount of dehydroascorbic acid than the freshly frozen strawberries or the test dose of crystalline ascorbic acid. Higher values for total ascorbic acid than for reduced ascorbic acid in the blood serum also have been reported by Borsook and co-workers (1937), Davey and co-workers (1952) and Linkswiler (1954). It is unfortunate that time did not permit the analysis of the dehydroascorbic acid and 2,3-diketogulonic acid of the blood serum of the subjects in this study. The relative concentrations of reduced ascorbic acid, dehydroascorbic acid, 2,3-diketogulonic acid of the blood after the administration of different sources of ascorbic acid (with different proportions of reduced ascorbic acid and dehydroascorbic acid in the test doses) is suggested for further study.

The pattern of this study differed from that reported by Einbecker et al. (1947; 1950), and direct comparisons of the experimental findings cannot be made. Einbecker et al. found that the increments in plasma ascorbic acid were com-

parable after test doses of crystalline ascorbic acid and frozen strawberries which supplied equivalent amounts of reduced ascorbic acid. In this study, the frozen strawberries supplied considerably less reduced ascorbic acid than the test dose of crystalline ascorbic acid, yet the plasma ascorbic acid curves were similar. It is possible that a greater amount of the ascorbic acid was in the form of dehydroascorbic acid in the strawberries used in this study than in the strawberries studied by Einbecker and co-workers.

Einbecker et al. (1947; 1950) found also that the increment in blood ascorbic acid concentration was less following a test dose of frozen strawberries than a test dose of crystalline ascorbic acid which was equivalent to the apparent total ascorbic acid of the strawberries. This was not the case in this study for a test dose of strawberries which had been frozen for 30 hours.

In this study, the test dose of strawberries which had been frozen and stored for four months contained an amount of total apparent ascorbic acid which was greater than the test dose of crystalline ascorbic acid. Since the plasma ascorbic acid concentrations of the subjects after the test dose of strawberries, frozen for four months, were comparable to those after a test dose of a smaller quantity of crystalline ascorbic acid, the results of the two studies, in this respect, do not appear to be in conflict.

The data reported in this study indicate that changes in the reduced ascorbic acid, dehydroascorbic acid and 2,3-diketogulonic acid concentrations occurred in strawberries throughout the period of frozen storage. Information on the ascorbic acid content of frozen strawberries based on the analysis of the total ascorbic acid content of the strawberries would appear to be more reliable than information based on analysis only of the reduced ascorbic acid concentration of the strawberries. Further study of substances which may form **osazones** with 2,4-dinitrophenylhydrazine in the determination of total ascorbic acid is needed.

SUMMARY AND CONCLUSION

Changes in the apparent ascorbic acid of strawberries during frozen storage were studied on strawberries of the Catskill and Robinson varieties. Fresh berries of the Catskill variety contained 83.0 and 81.6 milligrams of total apparent and reduced ascorbic acid, respectively, per 100 grams of strawberries; fresh berries of the Robinson variety contained 50.0 and 47.6 milligrams of total apparent ascorbic acid and reduced ascorbic acid, respectively, per 100 grams of strawberries.

Concentrations of total apparent ascorbic acid, reduced ascorbic acid, dehydroascorbic acid, 2,3-diketogulonic acid, reductones and reductic acid, reducing sugar and total solids were determined in strawberries which had been frozen for 30 hours and in strawberries during frozen storage at -27 to -30 degrees Centigrade. Analyses were made at monthly intervals for a period of ten months in strawberries of the Catskill variety and six months in strawberries of the Robinson variety. The concentrations of the various components in the strawberries which had been frozen for 30 hours were employed as base line to study the changes of these components during frozen storage. Strawberries of the Catskill variety which had been frozen for 30 hours contained

80.0 milligrams of total apparent ascorbic acid, 75.0 milligrams of reduced ascorbic acid, 16.3 milligrams of dehydroascorbic acid, 12.5 milligrams of 2,3-diketogulonic acid, 8.24 grams of reducing sugar, and 11.55 grams of total solids in 100 grams of berries. Strawberries of the Robinson variety which had been frozen for 30 hours contained 50.0 milligrams of total apparent ascorbic acid, 46.6 milligrams of reduced ascorbic acid, 6.5 milligrams of dehydroascorbic acid, 6.3 milligrams of 2,3-diketogulonic acid, 7.29 grams of reducing sugar, and 8.25 grams of total solids per 100 grams of strawberries. Reductones and reductic acid were not present in measurable quantities in the strawberries. The total ascorbic acid values for the strawberries were obtained by subtracting the concentration of 2,3-diketogulonic acid from the concentration of total apparent ascorbic acid. The total ascorbic acid concentration in strawberries which had been frozen for 30 hours was 67.5 milligrams per 100 grams of strawberries of the Catskill variety and 43.7 milligrams of strawberries of the Robinson variety.

At the end of the first month of frozen storage, there was a marked decrease in reduced ascorbic acid concentration of strawberries of both varieties in comparison with the concentrations of berries frozen for only 30 hours. An increase in the concentration of dehydroascorbic acid and 2,3-diketogulonic acid occurred during this period; there was

very little change in the total apparent ascorbic acid concentrations. The concentration of reducing sugar was decreased also in strawberries of both varieties after one month of frozen storage.

The concentration of 2,3-diketogulonic acid was low after the second and third month of frozen storage of the strawberries; however, an increase in the concentration of 2,3-diketogulonic acid occurred during the fourth month of frozen storage.

There was a loss of total ascorbic acid of the strawberries during the first month of frozen storage, but an increase in total ascorbic acid occurred during the second month.

Fluctuations in concentrations of the components of the strawberries occurred throughout frozen storage. Statistical analysis indicated that there was a significantly negative correlation between the concentrations of reduced ascorbic acid and dehydroascorbic acid in strawberries of the Robinson variety and a significantly inverse relationship between 2,3-diketogulonic acid and total ascorbic acid in strawberries of both varieties; this appeared to indicate that ascorbic acid was oxidized to 2,3-diketogulonic acid during the frozen storage. There was a highly significant relationship between concentrations of 2,3-diketogulonic acid and reducing sugar in strawberries of the Robinson variety

but not in strawberries of the Catskill variety.

The influence of the changes of apparent ascorbic acid in strawberries during frozen storage on the utilization of ascorbic acid in strawberries was studied with six healthy young women as subjects. Subjects were given 75 milligrams crystalline reduced ascorbic acid as a supplement to their customary diet for seven days before each test period. Test doses given in two successive days at four test periods were (a) 200 grams strawberries of Catskill variety, frozen for 30 hours; (b) an amount of crystalline reduced ascorbic acid equivalent to the amount of total apparent ascorbic acid contained in the test dose of 200 grams of strawberries frozen for 30 hours; (c) dehydroascorbic acid in the form of orange juice treated with activated charcoal, and (d) 250 grams of strawberries, frozen and stored for four months. The test dose of 200 grams strawberries, frozen for 30 hours, contained 169 milligrams of total apparent ascorbic acid, 148 milligrams of reduced ascorbic acid, 33 milligrams of dehydroascorbic acid, 25 milligrams of 2,3-diketogulonic acid, and 144 milligrams of total ascorbic acid. The test dose of crystalline ascorbic acid contained 169 milligrams of reduced ascorbic acid only; the test dose of orange juice treated with charcoal contained 169 milligrams of dehydroascorbic acid only. In the test dose of 250 grams of strawberries, frozen for four months, there were 183 milligrams

of total apparent ascorbic acid, 114 milligrams of reduced ascorbic acid, 54 milligrams of dehydroascorbic acid, 51 milligrams of 2,3-diketogulonic acid, and 132 milligrams of total ascorbic acid. Blood samples were taken before and one-half, one, two, three, and four hours after the administration of crystalline ascorbic acid; and before and one, two, three, four, and five hours after ingestion of the other test doses. A one-hour urine sample was collected before each test dose; a composite urine sample was collected for a four-hour period following the test dose of crystalline ascorbic acid and a five-hour period following the other test doses. The blood serum was analyzed for total apparent ascorbic acid and reduced ascorbic acid; the urine was analyzed for reduced ascorbic acid.

There was not a significant difference between the highest values of ascorbic acid in the blood serum of the subjects after the test doses of frozen strawberries and after the test doses either of crystalline reduced ascorbic acid or of dehydroascorbic acid. There were no significant differences in urinary excretion of ascorbic acid of the subjects following the various test doses.

The total ascorbic acid of the strawberries, frozen and stored for four months, appeared to be as well utilized as that of strawberries frozen for 30 hours only.

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