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Interpretation of chemical and biological analyses of vitamin C in apples held under various conditions of storage

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NOTE TO USERS

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INTERPRETATION OF CHEMICAL AND BIOLOGICAL ANALYSES OF VITAMIN C IN APPLES HELD UNDER VARIOUS CONDITIONS OF STORAGE

by

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INTRODUCTION AND PURPOSE

The unseasonal blizzard which began on November 11, 1940 and swept across Iowa, Nebraska, Kansas, Minnesota, Missouri, Illinois, and the Dakotas will probably go down in history as a major disaster to the horticultural industry of this region. If normal fall weather with the usual killing frosts had prevailed in 1940, the unseasonal cold weather on Armistice Day of that year would not have seriously injured fruit trees. The fall of 1940, however, was unusually warm and there was sufficient soil moisture to keep plants in an active growing condition up to the time of the first killing frost which occurred on November 7. Even then the temperature did not drop low enough to injure the leaves on most fruiting plants. In the freak storm of November 11, temperatures throughout Iowa dropped from a maximum of 50 degrees to a low of zero in less than 24 hours. All vegetation with sap freely flowing was vulnerable to the abrupt change in temperature. The disastrous freeze killed 95 per cent of the trees in commercial apple orchards in 45 counties in central, western, and southern Iowa (Edgecombe, 1941). As a result, in 1941 the Iowa apple crop was only 48,000 bushels, less than one-tenth

of the 1940 crop (Herrick, 1941, 1942).

The widespread destruction brought up the question of the replacement of apple trees. Orchardists, naturally, after their 1940 experience, were anxious to replant with hardy vigorous stock. In making recommendations for replanting orchards, horticulturists advised the use of the apple tree varieties Hibernal and Virginia Crab for stocks (Edgecombe, 1941; Clark, 1941). Varieties topworked on such stocks are better able to withstand adverse conditions of climate and are superior to trees grown on their own stems in respect to disease resistance, vigor, productiveness, and quality of fruit. While a hardy stock was certainly desirable, nutritionists felt that at the same time the potential food value of the fruit of different varieties should be considered. No recommendations, however, could be made concerning the nutritional quality of apple varieties because of a lack of information. It was this dearth of knowledge that catalyzed the present investigation dealing with the concentration of vitamin C in Iowa apples and which led to the study of the physiological availability of the ascorbic acid in apples.

Apples are not particularly rich in any known food nutrient. Nevertheless, on account of their pleasant eating qualities and the flavor and texture they impart

to the diet, they are extensively used, both in the raw and cooked forms. In production, apples lead all other fruits in the United States. The commercial production in 1942-43 was 129 million bushels (Agricultural Statistics, 1943).

Of the nutrients present in apples, vitamin C or ascorbic acid is probably one of the most important, even though apples are classed in the group of foods low in this vitamin. The Jonathan, a commercial variety grown extensively in Iowa, for example, contains approximately 6 to 8 mg. per cent of vitamin C. In spite of the fact that apples have not been rated high in vitamin C, in the quantities consumed by some families, they often contribute a significant part of the daily requirement. For example, a child eating three large apples of a variety containing approximately 7 mg. per cent of ascorbic acid would receive about one-half of his daily needs for this vitamin (National Research Council, 1945).

Recent researches, however, have indicated that apples may represent a better food source of ascorbic acid than hitherto believed. In England, West and Zilva (1944) reported that Bramley's Seedling contains as much as 30 mg. per 100 gm. Kidson (1944) in New Zealand found that the concentration of vitamin C in 11 varieties of apples grown in the Nelson district may range

from 2 mg. to 36 mg. per 100 gm. The best variety, the Sturmer, a commercial variety in that country, is richer in ascorbic acid than tomatoes, a fruit generally considered one of the few dependable contributors of vitamin C to the diet. There is a definite need for information about the nutritive value of varieties of apples grown in Iowa. If apples high in vitamin C exist in Iowa, they might well become the basis for breeding and selection studies for the development of varieties higher in vitamin C content than the standard varieties. Such a program is already in progress at the New York Experiment Station at Geneva.*

There is ample evidence in the literature to indicate that it is possible to improve the vitamin C content of foods through breeding. Investigators, for example, have already demonstrated in the case of cabbage that together with improvement in yield and market quality, an increase in ascorbic acid through breeding is feasible. Two new varieties have been developed containing 64 and 72 mg. per cent of vitamin C, whereas standard varieties range from 49 to 57 mg. (Maynard, 1944).

Also, a very interesting breeding program initiated at the Louisiana State University (Miller, 1944) and

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*Personal communication from Dr. L. A. Maynard to Dr. Pearl Swanson

designed to produce sweet potatoes of high food value may be mentioned. All parental stocks in this country and as many foreign varieties as could be obtained have been tested for desirable genetic characters and stocks with unusual genetic characters used as parental material in the breeding program. After six years of work, a seedling was grown which yielded 40 per cent more number one grade potatoes than the standard variety, Porto Rico Unit I. Of special importance to the nutritionist, the new variety contained more than twice as much vitamin A as Porto Rico Unit I and nearly one-third more ascorbic acid (26 vs. 33 mg. per cent).

The importance of nutrient enrichment programs through plant breeding cannot be ignored. Vitamin C is not universally distributed in foods as are some of the vitamins. It is found in relatively few foods, so that an effort must be made to include such foods in the diet each day. Maynard of Cornell University states:

> Since apples are widely grown, have good keeping qualities, and are a cheap fruit, improvement of their vitamin C content would have great practical importance. The possibility of an apple richer in vitamin C than a tomato and nearly as rich as citrus fruits is certainly intriguing (1944, p. 11).

Along with the lack of knowledge concerning the concentration of vitamin C in varieties of apples, there

is a paucity of information in regard to the effect of storage and pre-storage treatments on the nutritional value of the fruit. Winter varieties of apples are in prime condition after being held in cold storage for several months, but are they still as good a source of vitamin C as they were before they were stored? And does the special kind of storage treatment chosen bear any relation to the retention of the vitamin? It has been one of the aims of the present study to obtain a picture of the average concentration of vitamin C in a number of varieties of apples produced in Iowa when picked from the trees and after being held in different types of storage for varying periods of time.

Chemical methods for determining the concentration of vitamins in foods have been developed which have many advantages over the older biological methods. Although the chemical methods are time- and money-savers, they have disadvantages also. For instance, in estimating vitamin C, methods based on the reduction of an indophenol dye are widely used. However, there may be present in foods related compounds, recently designated as reductones, which also reduce this dye but which have no physiological significance. In the evaluation of the ascorbic acid in apples, it seemed important to attempt a partition of reducing materials present into

nutritionally active and inactive substances.

In addition, an appraisal of the ascorbic acid of a food material should take into account the chemical form in which the vitamin may be present in the food. Both reduced vitamin C or ascorbic acid and oxidized vitamin C or dehydroascorbic acid can be utilized by the body. In fresh respiring tissue they are present in an equilibrium mixture (Rosenberg, 1942). In a processed food, however, the dehydroascorbic acid may represent a step in the oxidative destruction of the vitamin. The usual indophenol method for the estimation of ascorbic acid measures only the reduced compound. Therefore, in the investigation herein presented, the analysis of the potential vitamin C value of apples has included a measurement of dehydroascorbic acid.

Even when the above-mentioned factors are taken into consideration, nutritionists no longer are satisfied with the results of chemical analyses alone when they consider the dietary value of a food in respect to vitamin C. Ascorbic acid is a very labile substance, easily destroyed in the presence of oxidizing agents particularly when concentrations of hydrogen ion, anion, and copper are favorable. Freeing of enzymes in macerated tissue also may result in loss of ascorbic acid. Can the vitamin C in apples go through the processes of

mastication and digestion unchanged? Although chemical analyses show that a certain variety of apple contains 10 mg. of ascorbic acid per 100 gm. of fruit, it does not necessarily follow that 10 mg. of ascorbic acid are available to the body from every 100 gm. of apple ingested. Tisdall has been quoted in Food Industries (vol. 14, 1942, p. 39) as saying that the mere act of chewing apples caused destruction of one-half of their vitamin C. In the same journal it was suggested that if nutrition standards are based on the original ascorbic acid content of certain raw foods before eating, it might be advisable to restudy the whole matter to determine how much of the vitamin is really available to the body. Such studies would prevent any unwise assumptions that food is nutritionally adequate when as a matter of fact this is not the case. For these reasons, a study of the physiological availability of the vitamin C in apples when eaten by the human being was undertaken to give both fundamental and practical significance to the investigation.

When it was decided to study the relative concentration of vitamin C in Iowa apples, it was realized that wide variations within a variety introduced definite problems in sampling. The results of investigations at the Missouri Agricultural Experiment Station

(Murneek, 1945) indicated some of the factors responsible for such variation. The studies showed that the vitamin C content of apples from outside branches was considerably higher than that of apples from inside branches of the same tree; that the exposed half of an apple was higher in vitamin C than the shaded half of the same apple; that a small apple had more vitamin C per unit of weight than a large apple from the same tree; and that the skin and outer edges of the cortex were richer in the vitamin than the inner portion. How to formulate a sample which would be representative, unbiased, and characteristic of the orchard from which it was taken, which could be replicated from year to year, and which would be comparable with samples derived from other sources constituted a major problem.

Before any of the problems suggested above could be attacked, it was necessary to develop analytical procedures satisfactory for the quantitative estimation of ascorbic acid, dehydroascorbic acid, and reductones in the apple tissue, and ascorbic acid in blood plasma. Considerable time was spent in evaluating methodology.

In summary, the investigation herein reported is described in units as follows:

I. Evaluation of chemical procedures adopted

II. Formulation of an adequate sample of apples for analysis

III. Varietal differences in the vitamin C concentration of apples

IV. Stability of vitamin C in apples under various conditions of storage

V. Concentration of "true" and "apparent" vitamin C in apples

VI. Availability of the vitamin C in apples to the human being.

Each topic is treated as an individual unit in this thesis.

This study was initiated at the time of the establishment of the national cooperative project sponsored by the Office of Experiment Stations, U. S. Department of Agriculture and dealing with the conservation of the nutritive values of foods. The present investigation became a part of that project. The Iowa station was designated as a "key" station and was assigned the preparation of an annotated bibliography to include all information available on the subject of vitamin C in apples. The literature has been reviewed and an annotated list of references appears at the close of the present dissertation.

EVALUATION OF CHEMICAL PROCEDURES ADOPTED

DETERMINATION OF ASCORBIC ACID IN PLANT TISSUE

Historical

In 1895, Theobald Smith reported that guinea pigs fed a cereal diet unfortified with succulent vegetables died of a peculiar hemorrhagic disease. More than 10 years later, Holst and Frohlich noted a similarity between this malady in the guinea pig and human scurvy. In 1918, Cohen and Mendel reported that with suitably chosen diets they had experimentally produced scurvy in guinea pigs. Four years later Sherman, La Mer, and Campbell published the description of a method for the quantitative determination of vitamin C. Guinea pigs were fed a basal diet free of vitamin C, but designed to furnish optimum quantities of all other essential nutrients. Relative amounts of vitamin C in foods were measured by determining how many grams of the food under test had to be supplied each day to prevent scurvy in the guinea pig. When less than the "minimum protective dose" was fed, the severity of the scurvy produced was given a rating based upon the weight curve, duration of life, symptoms, and autopsy findings.

Until about 1933, this test represented the only method available for the quantitative estimation of vitamin C in food materials. Many investigators studied the distribution of the vitamin in foods using this method, but it was a slow and expensive procedure. When a rapid chemical method became available, knowledge about the vitamin accumulated very quickly.

Clark and co-workers (Cohen, Gibbs, and Clark, 1924) were the first to report that certain fruit juices and animal fluids were able to decolorize a redox dye of the indophenol class (the 2,6-dibromo derivative). Zilva (1927, 1932) later noted that certain concentrates of vitamin C reduced the parent substance, phenolindophenol. He did not attribute this reaction to the vitamin itself, but to some associated "protective substance". It was Tillmans (1930) and his co-workers (1932a, b) who observed that the ability of foodstuffs to reduce 2,6-dichlorophenol-indophenol frequently, but not without exception, paralleled their antiscorbutic potencies. Harris and Ray (1933a) and Birch et al. (1933) worked out conditions for the preliminary extraction of foodstuffs by grinding the sample with trichloroacetic acid in a mortar, and titrating rapidly in an acid medium with a standardized solution of the dye. These workers thereby converted the principle of the Tillmans reaction

into what seemed a specific and accurate test for the quantitative estimation of ascorbic acid in foodstuffs. The reliability of the method was confirmed in many instances by direct comparison of values obtained by the chemical method against those determined by biological assays (e.g., Harris and Ray, 1933a, b, c; Lund et al., 1934; Ray, 1934; Olliver, 1936).

Many acids have been used as the extracting medium, but since a number of investigators (Fujita and Iwatake, 1935; Musulin and King, 1936; Ponting, 1943) showed that metaphosphoric acid was superior to other acids in inhibiting enzymic and autoxidation, it has come to be used very generally as the extracting medium.

In 1937-38, Mindlin and Butler described a procedure for the determination of ascorbic acid in blood plasma in which the decrease in the concentration of oxidized dye produced by the addition of an amount of plasma insufficient to cause complete reduction of the dye was measured by means of a photoelectric colorimeter. This method eliminated the subjective reading of an end-point and the need of an accurately standardized dye solution. Later that year Bessey (1938) published a modification of the method which allowed for the estimation of ascorbic acid and dehydroascorbic acid in turbid and colored solutions in the presence of other

reducing substances. Morell (1941) suggested the use of a Waring Blendor for the extraction of ascorbic acid from plant materials. This blending process eliminated the cumbersome process of grinding the sample in a mortar with sand, and insured the thorough disintegration of the sample and the uniform distribution of the ascorbic acid through the entire liquid phase. Morell also found that a 3 per cent solution of metaphosphoric acid prevented loss of ascorbic acid during the blending of vegetable tissue.

Workers describing photoelectric methods noticed a fading of the dye in strong acid solutions and buffered their extracts to a pH of 3.6 before testing. Loeffler and Ponting (1942) eliminated the buffering through the use of a large proportion of 1 per cent metaphosphoric acid to weight of sample. The pH of the resulting solution was sufficiently low to prevent losses during blending and yet high enough to prevent fading during the reaction with the dye.

Papers have been published by Becker and diGleria (1937) and Kirk and Tressler (1939) describing methods for the determination of ascorbic acid using potentiometric measurements. The titration curves obtained, however, revealed a continuous drift in potential throughout the whole course of the titration with the

dye, making it impossible to locate the end-point of the titration with accuracy. In 1942, Harris, Mapson,and Wang described a method for the potentiometric determination of vitamin C involving the use of a special mercury-platinum double electrode. A sharp end-point was obtained, and the method gave results in good agreement with the direct visual titration method.

The reaction with 2,6-dichlorophenolindophenol in metaphosphoric acid solution provides the most widely used general basis for ascorbic acid analysis. In 1936, however, Roe developed an independent approach in which ascorbic acid was decomposed to furfural for colorimetric estimation. In 1943, Roe and Kuether described another method not based on oxidation-reduction. The 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid was formed and treated with sulfuric acid to give a colored product in amounts proportional to those of the ascorbic acid originally present.

Description of Method Adopted for the Determination of Ascorbic Acid in Apples

An adaptation and modification (Morell, 1941) of the photometric determination of ascorbic acid in blood serum as reported by Mindlin and Butler (1937-38) and modified by Bessey (1938) to include colored or turbid

solutions and plant tissues formed the basis of the method used herein for the analysis of the ascorbic acid in apples. The method was further modified by the suggestion of Loeffler and Ponting (1942) that extraction be made with a 1 per cent metaphosphoric acid solution.

The five apples from which the composite sample was taken were weighed as a unit to the nearest 0.1 gm. on a large Torsion balance. Two hundred ml. of the acid were measured in a volumetric flask calibrated to deliver and poured into the cup of a Waring Blendor. The apple samples were cut quickly and immediately immersed in the acid. The time required to obtain the slices from the 5 apples was about one and one-half minutes. The remaining portions of the 5 apples were weighed immediately. The difference between this weight and that of the whole apples was the weight of the sample. This sampling procedure reduced to a minimum the losses of ascorbic acid by oxidation due to exposure to air and freeing of enzymes. The apple sample was blended with the acid for exactly five minutes and then filtered through fluted filter paper. The pH of the extract was 2.0-2.3.

Ten ml. aliquots of a solution of 2,6-dichlorophenolindophenol dye (16 mg. in 1 l. of redistilled water) were measured into polished colorimeter tubes.

One ml. of the filtered apple extract was added from a 1 ml. Ostwald pipette, the tube inverted twice and placed in the Klett-Summerson photoelectric colorimeter. A stop watch was started the instant that the extract was released from the pipette, and the reading was taken exactly 30 seconds after that. A few crystals of ascorbic acid were then added to completely decolorize the dye, and a second reading taken. The color of three aliquots from each extract was determined. In each instance, the last reading represented any color or turbidity present in the tissue extract and was subtracted from the original reading. The loss in color, estimated by comparison of the corrected reading with that of a "dye blank", was attributed to ascorbic acid. The blank consisted of 1 ml. of 1 per cent metaphosphoric acid (instead of apple extract) and 10 ml. of dye solution. Six tubes of dye blank were prepared every day that analyses were made, and the average figure used in the calculations.

A question arose concerning the time at which the reading should be made since there may be a fading of the dye progressive with time. Some workers have adopted the procedure of taking readings at several time intervals, extrapolating to zero time, and using this value in the calculation of the ascorbic acid present. Other

workers use the procedure of taking readings of dye blank, unknowns, and standards at a definite time interval. Whether the differences between values obtained at a definite time and those arrived at by extrapolation were important, was studied. Colorimeter readings of dye containing 1 per cent metaphosphoric acid (blank), apple extract, and ascorbic acid were made at 15, 30, and 45 second intervals (table 1). The greatest fading observed amounted to 1.1 scale divisions. This, however, has no significance, since the colorimeter is accurate only to within 1 scale division, and also variation between items making up any mean was often of this magnitude. It was satisfying to note that the color of the apple extract was stable over a period of time as long as 45 seconds. Since the data indicated that there be no gain in accuracy by taking readings at several time intervals, it was decided to determine the color of all solutions used in the investigation exactly 30 seconds after addition to the dye.

In the blending process, the ascorbic acid was uniformly distributed throughout the entire liquid phase. By determining the concentration of ascorbic acid per ml. of filtrate, therefore, and by knowing the total volume of the liquid, the amount of ascorbic acid in the sample could be calculated. The total volume of liquid

Table 1

Photocolorimeter readings showing the stability of color in dye solutions containing blank, apple extract, or pure ascorbic acid

Solution tested	Aliquots		Time intervals 15 seconds	30 seconds	45 seconds	Extrapolated values
Dye blank	I	1	197.0	196.0	195.0	
		2	195.0	194.0	194.0	
		3	198.0	197.0	196.0	
		4	195.0	193.5	192.5	
		5	198.0	196.0	195.5	
		6	197.0	196.0	196.0	
		Mean	196.6	195.3	194.8	197.5
	II	1	188.0	187.5	186.5	
		2	189.0	188.0	187.0	
		3	189.0	188.5	187.0	
		4	188.0	187.0	186.0	
		5	189.5	189.0	187.5	
		6	190.0	189.0	188.0	
		Mean	188.9	188.1	187.0	190.0
Apple extract	I	1	86.5	85.5	86.0	
		2	88.5	88.0	88.0	
		3	88.5	87.5	87.5	
		Mean	87.8	87.0	87.2	88.0
	II	1	60.5	60.0	60.0	
		2	60.0	60.0	60.0	
		3	60.0	60.0	60.0	
		Mean	60.2	60.0	60.0	60.3
Ascorbic acid solution	I	1	164.5	164.0	162.5	
		2	165.0	164.0	163.5	
		3	164.5	163.5	162.5	
		Mean	164.7	163.8	162.8	165.6
	II	1	140.5	139.5	138.5	
		2	138.0	137.5	137.5	
		3	136.5	135.5	135.0	
		Mean	138.3	137.5	137.0	138.8

was the sum of the volume of metaphosphoric acid used (200 ml.) plus the liquid present in the apple sample. The moisture content of all samples of apples was determined in triplicate. Composite samples representing 5 apples were cut quickly and placed in tared weighing bottles. They were dried in air for 3 hours at 40° C. and then in a vacuum oven for 45 hours at 60°.

In the calibration of the photocolorimeter, the quantity of ascorbic acid represented by a given number of divisions on the colorimeter scale was determined with standard solutions of ascorbic acid. About 50 mg. of crystalline ascorbic acid were accurately weighed on an analytical balance, dissolved and diluted to a volume of 100 ml. with 1 per cent metaphosphoric acid. This solution was in turn diluted to various concentrations. When 148 dilutions representing 51 weighings had been read in the colorimeter over a period of more than a year, the points were plotted, a regression line fitted to them, and an equation calculated. The regression line has been reproduced in figure 1.

The equation has been used in the calculation of the concentration of ascorbic acid in all extracts of apples analyzed in the present study. Some investigators in calculation have used a factor obtained by the analysis of a standard solution of ascorbic acid. The present

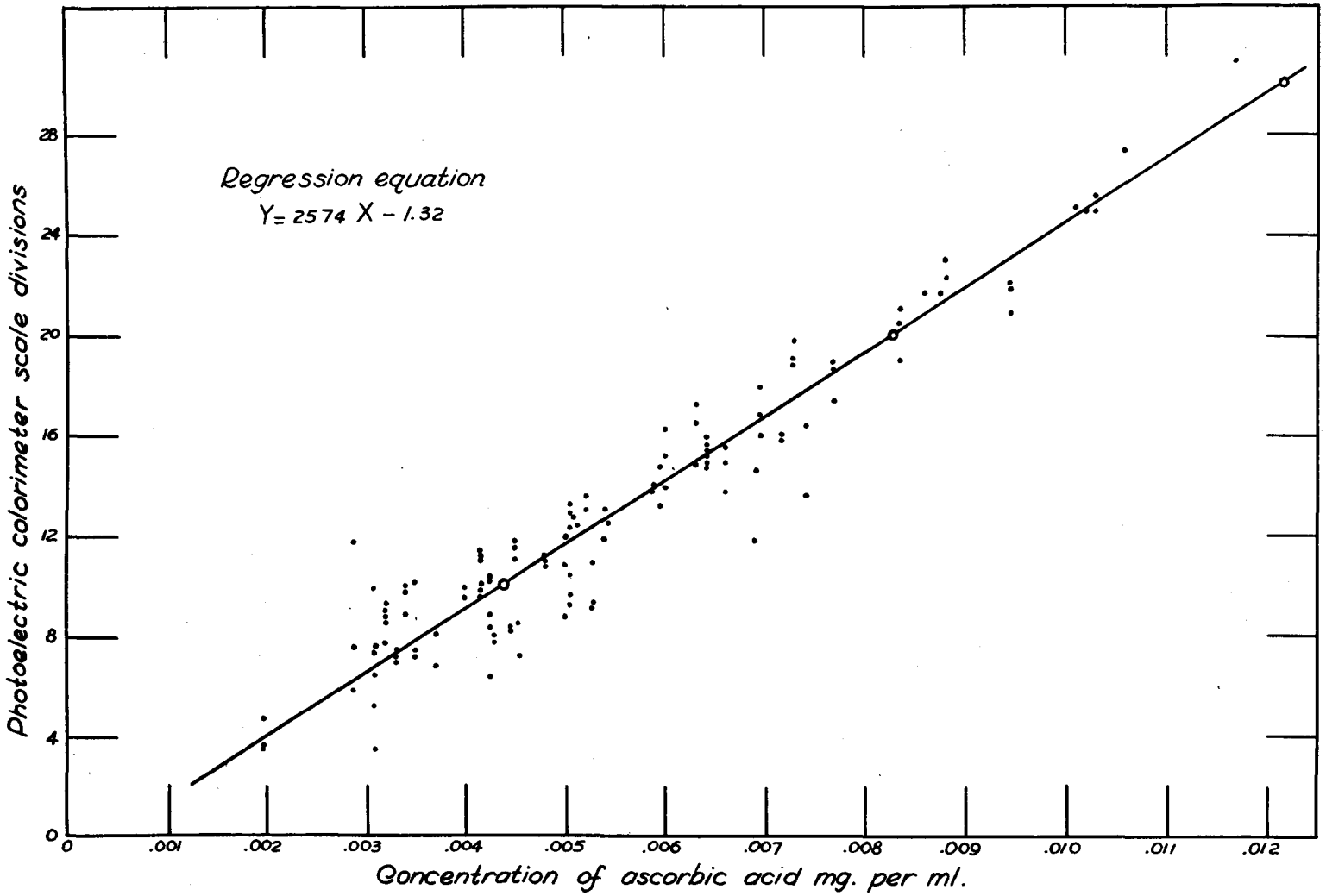

Fig. 1. Regression of photoelectric colorimeter scale divisions on mg. ascorbic acid per ml. of solution.

investigator, however, found that the factor so determined with different solutions of ascorbic acid was not constant. The regression line and equation indicate that the factor varies with the concentration of ascorbic acid in the sample, as is shown in table 2.

Table 2

Scale divisions on colorimeter corresponding to different concentrations of ascorbic acid and factors derived from the regression equation, showing variation in factor with variation in concentration of ascorbic acid

Scale divisions	Conc. of ascorbic acid	Factor
	mg. per ml.	
10	.004398	.000440
20	.008283	.000414
40	.016053	.000401
60	.023823	.000397
100	.039363	.000394
140	.054900	.000392

To test results calculated with the regression equation, solutions of crystalline ascorbic acid were treated as unknowns. The calculated concentration of ascorbic acid was in all cases in close agreement with the actual amount of ascorbic acid in the sample. Table 3 gives the colorimeter divisions, the calculated and actual amounts of ascorbic acid in each sample, and the percentage of the actual amount present which the

estimated values represent. Good recoveries were obtained with ascorbic acid solutions varying in concentration from .0087 to .0435 mg. per 100 ml.

Table 3

Recovery of ascorbic acid from standard solutions of pure ascorbic acid

Sample no.	Scale divisions	Estimated ascorbic acid	Actual ascorbic acid	Per cent recovered
		mg. per ml.	mg. per ml.	
1	21.1	.000871	.000871	100.0
2	22.0	.000906	.000863	104.9
3	25.4	.01038	.01028	101.0
4	27.5	.01120	.01126	99.5
5	44.1	.01718	.01726	99.5
6	54.4	.02165	.02104	103.0
7	59.4	.02359	.02378	99.2
8	66.9	.02604	.02590	100.5
9	88.3	.03435	.03453	99.5
10	111.9	.04352	.04316	100.8
			Mean	100.8

In an attempt to evaluate the accuracy of the method and the technique of the investigator, further recovery experiments were made. Two slices from the center of an apple were used for each test. One of the slices was analyzed in the usual manner. A known quantity of ascorbic acid was added to the metaphosphoric acid in which the second slice was to be blended and subjected to the blending process along with the apple sample. It had to be assumed that the concentration of ascorbic acid in

the two slices of apple was the same. The results obtained are shown in table 4.

The Problem of Interfering Substances

Difficulties due to the presence of reducing substances other than ascorbic acid accompany all methods based on the reduction of indophenol dye. Interfering reducing substances may influence final values and it is hard to distinguish their effect from that of the vitamin itself. Many of the substances responsible for non-specific reduction of the indophenol dye do not normally occur in plant or animal tissues, and if known to be present can be removed. One group of substances, often referred to as "reductones", however, may be encountered frequently. In the strict chemical sense, reductone is hydroxypyruvic aldehyde and results from the alkaline splitting of sugars. This particular substance probably is not formed during the heating of foodstuffs, but other reducing substances may be, and these, too, are commonly classified as reductones.

Reductones have been found in various caramelized and fermented products such as malt extracts, heated cereal grains, fermented juices, and in stored frozen food products. Mapson (1943a) has reported that

Table 4

Recovery of ascorbic acid from extracts in which apple tissue and known quantities of standard ascorbic acid solutions were blended together

Apple no.	Wt. of sample	Volume of extract	Ascorbic acid per gram of apple	Ascorbic acid from apple	Quantity of ascorbic acid added	Ascorbic acid in extract	Ascorbic acid recovered	Per cent recovery
	gm.	ml.	mg.	mg.	mg.	mg.	mg.	
1	43.4	236.8	.053					
1	45.0	242.1	----	2.39	4.35	6.74	6.49	103.8
2	46.7	239.6	.051					
2	48.8	245.3	----	2.48	4.35	7.02	6.83	97.3
3	34.5	229.6	.071					
3	41.3	235.4	----	2.93	4.17	7.10	7.08	99.6
4	32.2	226.9	.167					
4	35.0	229.3	----	5.84	4.17	10.01	9.73	97.1
5	42.2	236.2	.055					
5	36.8	231.6	----	2.02	1.89	3.91	3.96	101.2
6	39.3	233.2	.024					
6	37.9	232.0	----	0.91	4.08	4.99	4.87	97.6
7	43.7	236.9	.028					
7	38.7	232.7	----	1.08	4.08	5.16	5.07	98.2
							Mean	99.3

reductones also may be encountered in certain dehydrated foods which have been subjected to heat treatment. He has questioned the specificity of the usual indophenol methods for the estimation of ascorbic acid in such foods, particularly after storage. The reductones are difficult to distinguish chemically from ascorbic acid as they possess only slightly different stabilities in acid and alkaline solutions and are oxidized by copper and even by ascorbic acid oxidase. Mapson (1943b) developed a method involving the condensation of ascorbic acid with formaldehyde at a pH of 2.0, an acidity at which the reductones are only slowly condensed. The amount of reductones present is derived by extrapolation from a series of determinations made at intervals, and the actual ascorbic acid in the sample calculated by difference from the total reducing power.

Description of Method Adopted for the Estimation of Reductones

The method used in the present investigation was the one developed by Mapson, mentioned above.

A portion of the same filtered extract prepared for the estimation of ascorbic acid was used for the determination of reductones present. The extract was

adjusted to pH 2.0 by the addition of 10 per cent sodium citrate. Formaldehyde was added to a concentration of 8 per cent by measuring 2 ml. aliquots of formaldehyde into 25 ml. volumetric flasks and adding 23 ml. of apple extract. Aliquots (1 ml. in volume) of this mixture were added to 10 ml. of indophenol dye in colorimeter tubes at the following intervals: 10, 25, 40, 55, 70, 80, and 90 minutes, and the rate of reduction of the dye determined with the photoelectric colorimeter. The readings were subtracted from the reading of a suitable blank and plotted as ordinates against the times as abscissae. The line joining the points on the flat part of the curve so obtained was extrapolated back to the ordinate axis, cutting it at a point (X). The concentration of reductones present could then be calculated. The difference between the initial reading (A) and the value at point (X) was used for the estimation of the true ascorbic acid content of the extract.

To test the method, some gluco-reductone was prepared (Lugg, 1942) and analyzed. Solutions of crystalline ascorbic acid were also analyzed alone and mixed with gluco-reductone. The curves obtained (figure 2) show that at pH 2.0 ascorbic acid is almost completely condensed with formaldehyde in 40 minutes, whereas only

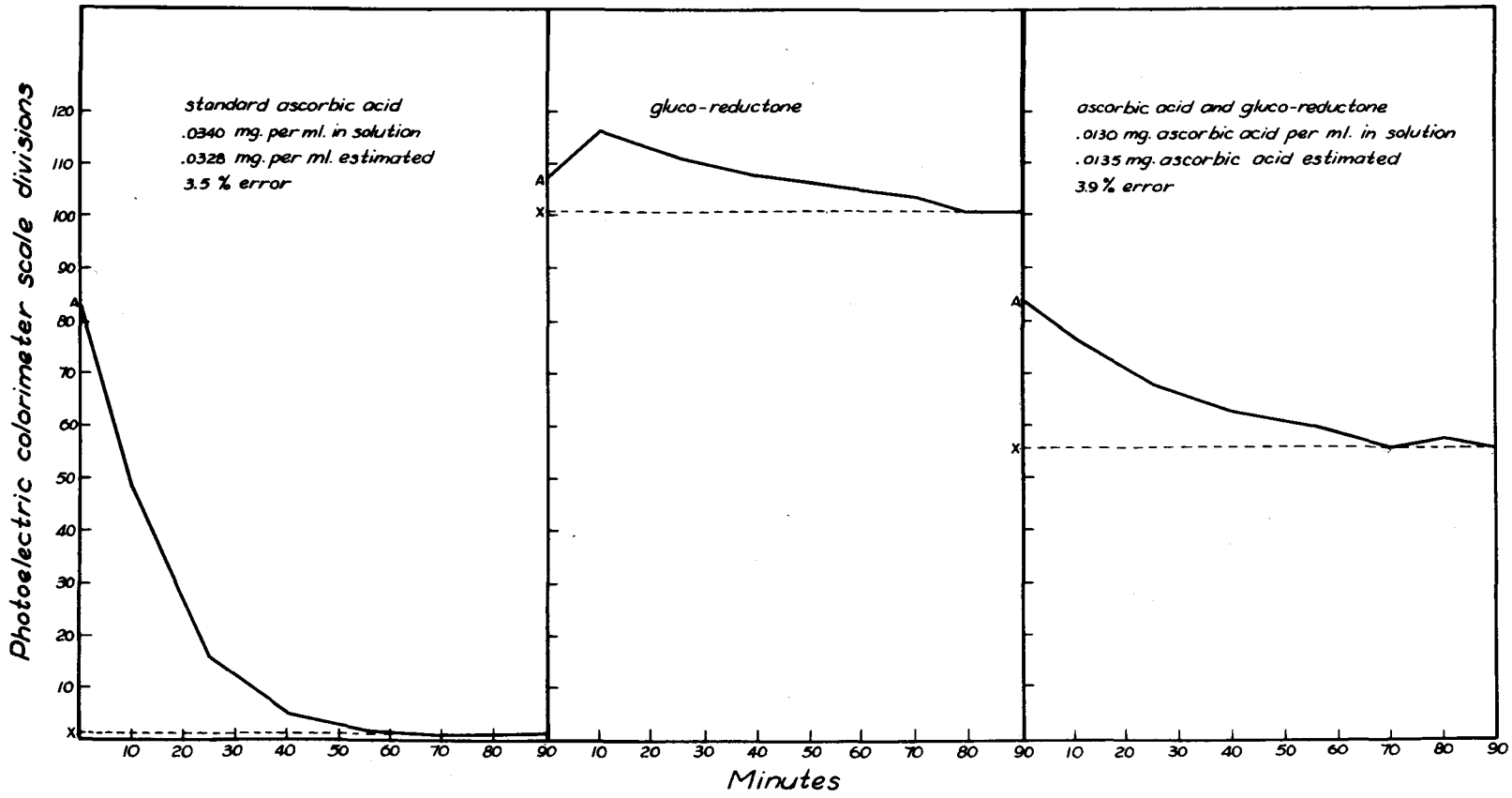

Fig. 2 Reaction of pure ascorbic acid, gluco-reductone, and a mixture of ascorbic acid and gluco-reductone with 8 percent formaldehyde at pH 2.0

a small proportion of gluco-reductone was condensed even in 90 minutes, thereby making it possible to determine quantitatively ascorbic acid in the presence of reductones.

DETERMINATION OF DEHYDROASCORBIC ACID

The early workers concerned with the estimation of ascorbic acid in foodstuffs realized the possibility of the presence of ascorbic acid in tissues in the reversibly oxidized form (dehydroascorbic acid). By direct titration only the reduced form of the vitamin was estimated. The dehydroascorbic acid present was accounted for by the determination of the amount of ascorbic acid in the extract before and after reduction with hydrogen sulfide and subsequent elimination of the sulfide by a stream of wet nitrogen. Bessey (1938) questioned the accuracy of the results obtained by the use of this procedure and King (1941) showed that certain ketones and aldehydes on treatment with hydrogen sulfide formed compounds which responded like ascorbic acid in oxidation-reduction procedures, leading to considerable error.

Roe and co-workers (1939, 1943) have developed a method for the determination of vitamin C not based on an oxidation-reduction reaction. An acid extract of

tissue was passed through norit, thus oxidizing ascorbic acid to dehydroascorbic acid. The bis 2,4-dinotrophenylhydrazine derivative of dehydroascorbic acid was formed by the addition of 2,4-dinitrophenylhydrazine, treated with sulfuric acid, and the resulting red color measured photometrically. An adaptation of these analytical principles to the determination of dehydroascorbic acid in the presence of ascorbic acid (Roe and Oesterling, 1944) with slight modifications was used in the present study.

Description of Method Adopted for the Estimation of Dehydroascorbic Acid

Approximately 10 grams of apple tissue (representing 5 apples) were blended in the Waring Blendor for three minutes with 200 ml. of a solution containing 5 per cent metaphosphoric acid and 1 per cent thiourea. (The purpose of the thiourea was to stabilize the ascorbic acid during the extraction and subsequent treatment.) The extract was then filtered and 5 ml. aliquots were measured into each of four small Erlenmeyer flasks. One of these was stoppered and set aside to be used as a blank. To each of the others was added 1 ml. of 2 per cent 2,4-dinitrophenylhydrazine made up in approximately 9 N sulfuric acid. These flasks were stoppered and held at 37° C. for three hours, then cooled, together with

the blank, in ice water. While the flasks were still in the ice bath, 6 ml. of 85 per cent sulfuric acid were added from a burette, a drop at a time, during not less than one minute. Finally 1 ml. of the 2 per cent 2,4-dinitrophenylhydrazine solution was added to the flask containing the blank. The flasks were then shaken thoroughly, removed from the ice water, and the contents transferred to a series of matched colorimeter tubes. Exactly 30 minutes after the addition of the acid, the concentration of the color of the solution was determined in the photoecolorimeter. The blank reading was subtracted from the mean of the readings of the other three samples in each set to compensate for the slight coloration which occurred when the 85 per cent sulfuric acid was added.

A calibration curve was made (reproduced in figure 3) with standard solutions of dehydroascorbic acid in concentrations ranging from 0.242 to 2.006 mcgm.per ml. The regression equation calculated from this line was used in the calculation of dehydroascorbic acid in the apples. To prepare the standard, approximately 50 mg. of crystalline ascorbic acid was accurately weighed and made up to a volume of 50 ml. with 5 per cent metaphosphoric acid. The solution was treated with a few drops

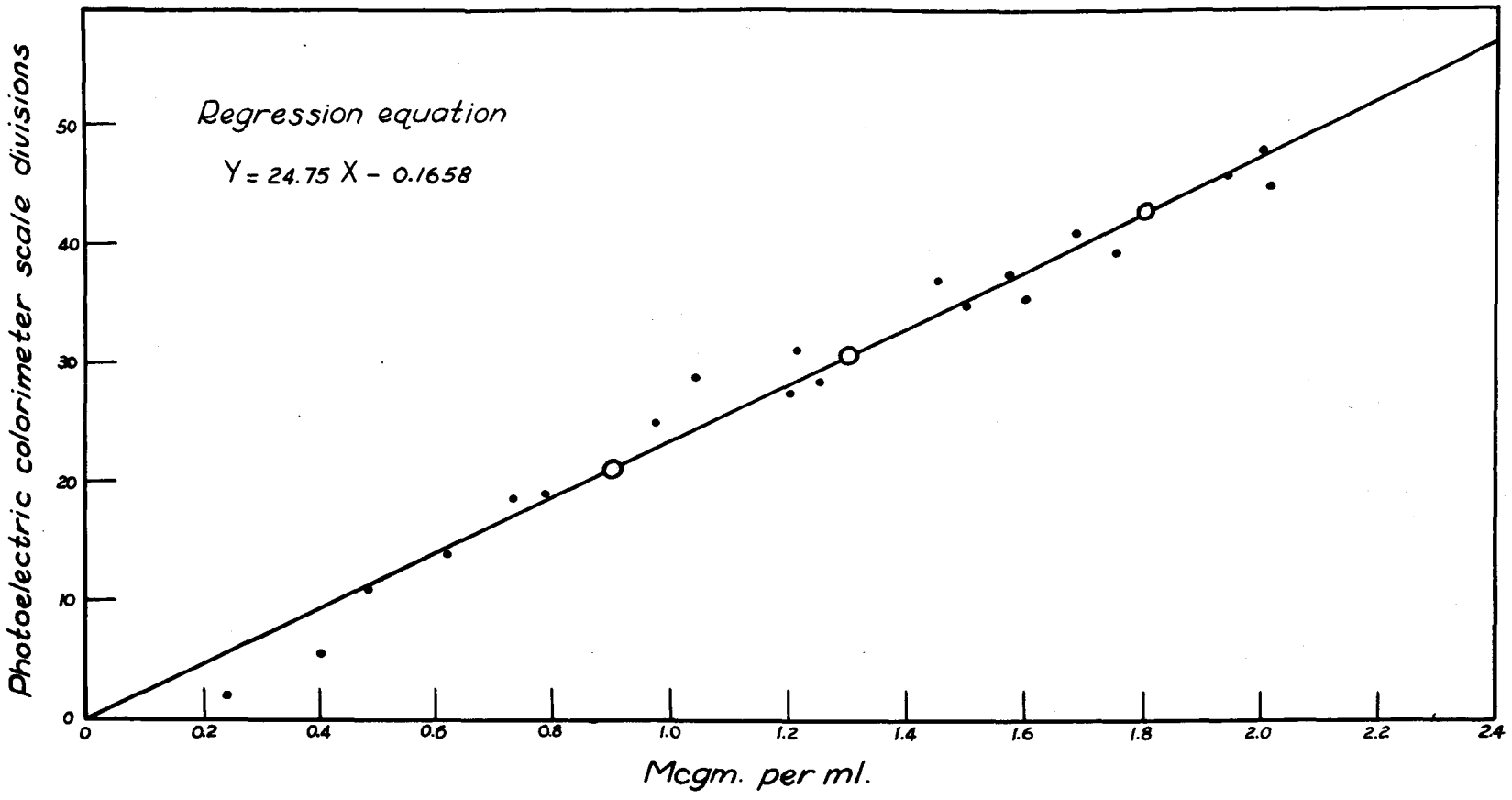

Fig.3 Regression of photoelectric colorimeter scale divisions on mcgm. of dehydroascorbic acid per ml. of solution.

of bromine, shaken until yellow, decanted from the excess of bromine to a large test tube, and aerated until colorless. Standards of appropriate concentrations were made by diluting with 5 per cent metaphosphoric acid containing 1 per cent thiourea.

To test the accuracy of the method, dehydroascorbic acid was added to an apple extract. The recoveries obtained are shown in table 5 below.

Table 5

Recovery of dehydroascorbic acid added to apple extracts

Dehydro-ascorbi acid from apple	Quantity of dehydro-ascorbic acid added	Dehydro-ascorbic acid in extracts	Dehydro-ascorbic acid re-covered	Per cent of added dehydro-ascorbic acid re-covered
mcgm.	mcgm.	mcgm.	mcgm.	
1.548	5.870	5.418	5.285	96.5
1.548	7.608	9.156	9.265	101.4

DETERMINATION OF ASCORBIC ACID IN BLOOD PLASMA

The plan of the experiment for determining the physiological availability of ascorbic acid in apples involved the analysis of plasma representing samples of

blood taken from human subjects at 30-minute intervals during the morning. The large number of samples required from each subject each day demanded that a micro-analysis be used for the determination of plasma ascorbic acid. The titration method of Farmer and Abt (1936) has been widely used for the determination of ascorbic acid in small amounts of blood. In this method, the end-point is determined by the appearance of an unstable, very faint pink color. Because of the possible error due to the subjective factor involved in detecting such a color change, the investigator searched for a method in which the measurements could be made by the photoelectric colorimeter. Such a method has been developed by Mindlin and Butler (1937-38) for use with the Evelyn colorimeter. The investigator spent a great deal of time attempting to modify the method for use with the Klett-Summerson instrument, but was unsuccessful. An opalescence of the deproteinized plasma was sometimes encountered which could not be explained either in terms of dietary factors, or treatment of the sample. When the opalescence did not occur, the results seemed satisfactory, although the values obtained were consistently higher than those obtained by the visual titration method.

Description of Method Adopted for the Estimation of Ascorbic Acid in Blood Plasma

The micro-technique of Farmer and Abt (1936) was used for the determination of ascorbic acid in blood plasma. Centrifuge tubes (1 ml.) were oxalated by drying after rinsing with a 2 per cent solution of lithium oxalate. Blood was collected in these tubes from the tip of a finger which was pierced with an automatic lancet. The blood was centrifuged for ten minutes after which two 0.2 ml. aliquots of the plasma were withdrawn with a micro-pipette, transferred to 3 ml. tubes containing 0.6 ml. of a mixture of 4 parts of 2.5 per cent metaphosphoric acid and 2 parts of redistilled water, and centrifuged for five minutes. The deproteinized plasma so obtained was either titrated immediately or tightly corked and held in the refrigerator (never for more than 4 hours) until the titration could be carried out.

A solution of dye was prepared by weighing 100 mg. of 2,6-dichlorophenolindophenol, dissolving in hot redistilled water, filtering, and diluting to 100 ml. when cool. This stock solution was stored in a brown bottle in the refrigerator. A 1:10 dilution was prepared each day and standardized by titrating against 2 ml. of a standard solution of ascorbic acid (approximately 0.06 mg. crystalline

ascorbic acid per ml.) from a 5 ml. microburette (U.S.B.S.) calibrated to 0.01 ml. The titration was corrected for the quantity of dye needed to produce the same faint pink color in 2 ml. of 2.5 per cent metaphosphoric acid. The factor obtained was used in the calculation of determinations of plasma ascorbic acid carried out the same day.

The 1:10 dilution of the stock dye was further diluted with an equal amount of redistilled water and used to fill the Farmer and Abt micro-pipette. For the titration, 0.2 ml. aliquots of deproteinized plasma were measured into the depressions of a titration tile. Dye was added from the burette while the plasma-dye mixture was stirred with a very fine glass rod. The first faint pink color was taken as the end-point of the titration. Six aliquots of deproteinized plasma representing one blood sample were titrated, and the average was used for calculating the plasma ascorbic acid value. A correction blank of 0.2 ml. of 2.5 per cent metaphosphoric acid was titrated in the same way as the plasma. An average of 12 blank titrations was used as the blank value in the calculations for each day.

The ability of the investigator to recover ascorbic acid from standard solutions by the method described above is shown in table 6.

Table 6

Recovery of ascorbic acid from standard solutions of pure ascorbic acid using the micro-method of Farmer and Abt

Concentration of ascorbic acid solutions used	Ascorbic acid recovered	Per cent recovery
mg. per ml.	mg. per ml.	
0.001512	0.001545	102.2
0.001429	0.001441	100.8
0.001717	0.001784	103.0
0.001524	0.001504	99.0
0.001540	0.001455	94.0
0.005842	0.005890	100.8
0.001425	0.001490	104.6
0.001484	0.001439	97.0

THE FORMULATION OF AN ADEQUATE SAMPLE OF APPLES FOR ANALYSIS

There is considerable variation in the concentration of ascorbic acid in different varieties of apples (Fish, Dustman, and Marsh, 1944; Kidson, 1944). The concentration of vitamin C in any one variety, likewise, is not at all constant. Kidson (1944) in New Zealand, reporting on the ascorbic acid content of apples from the Nelson district, gave values for Delicious apples from four sources as 1.9, 3.4, 4.7, and 5.1 mg. per 100 gm. Sturmer apples from five sources varied in ascorbic acid content from 17.9 to 35.9 mg. per cent. Murneek (1945) suggested that the variability in the concentration of ascorbic acid in apples may be due largely to various environmental factors to which they were exposed during growth and ripening, and that these, in addition to the hereditary variability (specific and varietal differences), should be taken into account in a proper assay of the vitamin C content of apples. One of the objects in the present investigation has been to secure an estimate of the average vitamin C concentration of certain varieties of apples grown in Iowa. In order to do this, considerable time was spent in determining how to formulate an adequate sample of

apples for analysis.

Before any sampling plan could be decided upon, certain preliminary analyses were necessary to determine sources of variation in samples. Information thus obtained suggested procedures for the formulation of a representative sample. These procedures were then tested with the use of samples large enough to yield reliable information. Upon these later tests, the recommendation for the sampling procedure described in a later portion of this section is based.

PRELIMINARY EXPERIMENTS

A number of studies reported in the literature have shown that the peel is several times as rich in vitamin C as the cortex (Fellers, Isham, and Smith, 1932; Zilva, Kidd, and West, 1930; Todhunter, 1943) and that the side exposed to the sunlight is higher than the shaded side (Murphy, 1938; Murneek, 1945). The quantity of tissue needed for a satisfactory analysis is so small that the whole apple cannot be used in the determination. To obtain a portion of an apple with the proper proportion of peel and cortex and with all sides of the apple represented was the first problem that presented itself.

Mean data derived from sectors of the fruit should give as reliable values as data based on the analysis of the whole fruit. After considerable discussion, it was arbitrarily decided to use a full transverse section through the center of each apple (as illustrated in the diagram below) approximately 2-5 mm. thick, depending on the size of the apple.

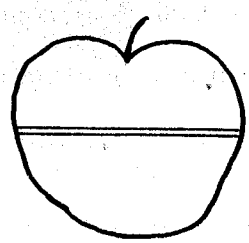

Figure 4. Diagram showing sector of apple used for analysis

A stainless steel knife was used for cutting and coring the apples. All apples analyzed in the investigation herein reported have been sampled in this manner. When the present study was initiated, no reports dealing with the portion which would be representative of a whole fruit could be located in the literature. However, while this manuscript was in preparation (December 1945), Schlaphoff and Leverton reported having studied the question of whether or not a portion of tomato could be analyzed for ascorbic acid in place of the whole fruit. They found

that the mean ascorbic acid content of 20 tomatoes that had been sampled by taking a horizontal slice from the approximate center of each was not significantly different from the mean derived from the analyses of the 20 whole fruits. If the ascorbic acid in apples is distributed in the same manner as it is in tomatoes, the section of apple removed in analyses herein reported may be assumed to be representative of the whole apple.

Before any attack on the problem could be made, it seemed important to obtain a picture of the variation in the concentration of ascorbic acid in individual apples under the specific method of analysis used. A number of Jonathan apples from a commercial pack were analyzed individually. In one lot of 10 apples, the amount of ascorbic acid varied from 1.8 to 4.9 mg. per 100 gm. of fruit. The fact that differences of this size were encountered was not surprising, as even larger variations from item to item in other foods have been reported. For example, when Leverton and Werner (1944) analyzed 174 individual potato tubers, the amount of ascorbic acid varied from 7.54 to 64.02 mg. per 100 gm. of fresh potato. Thus, the results of the studies described above indicate that the analysis of individual apples or of lots containing a relatively small number of apples would not yield

information of satisfactory precision.

In the next study the possibility of reducing the variation between successive samples by picking the samples in an orderly fashion was investigated. When lots of Edgewood apples were picked to represent all sides of eight trees in a plot, the average concentrations in two lots of 10 apples each were 2.77 and 2.92 mg. per cent. This relatively small difference in vitamin C content between two samples containing apples picked according to a definite plan may be compared with 1.94 and 3.08 mg. per cent, which were the average concentrations of ascorbic acid in two lots of 10 apples each, picked at random from a commercial pack of Jonathan apples. Murneek's results in his studies of environmental factors affecting ascorbic acid content of apples while maturing on the tree (1945) explain why a reduction in variation might be expected when lots are selected from all sides of a number of trees. He found that:

> Fruits developed to harvesting maturity on the outside portion of the tree, where there is better exposure to light, are almost invariably higher in ascorbic acid content than when grown on shaded branches. Within the apple itself, there is a higher concentration of this vitamin on the side exposed to sunlight than in the opposite or shaded (shaded by the fruit itself) side. This is not merely "skin deep", or due to the formation of red color, but within the

> flesh of the fruit, as is indicated by peeled apples and varieties that have no blush (red) color, such as the Golden Delicious. Within the range of commercially desirable sizes, the smaller fruits are somewhat higher in ascorbic acid than the larger ones. If a comparable tree or limb carries a relatively light crop, the vitamin content of the fruit is apt to be somewhat higher than in the case of a heavy yield. Ascorbic acid concentration in fruit borne on weak trees, of low vegetative vigor, evidently is higher than in fruit on vigorous trees. This may be associated with a comparatively low nitrogen or high carbohydrate content of such trees.

The last preliminary study was designed to find out if the reduction in variation that followed the orderly formulation of the sample was associated with the balancing of the sample with equal numbers of apples from all sides of each tree represented. The concentration of vitamin C in samples representing the two halves of each tree in a row was determined. The mean ascorbic acid content of the four groups of apples are shown in table 7.

The question arose as to whether or not the differences between the means shown in table 7 were great enough to be more than chance fluctuations in random sampling. The analysis of variance of the ascorbic acid concentration in the apples is shown in table 8.

When the F test was applied to the mean squares, the variance between the north and south sides of the trees was found to be significant, and that between the east

Table 7

Concentration of ascorbic acid in apples from the north and south halves of trees from the east and west ends of a row

Description of apple	Number apples in sample	Source of sample	Ascorbic acid in fresh fruit
			mg. per 100 gm.
Edgewood, preprime	10	North half, trees 1-4	2.22
	10	South half, trees 1-4	2.92
	10	North half, trees 5-8	3.63
	10	South half, trees 5-8	4.02

Table 8

Analysis of variance of concentrations of ascorbic acid in apples from the north and south halves of trees and from the east and west ends of a row

Source of variation	Degrees of freedom	Mean square
Between sides of tree (N-S)	1	2.97
Between ends of row (E-W)	1	15.75
Interaction	1	0.24
Error	36	0.50

and west ends of the row highly significant. The analysis indicates that individual trees should not be used to sample a plot and that it is important to pick apples from

different parts of the tree and from equal numbers of trees from the east and west ends of the row.

SAMPLING STUDY

On the basis of results obtained in the preliminary experiments, a sampling study was planned in which the concentration of ascorbic acid in large numbers of individual apples and lots of apples was determined. The results obtained were analyzed statistically and used as an indication of the character and size of sample which would best represent the vitamin C content of the apples on the trees from which the apples tested were picked. Willow Twig apples were chosen for this investigation because they were higher in vitamin C than any other variety available.

The apples used in experiments 1, 2, and 3 were all picked on the same day from the same 10 trees in a large commercial orchard near Louisiana, Missouri. The trees were as uniform in age, amount of crop, vigor in growth, exposure to sunlight, soil conditions, and freedom from disease as is generally observed in commercial orchards receiving good cultural treatment. The apples were mature and were chosen to be uniform in size and color and representative of the size and color of the fruits on the half

of the tree from which they were picked. The apples were taken directly to Ames by automobile and held in cold storage (32° F.) for 17 days. All of the samples were analyzed for ascorbic acid within the subsequent five-day period. The per cent of moisture determined in a group of five apples was 83.66.

The apples used in experiment 4 were picked at maturity from 10 trees in a large commercial orchard in Illinois, south of Jacksonville. In this case the apples were held in cold storage for 27 days and were then analyzed in the following three-day period. A moisture determination showed that the apples were 84.40 per cent water. All analyses are reported on the basis of fresh weight.

Experiment 1

The object of experiment 1 was to compare the variation in the concentration of ascorbic acid from apple to apple with that of apples representing different trees. A lot of 100 apples, 10 picked at random from each of the 10 trees, was used in the study. Each apple in the lot was analyzed for vitamin C. The apple lowest in ascorbic acid contained 10.87 mg. per cent; the one with the highest concentration had 23.56 mg. per cent (table 9). The mean concentrations of apples representing the 10

Table 9

Concentration of ascorbic acid in 100 apples (10 from each of 10 Willow Twig trees) analyzed individually

Tree number									
1	2	3	4	5	6	7	8	9	10
mg. per 100 gm.									
19.20	14.57	16.99	16.40	18.15	18.20	16.31	17.30	14.67	17.68
18.29	17.79	18.12	15.52	17.18	19.70	15.38	19.94	16.17	14.74
17.85	15.38	17.33	16.44	16.31	18.86	17.66	18.44	16.69	16.02
19.97	17.53	16.86	16.23	16.95	16.62	16.93	18.53	16.34	15.76
16.80	17.02	14.94	17.07	17.54	16.69	15.01	17.28	18.12	16.40
19.13	16.58	15.59	17.17	16.37	21.15	15.56	23.56	18.29	12.67
17.38	14.11	18.08	17.12	10.87	19.41	15.01	19.33	18.10	16.54
16.77	18.29	17.95	16.00	16.07	18.61	16.63	21.92	16.39	15.20
14.61	18.65	14.62	16.81	17.17	14.48	16.76	15.91	18.67	16.17
20.28	19.42	18.10	16.87	19.19	17.51	19.47	19.38	18.40	17.12
Means									
18.03	16.93	16.86	16.56	16.58	18.12	16.47	19.16	17.18	15.83
Mean of all, 17.17									

different trees ranged from 15.83 to 19.16 mg.

The analysis of variance (table 10) of these determinations indicated that apples from different trees differed significantly in vitamin C content.

Table 10

Analysis of variance of vitamin C determinations on 10 apples from each of 10 Willow Twig trees

Source of variation	Degrees of freedom	Mean square
Trees	9	9.72
Apples of same tree	90	2.48

By taking 10 apples at random from each of 10 trees instead of 100 apples at random, the efficiencyyof estimating the concentration of ascorbic acid in the apples on the 10 trees was increased 35 per cent.

Experiment 2

The results of one of the preliminary experiments indicated that there was a significant difference in the concentration of ascorbic acid in apples from the north and south halves of trees. To test this finding and at the same time examine the amount of variation between sets of apples from different trees, 10 apples were picked from

the north half and 10 from the south half of each of the same 10 trees (200 apples in all) from which the apples for experiment 1 were picked. These apples were analyzed as composite samples with five apples represented in each analysis (40 analyses, data presented in table 12).

The analysis of variance of the results obtained (table 11) showed that there was a highly significant difference between the ascorbic acid content of apples from the north and south halves of the trees. The apples from the north contained more ascorbic acid than those from the south. The variation from tree to tree also was highly significant, confirming the results of experiment 1.

Table 11

Analysis of variance of vitamin C determinations on four sets of five apples from each of 10 Willow Twig trees

Source of variation	Degrees of freedom	Mean square
Trees	9	2.77
Sides of trees (N-S)	1	6.56
Error	29	.33

The mean square associated with tree-side interaction was very low, indicating no differential response from tree to tree. By taking two determinations from the north half

Table 12

Concentration of ascorbic acid in 200 apples (4 sets of 5 apples from each of 10 Willow Twig trees) analyzed as composites

	Tree number									
	1	2	3	4	5	6	7	8	9	10
	mg. per 100 gm.									
North	18.06	18.27	19.88	17.84	19.07	19.07	18.00	18.19	18.13	17.54
	18.53	17.36	19.12	17.38	18.63	18.62	16.26	18.95	18.66	16.26
Mean	18.29	17.81	19.50	17.61	18.85	18.84	17.13	18.57	18.39	16.90
South	16.65	17.17	18.31	18.08	17.59	17.46	15.93	18.87	17.55	15.74
	17.29	17.56	19.11	17.81	17.25	17.24	16.87	17.68	18.34	15.12
Mean	16.97	17.36	18.71	17.94	17.42	17.35	16.40	18.27	17.94	15.43
Tree means	17.63	17.59	19.10	17.78	18.13	18.10	16.76	18.42	18.17	16.16
Mean of all, 17.79										

and two from the south half of 10 trees instead of 40 determinations at random from the 10 trees, the efficiency was tripled.

Experiment 3

The results of the first experiment show that although there are large differences in the concentration of ascorbic acid from apple to apple, these differences were considerably smaller than those found between the 10 apples from different trees. In experiment 2, the differences between the ascorbic acid content of apples from the north and south sides of the trees were even greater than those from tree to tree.

Apples used in the third experiment also were picked from the same 10 trees as were those used in experiments 1 and 2. The samples were formulated as described below in an effort to eliminate as much of the variation between samples as possible.

Ten samples were used in the study, each containing 20 apples, one apple from the north and one from the south of each of the 10 trees. Each sample was made up of four sub-samples. Sub-sample I was composed of 5 apples from the north sides of trees 6-10; sub-sample II, of five from the south sides of the same five trees; sub-sample III, of

five apples from the north sides of trees 1-5; and sub-sample IV, of five apples from the south sides of trees 1-5. Each sub-sample was treated as a composite in the vitamin C analysis, sectors being removed from each apple represented therein. When the results were analyzed statistically, the variation between the four sub-samples was highly significant with the sub-samples taken from the north sides of the trees containing more vitamin C than those from the south. The results support the findings of the first two experiments. The amount of variation from sample to sample (range from 17.36 to 18.85 mg.) was barely significant (table 13).

By taking an equal number (100) of apples from the north and south halves of the trees instead of 200 apples at random from the orchard, the efficiency was increased 46 per cent.

Experiment 4

In an attempt to evaluate the results of experiment 3, the study was repeated using 20 samples (20 apples in each sample) instead of 10 (table 14). The apples for the study were obtained from a different orchard than those used in experiments 1, 2, and 3. The difference in concentration of vitamin C between the four sub-samples again

Table 13

Concentration of ascorbic acid in 10 samples, each sample containing 20 apples (analyzed as 4 composites of 5 apples each) and made up of 1 apple from the north and 1 from the south of each of 10 trees

Sample number	Sub-sample number				Mean
	I(N)	II(S)	III(N)	IV(S)	
	mg. per 100 gm.				
1	19.58	17.85	18.98	18.08	18.62
2	18.57	18.24	18.68	17.18	18.17
3	18.21	16.44	19.00	17.19	17.54
4	19.47	17.90	19.93	18.11	18.85
5	18.81	18.41	18.19	17.68	18.27
6	18.84	18.53	18.54	18.14	18.51
7	18.76	17.86	18.51	17.24	18.09
8	18.20	15.40	18.68	17.19	17.36
9	17.03	16.07	18.66	18.67	17.61
10	18.15	16.78	17.50	17.29	17.43
Means	18.56	17.35	18.67	17.68	18.06

was highly significant. This time, however, the apples from the south and not those from the north, as in experiments 1, 2, and 3, contained the highest concentration of the vitamin. The investigator has no explanation for this apparent inconsistency. The data, however, again serve to emphasize the importance of having equal numbers of apples from the north and south sides of trees in a sample. There was no significant difference between the 20 samples, indicating uniformity in the samples. The size of the

Table 14

Concentration of ascorbic acid in 20 samples, each sample containing 20 apples (analyzed as 4 composites of 5 apples each) and made up of 1 apple from the north and 1 from the south of each of 10 trees

Sample number	Sub-sample number				Mean
	I(N)	II(S)	III(N)	IV(S)	
	mg. per 100 gm.				
1	19.67	17.59	20.72	19.55	19.38
2	20.59	20.84	18.66	18.26	19.59
3	18.46	19.45	20.50	20.06	19.62
4	20.06	18.72	20.24	20.82	19.96
5	20.41	18.30	19.73	22.00	20.11
6	20.52	21.61	20.19	20.97	20.82
7	20.73	21.52	20.26	20.18	20.67
8	19.22	18.23	19.60	20.64	19.42
9	16.80	19.55	19.26	19.40	18.75
10	21.01	19.54	20.22	21.38	20.54
11	19.51	19.67	18.15	19.05	19.09
12	17.58	18.83	21.17	22.24	19.95
13	17.31	21.35	19.69	19.95	19.57
14	18.95	19.47	19.11	22.04	19.89
15	18.30	19.49	19.71	21.57	19.77
16	19.62	20.28	18.02	18.41	19.08
17	18.25	19.12	19.01	20.44	19.20
18	16.77	17.90	20.66	21.26	19.15
19	18.09	20.79	20.77	20.09	19.93
20	20.93	21.36	19.46	22.06	20.95
Means	19.14	19.68	19.76	20.54	19.77

sample (20 apples) and the method of formulating the sample were such as to provide a measurement of ascorbic acid characteristic of the apples of the 10 trees represented.

DISCUSSION

The analyses described above illustrate several points that an investigator should take into consideration in the preparation of a sample of large fruits for analysis representative of the population from which it is derived. The great difference in the concentration of vitamin C of individual apples taken from the same tree and the even greater differences in the ascorbic acid content of apples produced by different trees show the importance of using a large number of apples in a sample and of an equal distribution within the sample of apples derived from different trees. In addition, the apples from any one tree probably should represent fruit picked from all sides of the tree. The difference in the concentration of ascorbic acid in apples from the north and the south sides of trees illustrates this point. Perhaps in the future the study should be enlarged to include east and west sides as well. In setting up the present study, it was thought that due to the influence of light, apples from the north and the south represented the extreme values.

If a sample is picked in an orderly fashion so that individual variation, tree variation, and side of tree variation are accounted for, the sample can be expected to

give a good estimate of the concentration of vitamin C in apples derived from the trees represented in the sample. The results in experiment 3, however, in which the differences between samples just bordered on significance, suggest the advisability of considering the inclusion of more trees in the group providing the sample. In other words, a properly designed sampling of apples (formulated as in experiment 3) representing more than 10 trees would increase the chances of drawing a sample that yields a good estimate of the concentration of ascorbic acid in the population sampled.

Comparison of samples formulated as in experiment 3 above permits a reliable evaluation of the effect of processing and storage procedures. It is appreciated that the samples as picked do not represent a random sample of the apples produced by the trees included in the study. It should be recalled, also, that trees were chosen to be as uniform in age, amount of crop, vigor in growth, exposure to sunlight, soil conditions, and freedom from disease as possible. The samples as picked are representative of the U. S. Fancy grade of apples. It is reasonable to assume that about 90 per cent of the apples that go in commercial storage are of the higher grades. It was necessary to set up samples of a high grade of apples on account of problems

involved in holding the fruit for the storage phase of the investigation. The relative advantages of a sample of high keeping quality and of one completely randomized were carefully considered.

The importance of the present study lies in the clean-cut demonstration of the need of an orderly formulated sample. It would be interesting in the future to compare results obtained in sampling procedures based on wholly randomized lots with those reported in the present experiment.

PROCEDURE ADOPTED AND RECOMMENDED FOR THE FORMULATION OF ADEQUATE SAMPLES OF APPLES FOR ANALYSIS

It might be well to describe at this point, the exact procedure followed in the formulation of all samples used in the analyses made in the present investigation. It, the author believes, should form the basis for a procedure that might be recommended to other workers in the field.

An east-west row or group of 10 apple trees of one variety was selected. The north halves of the first five trees starting at the west end of the group were designated as quadrant I, the south halves of the same trees, as quadrant II, the north halves of the five trees at the east end of the group, as quadrant III, and the south

halves of these five trees, as quadrant IV.

If, for example, the plan for the vitamin C study for the year required 400 apples of one variety, 40 apples were picked from each of the 10 trees selected. Twenty of the apples were from the south half of the tree and 20 from the north. Pickers were instructed to choose only those apples which were free from all skin injuries such as scab, sunburn, severe russeting, and insect damage. The apples were selected, furthermore, to be fairly uniform in size and color, and to be representative of the size and color of the apples on the half of the tree from which they were taken. When 20 apples had been picked from the north half of the first tree, inspected, put in a box, and labeled, the pickers moved to the south half of the tree and repeated the procedure there. Finally, there were 20 boxes of apples, one containing 20 apples from the north half and one containing 20 apples from the south half of each of the 10 trees. (Each sample for analysis finally contained 20 apples--one from each of these boxes.)

After picking, the apples were taken to the laboratory and laid in rows on a table, as illustrated in figure 5. The fruit was carefully hand cleaned to remove spray residue, and wrapped in oiled paper to reduce the incidence

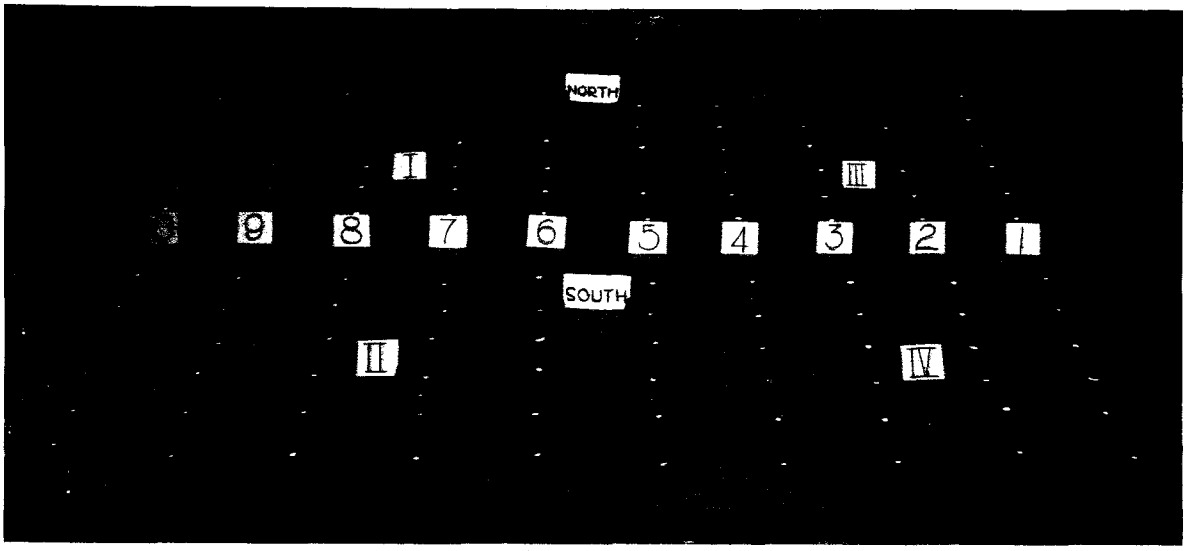

Figure 5. Arrangement of apples representing the north and south sides of 10 trees in an orchard, as used in the formulation of samples. Quadrants I to IV are indicated.

of scald during storage. One apple from each of the five rows representing quadrant I was then put in a bag and the bag labeled "sub-sample I". Sub-samples from each of the other three quadrants were made up in the same way. The four small bags, each containing five apples, and labeled I, II, III, and IV, were then put in a large bag and labeled "Sample I". The other 19 samples were made up in the same manner and stored and analyzed as the experimental plan for the year designated.

Sectors of the five apples in each sub-sample were analyzed as a composite for ascorbic acid content. The four values so obtained were then averaged, the average representing the concentration of ascorbic acid in the apples on the 10 trees from which the apples were taken.

VARIETAL DIFFERENCES IN THE VITAMIN C CONCENTRATION OF APPLES

During the past 20 years, many varieties of apples have been analyzed for ascorbic acid. The great majority of the varieties tested contain relatively small quantities of the vitamin. A number of investigators, however, have reported the finding of a variety containing 20 or more milligrams of ascorbic acid per 100 grams of fruit. One of the earliest of such reports came from England when in 1930b, Bracewell, Hoyle, and Zilva reported that of 10 varieties tested, Bramley's Seedling was markedly more active antiscorbutically than any of the others. More recently (1944), West and Zilva have reported the potency of this apple in terms of milligrams of ascorbic acid present. They found that when freshly picked, Bramley's Seedling apples contained about 31 mg. per 100 gm. of tissue. Kidson (1944), in New Zealand, analyzed 11 varieties of apples grown in the Nelson district and found one, the Sturmer, which contained as much as 36 mg. per cent of ascorbic acid. Of 150 varieties tested by Johansson (1939) in Sweden, four gave values of about 30 mg. Superior even to these was the White Winter Calville. Three consecutive crops of this variety were investigated and the mean value

of the results was about 50 mg. of ascorbic acid per 100 grams of fruit. In this country, Fish, Dustman, and Marsh in West Virginia reported in 1944 that the Red Duchess contained 20 mg. per 100 gm. As data secured in the present investigation will show, Iowa also has its high vitamin C apple, the Willow Twig. Most of the more popular varieties, however, contain only about 5 to 12 mg. per cent when freshly picked.

In the present study, 29 varieties of apples were analyzed as soon after picking as possible. It was the original plan that all the apples for this investigation were to come from the college orchard at Ames, but in 1944 and 1945 there were no apples in this orchard because of late spring frosts. The apples used in these two years were obtained from orchards in other parts of Iowa and from Illinois and Missouri.

Whenever possible, the samples were made up of 20 apples picked from the north and south sides of 10 trees as described in the unit on the formulation of an adequate sample of apples for analysis. As indicated earlier, such a sample yielded a good estimate of the concentration of ascorbic acid in the apples of the trees from which they were picked. Direct comparison of the vitamin C values of different varieties was, therefore, believed to be reliable.

In some instances, however, there were not as many as 10 trees of each variety in the orchard. In such cases, 20 apples were picked from a fewer number of trees. In 1943, the apples were analyzed on the same day that they were picked, but, because the apples came from distant orchards, this was impossible in 1944 and 1945. When it was necessary to hold the samples for a day or longer before analysis, they were kept at 32° F.

ASCORBIC ACID CONTENT OF SOME MIDWESTERN APPLES

The ascorbic acid content of all of the varieties of apples analyzed by this investigator are listed in table 15. Also given are the water contents, sources of the samples, the year or years of analysis, and the number of days after picking that the samples were held before analysis.

The data show that most of the varieties of apples grown in the Midwest contain approximately 6 to 9 mg. of vitamin C per 100 gm. of fresh fruit. In certain varieties, namely the Lansingberg, Northwestern Greening, Paradise Winter Sweet, and White Winter Pearmain, the concentration of ascorbic acid ranges between 3 and 4 mg. per cent. Of all the varieties studied, the Willow Twig

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Table 15

Concentration of ascorbic acid in different varieties of apples grown in the Midwest when freshly picked

Variety	Days held after picking	Ascorbic acid in fresh fruit	Water	Source of sample	Year
		mg. per 100 gm.	%		
Willow Twig	0	23.4	83.99	Ames, Iowa	1943
Red Willow	6	22.3	84.79	Mitchellville, Iowa	1945
Willow Twig	6	19.7	83.87	Patterson, Illinois	1944
Willow Twig	3	19.5	83.16	Hannibal, Missouri	1945
Willow Twig*	4	19.2	83.41	Hannibal, Missouri	1945
Willow Twig*	3	18.0	-----	Louisiana, Missouri	1945
Willow Twig*	4	14.6	-----	Pittsfield, Ill.	1945
Red Willow*	4	14.6	-----	Pittsfield, Ill.	1945
Edgewood	0	9.0	85.32	Ames, Iowa	1943
Jonathan	0	8.8	83.68	Ames, Iowa	1943
Turley	2	8.5	85.79	Mitchellville, Iowa	1944
Winter Banana	2	8.5	85.44	Manchester, Iowa	1944
Baldwin	6	8.2	83.92	Fort Madison, Iowa	1945
Secor	0	7.8	84.77	Ames, Iowa	1943
Yellow Newtown	3	7.7	84.75	Fort Madison, Iowa	1945
Jonathan	4	7.3	84.63	Columbus Junction, Missouri	1945
Golden Delicious	2	7.2	85.14	Mitchellville, Iowa	1944
Ralls	5	7.1	83.22	Fort Madison, Iowa	1945

*Samples, immature, picked three weeks before commercial picking date

Continued on next page

Table 15 (Continued)

Variety	Days held after picking	Ascorbic acid in fresh fruit	Water	Source of sample	Year
		mg. per 100 gm.	%		
Ben Davis	5	6.9	83.99	Mitchellville, Iowa	1945
Jonathan	1	6.8	84.17	Mitchellville, Iowa	1944
Grimes Golden	2	6.8	84.39	Fort Madison, Iowa	1945
Stayman Winesap	2	6.4	85.58	Mitchellville, Iowa	1944
Winesap	6	6.4	84.63	Fort Madison, Iowa	1945
Improved Ralls	7	6.3	85.07	Fort Madison, Iowa	1945
King David	4	6.3	83.05	Fort Madison, Iowa	1945
Harelson	7	6.3	87.10	Fort Madison, Iowa	1945
Ingram	6	6.3	85.52	Fort Madison, Iowa	1945
Arkansas Black	5	6.0	83.71	Fort Madison, Iowa	1945
Huntsman's Favorite	5	5.8	85.60	Fort Madison, Iowa	1945
York Imperial	3	5.8	86.32	Fort Madison, Iowa	1945
Arkansas (Black Twig)	5	5.7	84.25	Fort Madison, Iowa	1945
Red Rome	4	5.4	85.00	Patterson, Illinois	1944
Starking	1	5.3	84.78	Mitchellville, Iowa	1944
Winter Banana	5	5.3	86.78	Fort Madison, Iowa	1945
Paradise Winter Sweet	6	4.0	84.59	Fort Madison, Iowa	1945
Northwestern Greening	15	3.8	86.55	Mitchellville, Iowa	1945
Lansingberg	8	3.6	85.85	Fort Madison, Iowa	1945
White Winter Pearmain	6	3.2	85.39	Fort Madison, Iowa	1945

is outstanding in that it contains several times as much ascorbic acid as any of the other varieties. In 1943, the Willow Twig apples from the college orchard at Ames had about 23 mg. of ascorbic acid per 100 mg. of fruit. The following year, a sample from a commercial orchard south of Jacksonville, Illinois contained approximately 20 mg. per cent. In 1945, samples of Willow Twig apples from three sources were picked about three weeks before the commercial picking date. The concentration of ascorbic acid varied from 14.6 to 19.5 mg. per 100 gm. Another sample from one of these sources was picked on the commercial picking date. The concentration of vitamin C was approximately the same as in the earlier sample. The Red Willow, a sport of the Willow Twig, was also high in vitamin C. A sample of immature Red Willow apples contained the same amount of ascorbic acid (14.6 mg.) as immature Willow Twig apples from the same orchard. Moreover, Red Willow apples from central Iowa contained 22.3 mg. per cent of vitamin C. Samples of Willow Twig or Red Willow samples relatively low in vitamin C contained at least twice as much ascorbic acid as most of the other apple varieties tested. It is interesting that although the concentration of ascorbic acid in the Willow Twig obtained from different orchards and in different years

varied, this variety and its sport were the only ones that consistently showed a high concentration of vitamin C. The data indicate that Willow Twig apples are inherently high in vitamin C.

The variation of the concentration of ascorbic acid in the Willow Twig from orchard to orchard should be noted particularly (table 15). Similar variation was observed in other varieties. Samples of Jonathan apples were analyzed in three consecutive years. Each year the apples were obtained from a different orchard. The average concentrations of ascorbic acid were 8.8, 6.8, and 7.3 mg. per 100 gm. Winter Banana apples from two orchards contained 8.5 and 5.3 mg. per cent ascorbic acid.

It is interesting that even in as small an area as the one from which the apples analyzed in the present study were drawn, variation in concentration of vitamin C as high as 20 to 25 per cent occurs (table 15). Differences in environmental conditions probably are responsible. A number of investigators have studied the effects of such factors on the ascorbic acid content of apples. Conditions which may vary from place to place are soil, fertilization, irrigation or rainfall, spraying, and amount of sunlight. In 1933, Potter reported that Winesap apples from trees in plots receiving a complete fertilizer

appeared to be higher in vitamin C than those from trees in unfertilized plots. In 1939, however, Todhunter showed that in two seasons Winesap apples from fertilized plots were no higher in vitamin C than those from unfertilized plots. Moreover, the results of studies made by Kessler (1939) indicated that over-fertilizing of trees with nitrogen depressed the ascorbic acid content of the fruit.

A study of the effect of available moisture on the vitamin C content of apples (Todhunter, 1939) yielded evidence which was non-conclusive. There was no difference between the concentration of vitamin C in Rome Beauty apples from trees in plots receiving 30 or 60 acre inches of water per season, while Winesap apples from trees in plots receiving 60 acre inches of water appeared to be higher in vitamin C than those from plots receiving only 30 acre inches of water.

Spraying the trees seems to have no effect on the vitamin C content of apples (Fellers, Cleveland, and Clague, 1933).

The results of studies by several investigators are in agreement in regard to the effect of sunshine on the ascorbic acid content of the fruit. Zilva, Kidd, West, and Perry (1934), Johansson (1939), Kessler (1939), Murphy (1938), and Murneek (1945) all have shown that the side of

an apple exposed to the sun is higher in ascorbic acid than the shaded side.

Kessler (1939) and Murneek (1945) have reported that the concentration of ascorbic acid in apples from trees which were heavily loaded was less than in fruit from comparable trees carrying a light crop.

One or more of the factors mentioned above might be responsible for the differences found between samples of apples of the same variety from different localities.

COMPARISON OF ASCORBIC ACID CONTENT OF MIDWESTERN APPLES WITH ASCORBIC ACID CONTENT OF APPLES GROWN IN OTHER REGIONS

Some of the varieties of apples analyzed in the present study, and many more, have been tested by a number of other investigators. In table 16 the ascorbic acid values of certain varieties of apples determined by the writer (Anders) are listed with values for many varieties obtained by several other investigators using some modification of the indophenol titration method. If it is assumed that values given in table 16 are comparable, it seems that apples grown in other countries are higher in vitamin C than those grown in the United States. In this country, West Virginia would seem to produce apples richer in

Table 16

Concentration of ascorbic acid in different varieties of apples determined by a number of investigators using modifications of the indophenol titration method

Variety	Ascorbic acid in fresh fruit	Source of sample	Investigator
	mg./100 gm.		
Baldwin	8.2	Fort Madison, Iowa	Anders
Baldwin	13.7	Alnarp, Sweden	Johansson
Baldwin	6.0	Amherst, Massachusetts	Ruffley, Clague, Fellers
Ballarat	11.8	Umukuri, New Zealand	Kidson
Belle de Boskoop	16.4	Alnarp, Sweden	Johansson
Belle de Boskoop	10.8	Altenburg, Germany	Paech
Berlepsch	24.5	Altenburg, Germany	Paech
Blenheim	6.8	Paulinehof, Germany	Paech
Blenheim	24.7	Alnarp, Sweden	Johansson
Blenheim	24.9	Alnarp, Sweden	Johansson
Bramley's Seedling	31.8	Alnarp, Sweden	Johansson
Bramley's Seedling	18.8	East Malling, England	West and Zilva
Cox's Orange	17.5	Alnarp, Sweden	Johansson
Cox's Orange	10.2	Alnarp, Sweden	Johansson
Cox's Orange	8.3	New Zealand	Keys

Continued on next page

Table 16 (Continued)

Variety	Ascorbic acid in fresh fruit mg./100 gm.	Source of sample	Investigator
Delicious	1.9 - 4.5	Romney, West Virginia	Marsh
Delicious	6.0	Amherst, Massachusetts	Ruffley, Clague, Fellers
Delicious	3.7	New Zealand	Keys
Delicious	3.4	Mahana, New Zealand	Kidson
Delicious	5.1	Mahana, New Zealand	Kidson
Delicious	4.7	Mapua, New Zealand	Kidson
Delicious	1.9	Redwood's Valley, New Zealand	Kidson
Dougherty	7.5	Wakatu, New Zealand	Kidson
Duchess	15.4	Morgantown, West Virginia	Fish, Dustman, Marsh
Dunn's	7.3	Stoke, New Zealand	Kidson
Gelber Edel	31.8	Altenburg, Germany	Paech
Gewurzluiken	12.1	Salem, Germany	Paech
Golden Delicious	7.2	Mitchellville, Iowa	Anders
Golden Delicious	10.0	Morgantown, West Virginia	Fish, Dustman, Marsh
Golden Delicious	7.7	Alnarp, Sweden	Johansson

Continued on next page

Table 16 (Continued)

Variety	Ascorbic acid in fresh fruit	Source of sample	Investigator
	mg./100 gm.		
Granny Smith	10.2	Stoke, New Zealand	Kidson
Granny Smith	9.0	Tasman, New Zealand	Kidson
Granny Smith	7.2	Mahana, New Zealand	Kidson
Gravenstein	21.5	Alnarp, Sweden	Johansson
Gravenstein	14.5	Alnarp, Sweden	Johansson
Grimes Golden	6.8	Fort Madison, Iowa	Anders
Grimes Golden	2.6 - 3.3	Romney, West Virginia	Marsh
Grimes Golden	14.8	Morgantown, West Virginia	Fish, Dustman, Marsh
Johathan	8.8	Ames, Iowa	Anders
Jonathan	6.8	Mitchellville, Iowa	Anders
Jonathan	7.3	Columbus Junction, Missouri	Anders
Jonathan	10.7	Morgantown, West Virginia	Fish, Dustman, Marsh
Jonathan	14.9	Alnarp, Sweden	Johansson
King David	6.3	Fort Madison, Iowa	Anders
King David	8.8	Alnarp, Sweden	Johansson
London Pippin	9.3	Redwood's Valley, New Zealand	Kidson

Continued on next page

Table 16 (Continued)

Variety	Ascorbic acid in fresh fruit mg./100 gm.	Source of sample	Investigator
Maiden Blush	7.4	Morgantown, West Virginia	Fish, Dustman, Marsh
Maiden Blush	11.5	Alnarp, Sweden	Johansson
McIntosh	2.8	Alnarp, Sweden	Johansson
McIntosh	6.9	Morgantown, West Virginia	Fish, Dustman, Marsh
Melba	8.5	Morgantown, West Virginia	Fish, Dustman, Marsh
Northern Spy	11.0	New York	Curran, Tressler, King
Northern Spy	10.0	Amherst, Massachusetts	Ruffley, Clague, Fellers
Ontario	27.7	Amstetten, Germany	Paech
Ontario	36.0	Alnarp, Sweden	Johansson
Ontario	34.4	Alnarp, Sweden	Johansson
Red Duchess	20.0	Amherst, Massachusetts	Ruffley, Clague, Fellers
Rhein, Winterrambour	10.4	Trier, Germany	Paech
Rhode Island Greening	3.0	Amherst, Massachusetts	Ruffley, Clague, Fellers

Continued on next page

Table 16 (Continued)

Variety	Ascorbic acid in fresh fruit	Source of sample	Investigator
	mg./100 gm.		
Rokewood	8.4	Riewaka, New Zealand	Kidson
Rome Beauty	2.7	Mahana, New Zealand	Kidson
Rome Beauty	6.2	Mahana, New Zealand	Kidson
Rome Beauty	2.9	Kearneysville, West Virginia	Marsh
Rome Beauty	9.2	Morgantown, West Virginia	Fish, Dustman, Marsh
Starking	5.3	Mitchellville, Iowa	Anders
Starking	2.6	Kearneyville, West Virginia	Marsh
Statesman	9.2	Brightwater, New Zealand	Kidson
Statesman	4.1	Stoke, New Zealand	Kidson
Stayman Winesap	6.4	Mitchellville, Iowa	Anders
Stayman Winesap	14.5	Morgantown, West Virginia	Fish, Dustman, Marsh
Stayman Winesap	3.7	Kearneysville, West Virginia	Marsh
Sturmer	19.5	New Zealand	Keys
Sturmer	21.2	Umukuri, New Zealand	Kidson
Sturmer	25.2	Stoke, New Zealand	Kidson
Sturmer	23.5	Wakatu, New Zealand	Kidson
Sturmer	25.1	Tasman, New Zealand	Kidson
Sturmer	17.9	Hope, New Zealand	Kidson

Continued on next page

Table 16 (Continued)

Variety	Ascorbic acid in fresh fruit	Source of sample	Investigator
	mg./100 gm.		
Sturmer	31.0	Stoke, New Zealand	Kidson
Sturmer	35.9	Brightwater, New Zealand	Kidson
Sturmer	32.0	Brightwater, New Zealand	Kidson
Wagener	7.7	Morgantown, West Virginia	Fish, Dustman, Marsh
Washington	9.1	Redwood's Valley, New Zealand	Kidson
Wealthy	8.0	Morgantown, West Virginia	Fish, Dustman, Marsh
Wealthy	6.6	Alnarp, Sweden	Johansson
Wealthy	12.9	Alnarp, Sweden	Johansson
Wellington	23.5	Alnarp, Sweden	Johansson
Wellington	22.6	Alnarp, Sweden	Johansson
White Winter Calville	36.7	Alnarp, Sweden	Johansson
White Winter Calville	49.2	Alnarp, Sweden	Johansson
Winesap	6.4	Fort Madison, Iowa	Anders
Winesap	5.8	Wenatchee district, Wash.	Todhunter
Winter Banana	5.3	Fort Madison, Iowa	Anders
Winter Banana	3.5 - 7.0	Wenatchee district, Wash.	Todhunter

Continued on next page

Table 16 (Continued)

Variety	Ascorbic acid in fresh fruit mg./100 gm.	Source of sample	Investigator
York Imperial	5.8	Fort Madison, Iowa	Anders
York Imperial	8.4	Morgantown, West Virginia	Fish, Dustman, Marsh
York Imperial	1.7	Kearneysville, West Virginia	Marsh
York Imperial	3.0	Amherst, Massachusetts	Ruffley, Clague, Fellers

vitamin C than other states growing the same varieties. Apples grown in the Midwest and the Pacific Northwest compare very favorably.

Whether differences in values for the same varieties as listed in table 16 represent true regional differences should be questioned, however. Several reasons for this statement might be cited. Fish et al. (1944) have reported considerable loss of vitamin C in apples held for short periods (3-24 days) of time after picking. While certain of the investigators have exercised particular care in the handling of the samples from the time of picking to analysis, and have attempted to analyze the apples on the picking date, many workers have not mentioned nor seemed to be cognizant of the importance of the lapse of time between the picking date and the date of analysis, thus introducing a possible variable.

A large part of the variation found between values reported for the same variety by different investigators may be associated with the composition of the analytical sample. Some investigators have reported values based on the analysis of two or three or even only one apple, while others may use as many as twenty for the analytical sample. Very few take into account tree differences, and no record has been found of any attempt to formulate a

sample representing the fruit of a group of trees as has been done herein.

Variation may also reflect analytical procedures. Precautions must be observed to prevent losses during the extraction of the vitamin from the tissue. The recent introduction of the Waring Blendor has eliminated many errors from this source. However, it has not yet been adopted by all workers. The method of analysis employed may affect results also. Titration with indophenol dye, for example, while popular, is fraught with many possible sources of error. Factors such as the presence of interfering substances, acidity of medium, and rapidity of titration all affect the end point, and must be controlled rigidly. Again, certain investigators, recognizing the non-specificity of the indophenol titration, have sought to reduce the effect of other reducing substances by some modification of the method.

It is impossible to draw any conclusions in regard to differences in the concentration of vitamin C in a single apple variety as long as the data reported by different investigators contain a number of variables. It might well be that a large part of the variation noted would disappear if the procedure used by the different analysts could be standardized to eliminate certain of the

factors mentioned above which are known to affect the concentration of ascorbic acid in apples.

DISCUSSION

Instead of regarding apples in general as a poor source of vitamin C in the diet, varietal differences in the concentration of ascorbic acid in apples should be recognized. Although it is true that most apple varieties commonly grown and used in this country are not high in ascorbic acid, there are several which are comparable to the tomato in this respect. The Willow Twig, which the present investigator found to be consistently high in vitamin C, is an important commercial variety in the states of Illinois, Iowa, Nebraska, Kansas, Missouri, and Oklahoma. A recent survey in Calhoun County, Illinois, where Willow Twig is extensively grown, indicated that this variety and the Red Willow continue to be popular with apple growers. Willow Twigs, in Calhoun County, rank second in the number of trees, and are second to Jonathan. The Willow Twig is highly esteemed by the bakery and restaurant trade of midwestern cities for the making of apple pie. In its normal market season (April, May, and June) it usually surpasses in quality and market value any

other midwestern variety offered on the markets.

The fact that an apple as rich in ascorbic acid as the Willow Twig is widely grown in the Midwest is of importance from the standpoint of the possibility of this apple contributing a share of the daily requirement of vitamin C to the diet. Two medium-sized Willow Twig apples would supply an adult with at least one-half of his requirement for the day. Additional varieties high in ascorbic acid might eventually be grown in Iowa if horticulturists become interested in improving the nutritional quality of the fruit. It would seem that a breeding program with the object of increasing the vitamin C value of apples could well be initiated. Nutritionists are cognizant of the expense and time involved in the breeding of fruit trees, not to mention the problem of educating the public to use other than the old standard varieties. Inasmuch as the generation process in tree fruits is slow, Dove and Murphy (1936) tried to discover a method of testing seedlings to get an indication of the approximate ascorbic acid content of the apples long before the trees would bear fruit. They found that the ascorbic acid content of the leaves of McIntosh and Northern Spy trees was related to the vitamin C content of the apples. These investigators suggested that a great

deal of time could be saved by selecting seedlings to be grown to trees, other things being equal, on the basis of the concentration of the ascorbic acid in their leaves.

New varieties of apples high in ascorbic acid are for the future. At the present, advantage should be taken of the apples rich in vitamin C which are now available. Inasmuch as no effort has been made as yet to inform the public of the findings of the present investigation, it is worthy of note that consumers in the locality of Ames already have been asking grocers for Willow Twig apples, "the variety high in vitamin C".

STABILITY OF VITAMIN C IN APPLES UNDER VARIOUS CONDITIONS OF STORAGE

In the previous section of this treatise, the concentration of vitamin C in several varieties of apples was investigated in the freshly picked fruit. Inasmuch as more apples are consumed after being stored for varying periods, it is important to know something about the vitamin C content of stored apples. The present investigation included a study of the stability of the ascorbic acid in apples during storage.

Samples of apples for analysis were formulated according to the procedure recommended in the section dealing with the formulation of an adequate sample of apples for analysis. It has been shown that samples made up in this manner were uniform in ascorbic acid content. It may be assumed, then, that all the samples of any one variety when placed in storage contained the same concentration of ascorbic acid as the sample of that variety which was analyzed on the picking date. Differences in vitamin C content between the "picking date" sample and stored samples analyzed subsequently were, therefore, ascribed to storage treatment.

Every sample of apples used for the determination of

vitamin C content had a "twin". These duplicate samples were used for the determination of other physiological changes that accompany ripening and result from storage treatments. The indices, hardness, ground color, flavor, and texture were used as measuring units of ripening. These studies were carried out under the supervision of H. H. Plagge of the Pomology Subsection of the Iowa Agricultural Experiment Station.*

The ground color of the fruit was compared with a standard color chart developed by Plagge (unpublished modification and enlargement of ground color chart by Plagge, Maney, and Gerhardt, 1926). The stages in ground color range from full green through various yellow-greens to a yellow-orange. The stages are designated by numbers, starting with the very greenest color as number one, and ending with the yellow-orange as number eight. The stage of ground color of each sample is recorded in tables given in this section.

The improved Magness and Taylor fruit pressure tester described by Haller (1941) was used for measuring the hardness of the apples. The results of the tests were expressed in pounds of pressure. The test was made on (small) peeled portions of fruits to eliminate the

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*The author appreciates the cooperation of Dr. Plagge in allowing her to use these data.

factor of relative tenderness or toughness of the skin. Three tests, spaced equidistantly along the fruit's surface, were made on each apple. The means of the 60 tests thus carried out on each sample of 20 apples are recorded in the tables below.

The third index of ripening was the flavor and texture of the fruit determined organoleptically. Stages of ripeness were expressed in terms of numbers ranging from one to four. Number one indicates mature fruit as on the picking date (but not ripe enough to eat); number two, fruit that is prime eating or ripe; and number three, fruit that is postprime or overripe. The results of the organoleptic tests are listed in the columns headed "degree of ripeness" in tables presented in this section.

When the samples were taken out of storage for analysis, they were examined for apple scald, soggy breakdown, mealy breakdown, and other functional diseases, as well as for storage rots. All apples were in good condition when removed from storage, with no evidence of the disorders mentioned above.

The stability of vitamin C in nine varieties of apples was studied. All the apples used in 1943-44 were obtained from the station orchard at Ames. In 1944, however, there were no apples in this orchard, and the

samples were obtained from commercial orchards in Iowa and Illinois. The apples were tested for vitamin C on the same day they were picked in 1943. Some brief delays were inevitable the following year, but analyses were carried out as soon after the samples were picked as possible. In all cases but two, the samples were analyzed within three days of the picking date. Five of the samples (Jonathan, Golden Delicious, Stayman Winesap, Turley, and Starking) were placed in storage at 32° F. on the picking date. Inasmuch as data obtained in 1943 indicated that there was no loss of vitamin C in apples stored at 32° F. for two or three days after picking, the first sample of each variety analyzed probably contained approximately the same concentration of vitamin C as it did on the picking date.

The apple varieties studied are listed below (table 17), along with the sources, picking dates, and dates of analysis of the freshly picked apples.

EFFECT OF STORAGE AT ROOM TEMPERATURE ON THE ASCORBIC ACID CONTENT OF APPLES

Samples of Jonathan and Golden Delicious apples were analyzed for ascorbic acid when picked. Two comparable samples of each variety were held at 65-70° F. and

Table 17

Varieties of apples used in the study of the stability of vitamin C during storage, with source, picking date, and date of first vitamin C analysis of each

Variety	Year	Date picked	Date analyzed	Source
		Oct.	Oct.	
Edgewood	1943	15	15	Ames, Iowa
Golden Delicious	1944	8	10	Mitchellville, Iowa
Jonathan	1943	2	2	Ames, Iowa
Jonathan	1944	4	7	Mitchellville, Iowa
Red Rome	1944	16	20	Patterson, Illinois
Starking	1944	6	7	Mitchellville, Iowa
Stayman Winesap	1944	21	23	Mitchellville, Iowa
Turley	1944	9	11	Mitchellville, Iowa
Willow Twig	1943	14	14	Ames, Iowa
Willow Twig	1944	16	22	Patterson, Illinois
Winter Banana	1944	11	14	Manchester, Iowa

analyzed when the prime and postprime stages of ripeness had been reached. The results of the analyses are given in table 18.

The data show that at room temperature the fruit ripens to the prime eating stage very rapidly, and that a large percentage--approximately 75 per cent--of the ascorbic acid is lost in a few weeks.

VII

Table 18

Ascorbic acid and water content, and stage of ripeness of apples when freshly picked and after storage at 65-70° F.

Variety	Time in storage		Conc. of ascorbic acid	Ascorbic acid lost	Water content	Hardness	Stage in ground color	Degree of ripeness
	mos.	days	mg. per 100 gm.	%	%	pounds pressure		
Jonathan	0	0	6.8	--	84.17	17.87	3	1.3
	0	18	1.3	80	84.38	13.30	5	2.7
	1	7	2.9	58	84.40	10.73	7	3.3
Golden Delicious	0	0	7.2	--	85.14	15.01	2	1.0
	0	14	1.7	76	86.79	9.41	6	2.7
	1	3	1.3	83	86.15	8.04	6	3.7

EFFECT OF COMMON STORAGE ON THE ASCORBIC ACID CONTENT OF APPLES

Samples of Jonathan and Willow Twig apples were placed in an air-cooled or common storage room having a temperature range of 35-60° F. Samples were analyzed when they became prime and postprime in ripeness. The data obtained are recorded in table 19.

Comparison of the changes in the two varieties studied shows that the Jonathan apples were postprime after being held about three and one-half months in common storage, whereas the Willow Twig apples were still prime after four months of storage. In contrast to the Jonathan, the Willow Twig apples lost no ascorbic acid during the storage period.

EFFECT OF DEFERRING STORAGE ON THE ASCORBIC ACID CONTENT OF JONATHAN APPLES

Three samples of Jonathan apples were used to determine the effect of deferred storage on the concentration of ascorbic acid. One sample was analyzed on the picking date and the other two were placed in storage at 50-55° F. After 23 days, the apples had reached the prime eating stage. One of the samples was analyzed for ascorbic acid content and the other was transferred to a

Table 19

Ascorbic acid and water content, and stage of ripeness of apples when freshly picked and after storage at 35-60° F.

Variety	Time in storage		Conc. of ascorbic acid	Ascorbic acid lost	Water content	Hardness	Stage in ground color	Degree of ripeness
	mos.	days	mg. per 100 mg.	%	%	pounds pressure		
Jonathan	0	0	8.8	--	83.68	19.26	3	1.0
	2	8	3.3	63	83.78	13.42	4	2.7
	3	18	2.8	68	83.75	13.45	5	3.0
Willow Twig	0	0	23.4	--	83.99	21.53	2	1.0
	4	6	24.4	--	83.92	14.52	3	2.3
	6	26	25.0	--	83.45	14.11	5	3.7

storage room maintained at 32° F. and analyzed when it became postprime. The results of the tests are reported in table 20.

The results of this experiment indicate that a large loss of ascorbic acid in Jonathan apples under deferred storage treatment occurs during the first few weeks while the fruit is ripening to the prime eating stage. Transferring the fruit to cold storage when it became ripe did not inhibit the ripening process nor the rate of destruction of vitamin C. Samples of Jonathan apples which received the deferred storage treatment became postprime at approximately the same time as those held in common storage, and in both cases approximately two-thirds of the ascorbic acid originally present had been lost.

EFFECT OF COLD STORAGE ON THE ASCORBIC ACID CONTENT OF APPLES

A number of investigators have reported that the concentration of ascorbic acid in apples decreases during common storage, but that only small losses of the vitamin occur when the fruit is held in cold storage. In the present investigation the stability of vitamin C in nine varieties of apples held at 32° F. was studied.

Table 20

Ascorbic acid and water content, and stage of ripeness of Jonathan apples when freshly picked and after deferred storage treatment

Storage treatment			Conc. of ascorbic acid	Ascorbic acid lost	Water content	Hardness	Stage of ground color	Degree of ripeness
Time		Temp.						
mos.	days	°F.	mg. per 100 gm.	%	%	pounds pressure		
0	0	--	8.8	--	83.68	19.26	3	1.0
0	23	50-55	3.6	59	84.05	14.43	4	2.0
3	6	32	2.9	67	83.62	13.33	5	3.0

Concentration of Ascorbic Acid in Apples at Regular Intervals in the Storage Period

Ten samples of Jonathan apples were obtained for the study of changes in ascorbic acid content during cold storage. One sample was analyzed on the picking date; two were analyzed two days later; and the remaining seven, at monthly intervals. The data are presented in table 21.

Jonathan apples stored at 32° F. for seven months contained less than one-half of the ascorbic acid present on the picking date. It should be noted that the greatest decrease occurred during the first month of storage. More than one-third of the ascorbic acid originally present was lost by November 1. The samples analyzed after only two days of storage, however, contained approximately the same concentration of ascorbic acid as they had when freshly picked. It would be interesting to analyze samples every few days throughout the first month of storage to determine whether the drop in concentration of ascorbic acid is gradual or sudden.

The apples were in prime condition (degree of ripeness, 2 to 2.7) when they had been in cold storage for three to four months and had lost 60 per cent of the ascorbic acid originally present.

The ascorbic acid content of Willow Twig apples was

Table 21

Ascorbic acid and water content, and stage of ripeness of Jonathan apples when picked and after storage at 32° F.

Time in storage		Conc. of ascorbic acid	Ascorbic acid lost	Water content	Hardness	Stage in ground color	Degree of ripeness
mos.	days	mg. per 100 gm.	%	%	pounds pressure		
0	0	8.8	--	83.68	19.26	3	1.0
0	2	8.9	--	-----	-----	-	---
0	2	8.4	5	-----	-----	-	---
1	0	5.2	41	83.41	18.65	3	1.5
2	0	4.3	51	84.54	17.32	3	1.7
3	3	4.2	52	84.22	15.00	3	2.7
3	27	3.6	59	84.63	15.33	4	2.7
5	0	4.4	50	84.72	12.29	4	3.0
6	0	3.6	59	83.60	12.07	4	3.7
7	0	4.2	53	85.07	12.48	5	4.0

determined on the picking date, after two days in cold storage, and at six weeks intervals throughout the storage season. The results of the tests are given in table 22.

In contrast to the large loss of ascorbic acid which was noted in the Jonathan apples, there was no decrease in the concentration of ascorbic acid in Willow Twig apples stored at 32° F. for as long as seven months. In fact, a synthesis of the vitamin seemed to occur as the storage period progressed.

Willow Twig apples were subjected to the same storage treatment the following season. The data (table 23) again showed that there was as much ascorbic acid in Willow Twig apples at the end of a seven months storage period as on the picking date. The apparent synthesis again occurred.

In figure 6 the changes in ascorbic acid content, hardness, and degree of ripeness in the Jonathan and Willow Twig are shown graphically. The apples become softer as they ripen. The large drop in ascorbic acid content in the Jonathan is not accompanied by a sudden change in hardness nor ripeness of the fruit. There appears to be no definite relationship between the changes in ascorbic acid content and the physiological

Table 22

Ascorbic acid and water content, and stage of ripeness of Willow Twig apples when freshly picked and after storage at 32° F. (1943-44)

Time in storage		Conc. of ascorbic acid	Water content	Hardness	Stage in ground color	Degree of ripeness
mos.	days	mg. per 100 gm.	%	pounds pressure		
0	0	23.4	83.99	21.53	2	1
0	2	24.9	-----	-----	-	---
1	15	24.7	84.31	19.43	2	1.3
2	28	25.6	84.79	18.01	3	1.7
4	12	27.7	83.68	17.24	3	2.0
5	25	26.0	83.48	16.83	3	2.7
7	9	28.0	84.53	15.28	3	3.0

Table 23

Ascorbic acid and water content, and stage of ripeness of Willow Twig apples when freshly picked and after storage at 32° F. (1944-45)

Time in storage		Conc. of ascorbic acid	Water content	Hardness	Stage in ground color	Degree of ripeness
mos.	days	mg. per 100 gm.	%	pounds pressure		
0	0	19.7	83.87	19.53	2	1.0
1	13	20.5	83.77	18.34	2	1.3
3	1	19.3	84.19	15.47	3	2.0
4	20	21.1	83.65	14.41	3	2.0
7	9	23.3	83.29	13.46	5	3.0

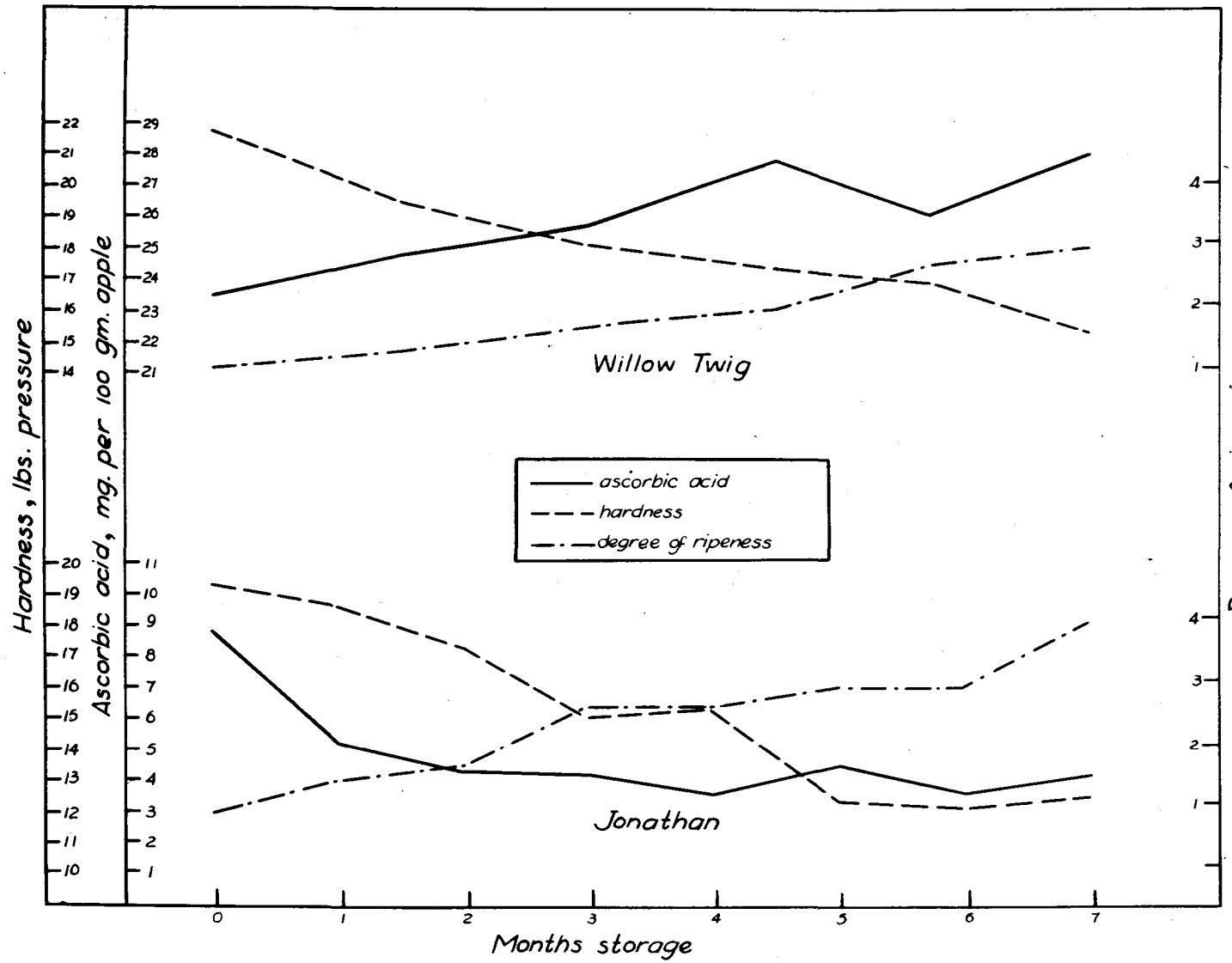

Fig.6 Changes in ascorbic acid content, hardness, and degree of ripeness in Willow Twig and Jonathan apples held in cold storage at 32°F.

characteristics studied. The greatest change in the concentration of vitamin C seems to take place before the physiological alterations occur.

Concentration of Ascorbic Acid in Apples at Maturity and at Prime and Postprime Eating Stages

Information concerning changes in concentration of ascorbic acid in other varieties of apples was desirable. It was impossible, however, with the laboratory facilities and personnel available, to follow the changes at monthly or six-weekly intervals. Jonathan apples, therefore, and six additional varieties were analyzed in 1944-45 at three stages of ripeness: (1) at maturity, (2) at the prime eating stage, and (3) at the postprime stage. These data are given in table 24, those relating to Willow Twig, in table 25.

All of the varieties stored at 32° F., except the Willow Twig, contained less ascorbic acid when prime than when they were picked. The percentage of the vitamin lost, however, varied from one variety to another. For example, the Jonathan and Winter Banana apples both reached the prime eating stage in about two and one-half months. The Jonathan had lost two-thirds of the ascorbic acid present on the picking date, whereas the Winter Banana

Table 24

Ascorbic acid and water content, and stage of ripeness of apples when freshly picked and after storage at 32° F.

Variety	Time in storage		Conc. of ascorbic acid	Ascorbic acid lost	Water content	Hardness	Stage in ground color	Degree of ripeness
	mos.	days	mg. per 100 gm.	%	%	pounds pressure		
Jonathan	0	0	6.8	--	84.17	17.87	3	1.3
	2	12	2.3	66	86.02	15.41	5	2.0
	4	12	3.0	56	84.60	15.83	6	2.3*
Edgewood	0	0	9.0	--	85.32	18.37	3	1.0
	3	5	4.5	50	85.38	15.51	4	2.3
	5	0	2.8	68	85.66	12.77	5	3.0
Golden Delicious	0	0	7.2	--	85.14	15.01	2	1.0
	2	8	4.1	43	86.66	11.59	4	2.0
	5	23	3.9	46	86.59	9.65	5	3.0
Red Rome	0	0	5.4	--	86.75	22.16	2	1.0
	1	23	4.6	16	86.75	16.72	4	2.0
	4	1	4.1	24	86.75	14.42	4	2.7*
Starking	0	0	5.3	--	84.78	17.06	3	1.3
	2	26	2.8	47	85.28	14.52	4	2.0
	6	25	2.1	61	86.22	13.17	6	3.0

*Not postprime

Continued on next page

Table 24 (Continued)

Variety	Time in storage		Conc. of ascorbic acid	Ascorbic acid lost	Water content	Hardness	Stage in ground color	Degree of ripeness
	mos.	days	mg. per 100 gm.	%	%	pounds pressure		
Stayman	0	0	6.4	--	85.58	20.14	1	1.0
Winesap	4	10	2.8	57	85.72	14.90	4	2.0
	7	6	3.0	54	85.59	13.07	4	3.0
Turley	0	0	8.5	--	85.79	19.84	2	1.0
	4	1	6.2	27	85.38	16.76	4	2.7
	6	14	4.8	44	85.34	13.84	5	3.0
Winter Banana	0	0	8.5	--	85.44	24.15	3	1.0
	2	18	7.7	9	85.82	18.15	4	2.0
	6	4	6.8	19	86.09	13.57	6	3.7

had lost only nine per cent. The Stayman Winesap and Turley apples became prime after storage at 32° F. for four months, with losses of ascorbic acid content of 57 and 27 per cent, respectively. There were also large differences in the percentage of ascorbic acid lost in different varieties when the postprime stage of ripeness was reached. The Winter Banana had lost only 19 per cent of the ascorbic acid present when freshly picked, while the Edgewood showed a loss of 68 per cent.

The effect of storage on the ascorbic acid content of apples seems to depend not only on the storage period and temperature, but also on inherent varietal characteristics.

DISCUSSION AND SUMMARY

In 1944, Fish, Dustman, and Marsh reported results of storage studies of apples grown in West Virginia. Wealthy and McIntosh apples were analyzed on the picking date and after storage at 3° and 20° C. for 4, 8, 15, and 30 days. Losses of ascorbic acid occurred more rapidly and were greater in the samples stored at the higher temperature. Jonathan apples analyzed on the picking date and after 3 days storage at 3° C. showed a

17 per cent loss of ascorbic acid, and after 24 days, a 51 per cent loss. In the present study, freshly picked Jonathan apples stored at 32° F. for two days had lost approximately 2 per cent, and after 30 days at the same temperature, 41 per cent of the ascorbic acid present on the picking date.

West and Zilva (1944) reported that vitamin C was synthesized in stored Bramley's Seedling apples. The fruit upon which this observation was based, however, was picked in July and August, long before it had reached picking maturity, and the data, therefore, are not comparable to those reported herein. Apples of the size and immaturity used in the English study have no practical value, as they cannot ripen sufficiently in storage to be used as food.

Johansson in Sweden (1939) found that the Bramley contained approximately two-thirds as much vitamin C after six months storage at 2-4° C. as when freshly picked. The same investigator reported that the ascorbic acid in the White Winter Calville, an apple variety high in vitamin C (50 mg. per 100 gm.) appeared to be relatively stable during storage.

In 1942, Keys in New Zealand studied the effect of storage on the concentration of ascorbic acid in apples.

Sturmer apples containing 20 mg. of ascorbic acid per 100 gm. were held in cold storage for eight months with no loss of the vitamin.

The author believes that it is important to report the stage of ripeness of the fruit at each analysis, as well as the period of time it has been held in storage, inasmuch as all varieties do not ripen at the same rate. From a practical standpoint, it is more interesting to know the concentration of ascorbic acid in different varieties of apples at the prime and postprime eating stages than after arbitrary periods of storage. In table 25, the concentrations of ascorbic acid in different varieties of apples (subjected to different storage treatments) at three stages of ripeness are given.

In general, the samples held at room temperature, in common storage, or in deferred storage contained less ascorbic acid at a given stage of ripeness than comparable samples of the same variety held in cold storage. In those varieties in which considerable losses of vitamin C occurred, the change took place in the early weeks of the storage period, i.e., before the prime eating stage was reached. Data presented by Murneek (1943) based on pooled values of five varieties of apples show the same trend in respect to losses of vitamin C during the storage period.

Table 25

Concentration of ascorbic acid in several varieties of apples when mature, and after holding in different kinds of storage until prime and postprime

Variety	Storage temperature	Concentration of ascorbic acid at different stages of ripeness		
		Mature	Prime	Postprime
	°F.	mg. per 100 gm. fresh fruit		
Edgewood	32	9.0	4.5	2.8
Golden Delicious	32	7.2	4.1	3.9
	65-70	7.2	1.7	1.3
Jonathan	32	8.8	4.3	4.4
	32	6.8	2.3	---
	35-60	8.8	3.6	3.3
	65-70	6.8	1.3	2.9
Red Rome	32	5.4	4.6	---
Starking	32	5.3	2.8	2.1
Stayman Winesap	32	6.4	2.8	3.0
Turley	32	8.5	6.2	4.8
Willow Twig	32	23.4	27.7	28.0
	32	19.7	19.3	23.3
	35-60	23.4	24.4	25.0
Winter Banana	32	8.5	7.7	6.8

The relative stability of the vitamin in three varieties should be noted, i.e., Willow Twig, Turley, and Winter Banana. The three experiments with the Willow Twig indicate that not only the apparent retention of the vitamin, but its synthesis during storage is real. This observation is supported by data presented in a later section, showing that Willow Twig apples at maturity contain little dehydroascorbic acid, and when postprime, negligible quantities of non-specific reducing materials.

The discovery that the Willow Twig apple is high in ascorbic acid, and that it contains as much of the vitamin at the prime and postprime stages as on the picking date, whether held in cold or common storage, has far-reaching significance. The Willow Twig has long been known to apple growers and to horticulturists as a variety of apple which has a long storage life. It ripens slowly in storage, reaching and sustaining the prime stage at approximately the time (March through May) that most varieties are becoming overripe and are no longer marketable. The Willow Twig is the only variety grown extensively in the Midwest which is still in good condition in late spring and early summer. It is, therefore, a potential source of vitamin C during most of the months of the year.

CONCENTRATION OF "TRUE" AND "APPARENT" VITAMIN C IN APPLES

DEHYDROASCORBIC ACID IN APPLES

The studies reported thus far in the present investigation have been concerned only with the concentration of the reduced form of ascorbic acid in apples. The oxidized form, dehydroascorbic acid, also is physiologically active (Gould and Shwachman, 1943), but is not detected by the chemical method for the determination of ascorbic acid involving the reduction of indophenol dye. The dehydroascorbic acid present in certain of the freshly picked samples analyzed for ascorbic acid was determined by the Roe and Kuether method (described in the section on methods). The concentration of dehydroascorbic acid in the samples analyzed (table 26) ranged from 0.80 to 1.50 mg. per 100 gm. of apple tissue, and represented from 5.1 to 19.8 per cent of the total ascorbic acid present.

It is interesting that the percentage of the total ascorbic acid present in the oxidized form was highest in the variety lowest in reduced ascorbic acid, and lowest in the variety containing the largest quantity of reduced ascorbic acid. It would appear from the data obtained that perhaps the varieties considered as extremely poor

Table 26

Concentration (mg. per 100 gm. fresh tissue) of dehydroascorbic acid in several varieties of apples

Variety	Dehydro-ascorbic acid	Reduced ascorbic acid	Total ascorbic acid	Per cent of total ascorbic acid present in the oxidized form
Arkansas (Black Twig)	1.20	5.74	6.94	17.3
Huntsman's Favorite	1.10	5.81	6.91	15.9
Jonathan	0.98	7.26	8.24	11.8
King David	0.90	6.30	7.20	12.5
Red Willow	1.20	22.27	23.47	5.1
White Winter Pearmain	0.80	3.22	4.02	19.8
Willow Twig	1.50	19.22	20.72	7.2
Winesap	1.20	6.39	7.59	15.8

sources of vitamin C on the basis of reduced ascorbic acid only, may be somewhat higher than has been hitherto supposed. Eheart (1941) also observed that varieties low in vitamin C contained a large proportion of the dehydro form.

No studies were carried out in the present investigation of changes in the concentration of dehydroascorbic acid in apples during storage. Such a project is now under way in the laboratory in which the data herein reported were obtained. Acquiring such information is important. In fresh tissue, ascorbic acid and dehydroascorbic acid probably exist in a state of equilibrium:

ascorbic acid $\rightleftharpoons$ dehydroascorbic acid

During storage, with aging of tissue, it is possible that the reaction proceeds to the right, with the ultimate degradation of the unstable dehydroascorbic acid into oxidation products which are not active antiscorbutically.

NON-SPECIFIC REDUCING SUBSTANCES IN APPLES

There is much evidence in the literature that the chemical method used in the present investigation for the estimation of the concentration of ascorbic acid in apples gives results accurately agreeing with the biological estimation of ascorbic acid in common foodstuffs.

In a few exceptional instances, however, interference from non-specific reducing substances has been noted (Harris, 1933).

Some of the apple samples analyzed in the present study were tested by the method developed by Mapson (1943b) for the presence of substances other than ascorbic acid which also have the property of reducing the indophenol dye. The principles on which the method is based are cited in the section, "EVALUATION OF CHEMICAL PROCEDURES ADOPTED", where the description of the procedure also is given.

Curves obtained when solutions of pure ascorbic acid, gluco-reductone, and a mixture of ascorbic acid and gluco-reductone were analyzed by this method are shown in figure 2. The curve illustrating the reaction of a standard solution of ascorbic acid with 8 per cent formaldehyde at a pH of 2.0 shows that a correction factor of one scale division should be applied to experimental data. Also, the photoelectric colorimeter used in the analysis is accurate only to one scale division. Each of the four sub-samples which made up the sample of apples for analysis was tested for the presence of reductones. The curves shown in figure 7 represent the average of the four values for each variety analyzed. A curve, representing an "unknown", with an extrapolated value of

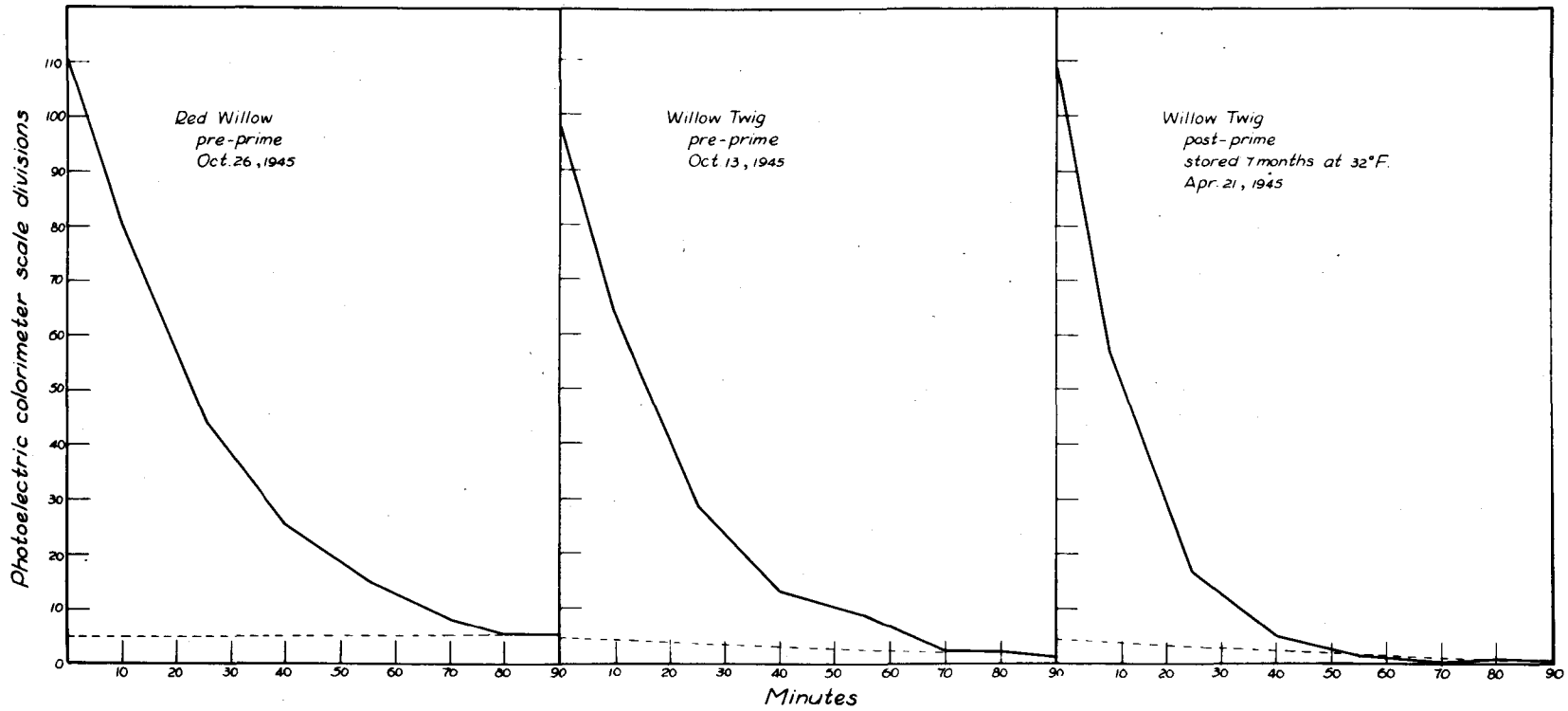

Fig. 7. Reaction of reducing materials in apples with 8 percent formaldehyde at pH 2.0

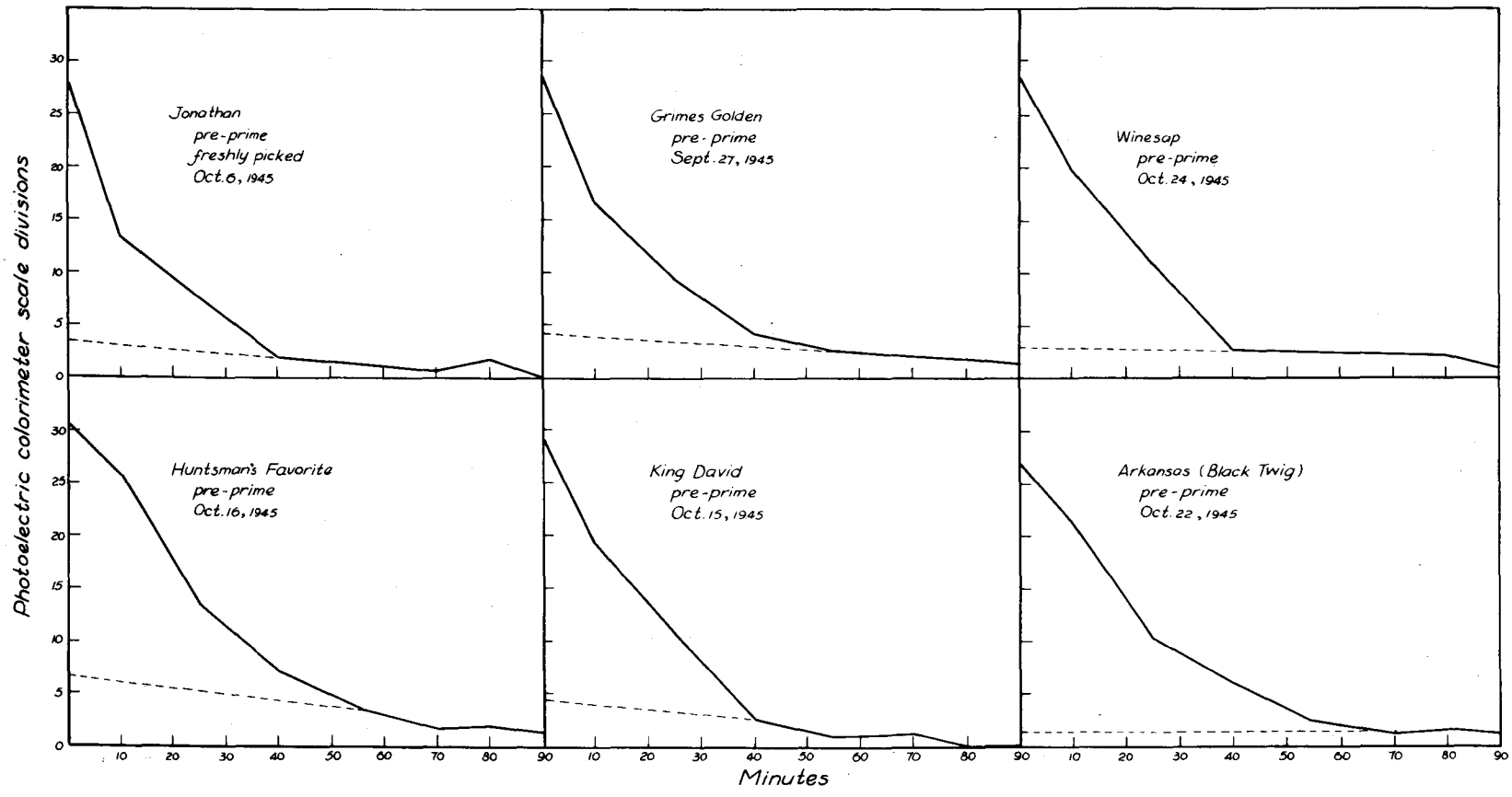

Fig. 7. Reaction of reducing materials in apples with 8 percent formaldehyde at pH 2.0 continued

approximately one scale division would be interpreted as arising from a solution containing no reductones. On the basis of this reasoning, the values reported for the concentration of ascorbic acid in the Willow Twig and Arkansas (Black Twig) varieties represent largely true ascorbic acid, and are not discolored by the simultaneous occurrence of non-specific reducing substances. Again, the Willow Twig proved an interesting variety. Not only is it exceptionally high in ascorbic acid, but this ascorbic acid is stable during a long storage period, and no non-specific reducing materials develop during storage. The shape of the curve when data obtained in the analysis of a sample stored for seven months are plotted (figure 7) is almost identical to the one representing a standard ascorbic acid solution (figure 2).

Several other varieties of apples were tested for the presence of reductones when freshly picked. Curves in figure 7 indicate that all varieties contain traces of non-specific reducing substances. Due to low values for total reducing materials, the quantity of reductones, in general, was equivalent to approximately 10 to 25 per cent of the total reducing substance. The concentration of reductones apparently is highest in the Huntsman's Favorite where it seems to be equivalent in reducing value

to 1.4 mg. of ascorbic acid per 100 gm. of fresh tissue. The true ascorbic acid content of the apples, therefore, falls from 5.6 to 4.2 mg. per cent.

Studies on stored apples are being extended at the present time by other workers in the laboratory.

SUMMARY

The results of studies reported in this section indicate that for an accurate estimation of the potential nutritive value of apples in respect to vitamin C, measurements should includeascorbic acid, dehydroascorbic acid, and reductones. In certain varieties, such as the Willow Twig and Arkansas, there appears to be no need to distinguish the character of reducing substances present, inasmuch as no indication has been found of the presence of non-specific reducing materials. The apparent concentrations of ascorbic acid and dehydroascorbic acid, therefore, are a true measure of the nutritive value of these apples. In other varieties, however, some of the reducing substances heretofore thought to be vitamin C may be reductones, and the true vitamin content is somewhat lower than the ascorbic acid analyses indicate. The data are summarized in table 27.

Table 27

Concentrations (mg. per 100 gm. of fresh tissue) of ascorbic acid, reductones, and dehydroascorbic acid in several varieties of apples when freshly picked

Variety	Conc. of ascorbic acid	Conc. of reductones	Conc. of dehydroascorbic acid	Conc. of true vitamin C
Arkansas	5.7	0.1	1.2	6.8
Huntsman's Favorite	5.8	1.4	1.1	5.5
Jonathan	7.3	0.9	1.0	7.4
King David	6.3	1.0	0.9	6.2
Red Willow	22.3	1.1	1.2	22.4
White Winter Pearmain	3.2	0.6	0.8	3.4
Willow Twig	19.2	0.6	1.5	20.1
Winesap	6.4	0.7	1.2	6.9

It is interesting that in almost every instance, the concentration of true antiscorbutic material is practically the same as the concentration of reduced ascorbic acid. The correction for reducing substances other than ascorbic acid is balanced by the small quantities of dehydroascorbic acid present.

DETERMINATION OF THE AVAILABILITY OF ASCORBIC ACID IN APPLES TO HUMAN BEINGS

The availability of the reduced ascorbic acid in apples to human beings was measured by the increase of blood plasma ascorbic acid after ingestion of the fruit. Todhunter, Robbins, and McIntosh (1942), having studied the rate of increase in the concentration of plasma ascorbic acid after the ingestion of the pure vitamin, reported that ascorbic acid ingested was quickly reflected by an increase in plasma ascorbic acid. A test based on this observation was developed and used to estimate the availability of ascorbic acid in orange juice, orange sections, strawberries, and raw cauliflower (Todhunter, Robbins, and McIntosh, 1942). In 1945, Hauck studied the availability of vitamin C in cabbage using the plan proposed by Todhunter. The pattern established by Hauck was followed in the investigation herein reported. The procedure used for collecting the blood and for determining the plasma ascorbic acid content is described in the section, "EVALUATION OF CHEMICAL PROCEDURES ADOPTED".

EXPERIMENTAL PERIODS

The study, conducted in May and June of 1945, consisted of three experimental periods of three days each.

Period I

Period I was designed to give a picture of natural variations in the concentration of ascorbic acid in blood plasma when the subjects were essentially in a fasting state as far as ascorbic acid was concerned. The fasting level of plasma ascorbic acid was determined every day that the subject participated in the experiment. Immediately after the fasting sample of blood was taken, the subject ate a standard vitamin C-free breakfast consisting of three small slices of whole wheat bread toasted, 10 gm of butter, and one cup of black coffee. The subjects ate no other food during the course of the morning. Additional blood samples were taken one, two, three, and four hours from the time the subject started to eat.

Period II

In Period II, the effect of the ingestion of the pure vitamin on the content of plasma ascorbic acid was studied. During this period, the subjects received a tablet containing 50 mg. of pure ascorbic acid (Merck)

with the vitamin C-free breakfast. Blood samples were taken every half-hour for the four hours following the meal to establish an absorption curve.

Period III

In period III, the 50 mg. of ascorbic acid given in period II as a supplement to the standard vitamin C-free breakfast was replaced by an equivalent amount of ascorbic acid in the form of apple tissue. Willow Twig apples were used because they were higher in vitamin C than any other variety available. An absorption curve again was obtained based on blood analyses at one-half hour intervals.

As a check on how well the apples were digested, fecal collections were made during this period. Each subject was given carmine (either suspended in water or as a cupsule) to mark the food eaten at the test meal. As long as carmine was excreted, stools were collected in waxed cartons. The collections were washed with tap water and the quantity of residue examined and weighed.

DETERMINATION OF THE CONCENTRATION OF ASCORBIC ACID IN THE APPLES FED

The procedure described below was used to estimate the concentration of ascorbic acid in the apples consumed

by the subjects.

Five boxes of Willow Twig apples were obtained for use in the study and stored at 32° F. Four groups of five apples, each composed of one apple from each of the five boxes, were analyzed as composites. This procedure was repeated at four intervals during the investigation. The concentration of ascorbic acid was calculated, and the mean of the 16 analyses was used in determining the quantity of apple to be ingested that would provide 50 mg. of ascorbic acid. The results of the analyses are presented in table 28.

Table 28

Concentration of ascorbic acid (mg. per 100 gm.) in Willow Twig apples fed to subjects

Sample number	Sub-sample A	B	C	D	Average
1	21.50	20.51	20.73	17.37	20.03
2	20.53	21.97	21.77	18.74	20.75
3	17.53	21.38	19.48	21.44	19.96
4	19.99	22.20	20.33	22.88	21.35
			Mean of all		20.52

In analyzing individual apples in experiment I of the sampling study, the investigator noted considerable

variation in the concentration of ascorbic acid from apple to apple. To reduce the possibility of widely different amounts of ascorbic acid being present in the quantity of apple prescribed for consumption, the following plan for eating the apples was adopted. Each subject was given five apples, one from each of the five boxes on which the ascorbic acid analysis was based, and instructed to eat a wedge-shaped section of approximately 50 gm. from each apple. She was supplied with a dietetic scale on which she weighed the whole apple, and the portion remaining after a wedge had been eaten. In the 250 gm. portion derived from the five apples, there were approximately 50 mg. of ascorbic acid. Subjects became adept in judging the size of the portions that would yield 250 gm.

SUBJECTS

Six healthy women, all graduate students in nutrition, served as subjects in the experiment. Information regarding their age, weight, and height is recorded in table 29. No one participated during her menstrual period or when suffering from a cold. All of the subjects were engaged in part or full time laboratory work. No attempt was made to control the activities nor the diets of the

Table 29

Age, height, and weight of subjects

Subject	Age	Height	Weight
	yr.	in.	lb.
A.A.	25	67	125
D.E.	24	63	141
M.H.	23	62	120
M.K.	26	65	175
M.N.	27	64	140
W.W.	23	67	171

subjects. Records were kept, however, of activity and of food consumed on each day preceding a test day. The approximate quantities of the ascorbic acid in the diets of the subjects was estimated from food composition tables. The records of food consumption submitted indicated that, on the average, all subjects received diets adequate in vitamin C, when compared with the 75 mg. per day recommended by the National Research Council as the daily allowance.

RESULTS AND DISCUSSION

Individual plasma ascorbic acid values representing each subject in each phase of the experiment are listed in the Appendix. Data representing the average responses

of each subject during the three periods of the experiment are given in table 30. The average concentrations of ascorbic acid in the plasma of the six subjects before the ingestion of breakfast were 0.81, 0.60, 0.47, 0.61, 0.61, and 0.46. These values are all within the normal range that follows the consumption of a diet adequate in vitamin C. Four of the subjects had been studied by Hauck (1945) during the previous winter season. It is interesting that the average fasting levels reported by this investigator were considerably lower than those determined in the present study. The difference may be an example of seasonal variation in plasma ascorbic acid values.

Graphical representations of each subject's daily response to the ingestion of the standard vitamin C-free meal are shown in figure 8. It may be seen that the day by day data for each subject vary considerably. Causes for the irregularities probably should be investigated. The author believes that some of the variation probably is attributable to the activity of the subject during the hours of the test. It is unfortunate that no record was kept of this activity. If an experiment similar to the present one were carried out in which the activity of the subjects could be controlled, the variation might be

Table 30

Average concentration (mg. per cent) of ascorbic acid in plasma at intervals in experimental periods I, II, and III in six subjects

Period	Time in hrs.*	Subjects						Av.
		A.A.	D.E.	M.H.	M.K.	M.N.	W.W.	
I (no ascorbic acid)	0	0.76	0.49	0.43	0.53	0.53	0.48	0.54
	1	0.78	0.43	0.41	0.59	0.59	0.46	0.54
	2	0.76	0.50	0.51	0.56	0.65	0.51	0.58
	3	0.72	0.56	0.46	0.57	0.67	0.42	0.57
	4	0.77	0.43	0.38	0.49	0.61	0.40	0.51
II (50 mg. crystalline ascorbic acid)	0	0.81	0.71	0.48	0.67	0.66	0.44	0.63
	½	0.73	0.69	0.46	0.59	0.73	0.55	0.62
	1	0.83	0.69	0.69	0.80	0.84	0.60	0.74
	1½	1.02	0.79	0.70	0.91	1.04	0.80	0.88
	2	0.91	0.92	0.77	0.92	1.12	0.75	0.90
	2½	1.06	0.84	0.81	0.82	1.05	0.78	0.89
	3	1.05	0.73	0.56	0.80	0.79	0.69	0.77
	3½	0.90	0.69	0.60	0.74	0.84	0.62	0.73
	4	0.73	0.60	0.34	0.64	0.73	0.63	0.61
Maximum increase		0.25	0.21	0.23	0.25	0.46	0.36	
III (50 mg. ascorbic acid as apple)	0	0.86	0.61	0.51	0.63	0.64	0.47	0.62
	½	0.78	0.70	0.54	0.64	0.61	0.62	0.65
	1	0.99	0.72	0.72	0.81	0.67	0.66	0.76
	1½	1.10	0.79	0.74	0.75	0.73	0.88	0.83
	2	0.96	0.93	0.92	0.87	0.93	0.79	0.90
	2½	1.19	0.76	0.79	0.79	0.74	0.91	0.86
	3	0.95	0.71	0.75	0.81	0.75	0.75	0.79
	3½	0.81	0.68	0.62	0.64	0.70	0.62	0.68
	4	0.76	0.70	0.39	0.64	0.62	0.48	0.60
Maximum increase		0.33	0.32	0.41	0.24	0.29	0.44	

*0 time represents fasting level

reduced. However, when the range of the deviation within a day is calculated, the plasma ascorbic acid on the average varies approximately 0.15 mg. per cent. Hauck's subjects showed a variation of 0.13 mg. per cent. This, therefore, seems to be the physiological variation that may be expected.

When average curves are compiled for each subject (note the solid lines in figure 11), the curves assume a reasonable uniformity as to shape. The curve representing the average plasma ascorbic acid values of all subjects (figure 12) during the course of the morning after the ingestion of a vitamin C-free breakfast is strikingly like the curve recorded by Hauck. The base line, so important in this type of experiment, therefore, seems to be fairly well established.

The day by day responses of all subjects to the addition of 50 mg. of crystalline ascorbic acid to the standard meal are shown in figure 9. When ascorbic acid was consumed with the breakfast, there was a rise in plasma ascorbic acid detectable in some cases in the blood taken one-half hour after the subject started to eat, but usually not definite until the one-hour sample was analyzed. The highest peak was most often reached at the two or two and one-half hour interval. Variation in

individual day by day curves again look erratic on first inspection. It must be borne in mind, however, that the quantity of ascorbic acid actually determined in the analysis is extremely small (0.0003 mg. to 0.001 mg.) and that in calculating the mg. of ascorbic acid per 100 ml. of plasma, this figure is multiplied by 1000. An error, then, in which an extra 0.0005 ml. of dye was used in the titration, for example, would cause the estimate of ascorbic acid to be 0.06 mg. per 100 ml. of plasma too high.

On the whole, a rise and drop in plasma ascorbic acid is evident subsequent to ingestion of the vitamin. Irregularities more or less disappear when the average response is plotted (figure 11). The effect of the ingestion of the ascorbic acid is distinct and assumes a typical absorption pattern.

The curves drawn from the data obtained in period III (figure 10), when the subjects were ingesting ascorbic acid as apple, again seem irregular. However, it should be noted that the curves representing the average response of each subject (figure 11) during the three-day apple period follow the general contour of those for period II in which crystalline ascorbic acid was given. The curves representing the changes in ascorbic acid in

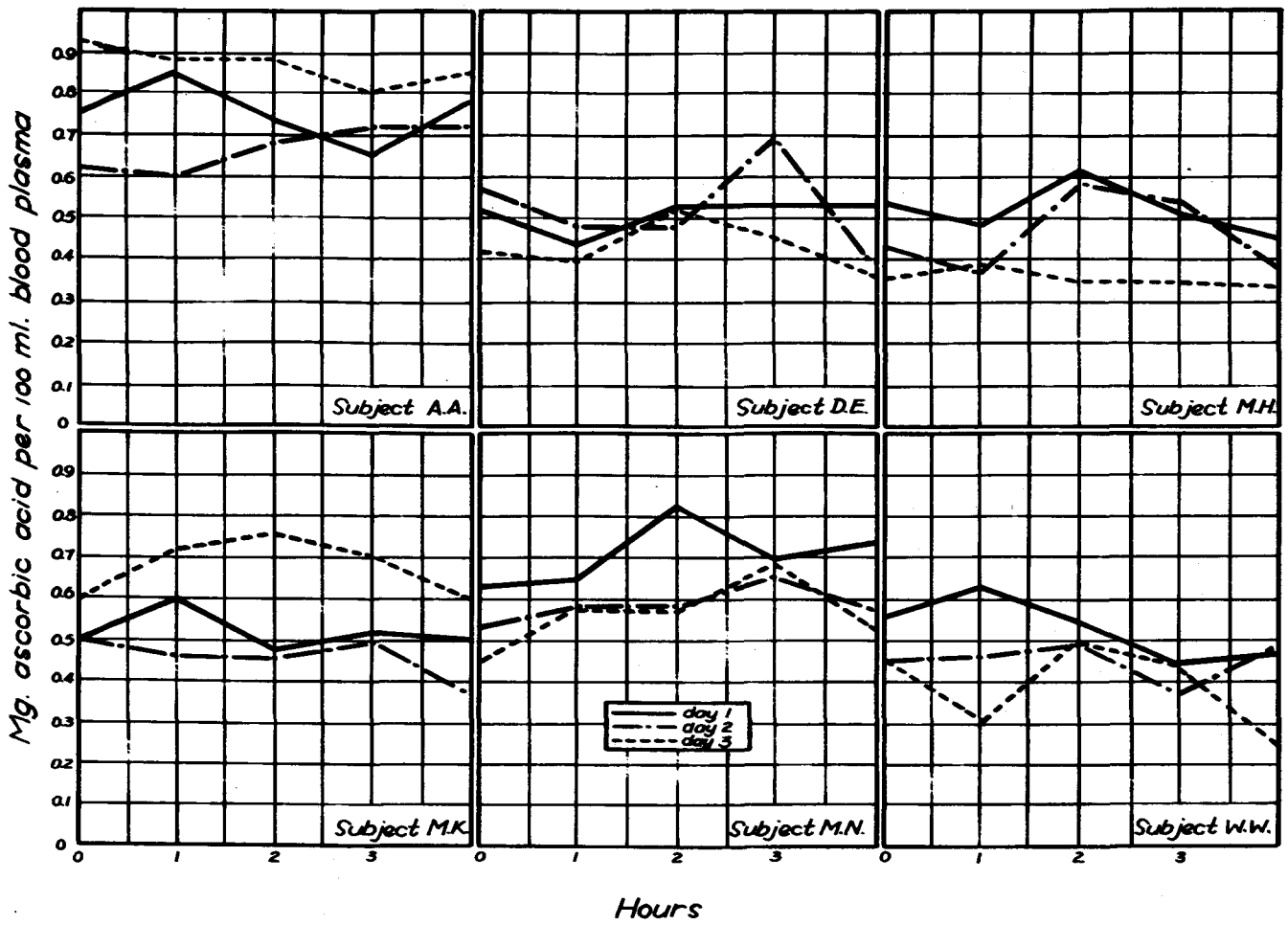

Fig. 8. Concentration of ascorbic acid in plasma in six subjects immediately preceding and at intervals following the ingestion of a standard vitamin C-free meal.

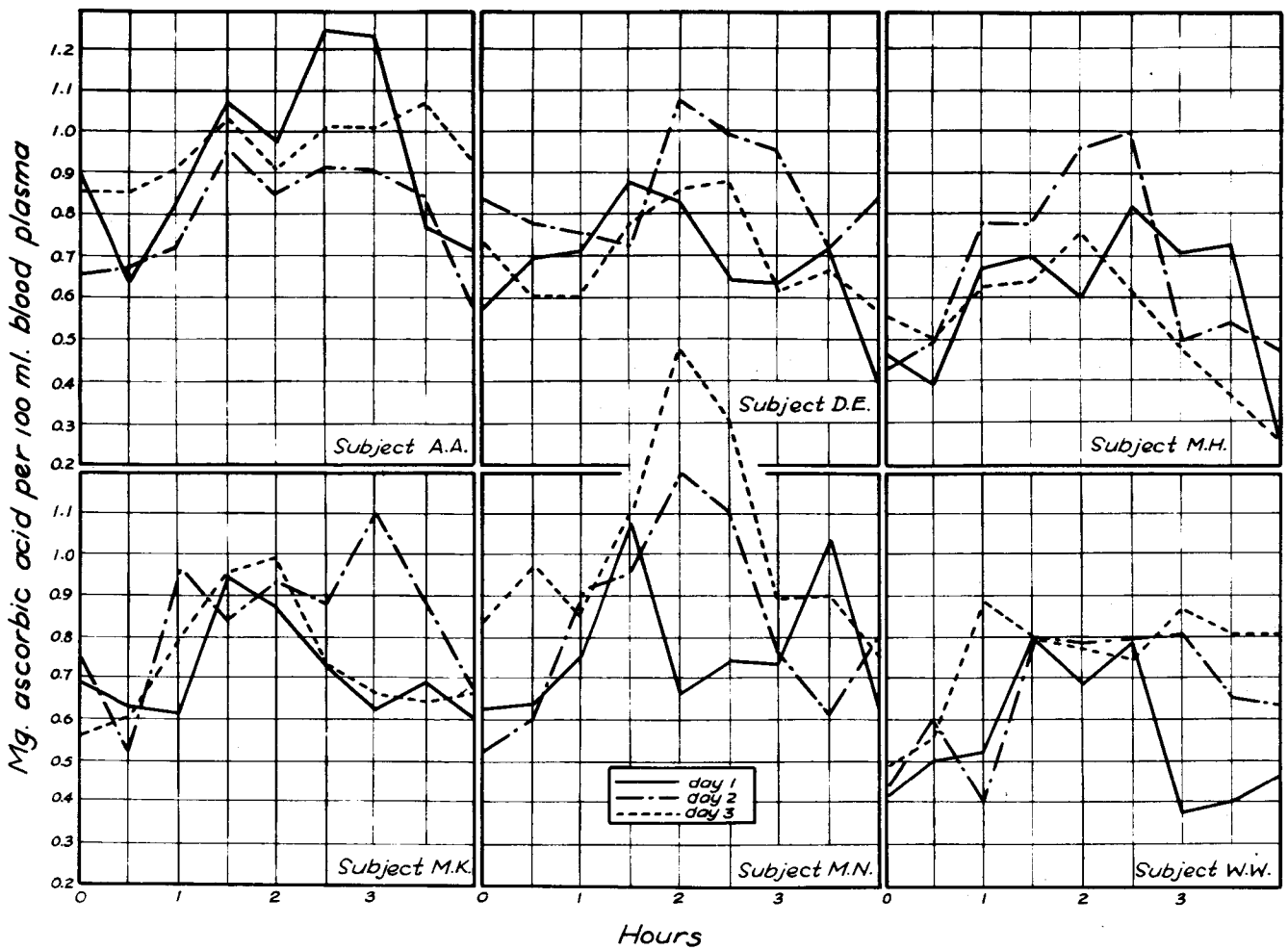

Fig.9. Concentration of ascorbic acid in plasma in six subjects immediately preceding and at intervals following the ingestion of a standard vitamin C-free meal plus 50 mg. of crystalline ascorbic acid.

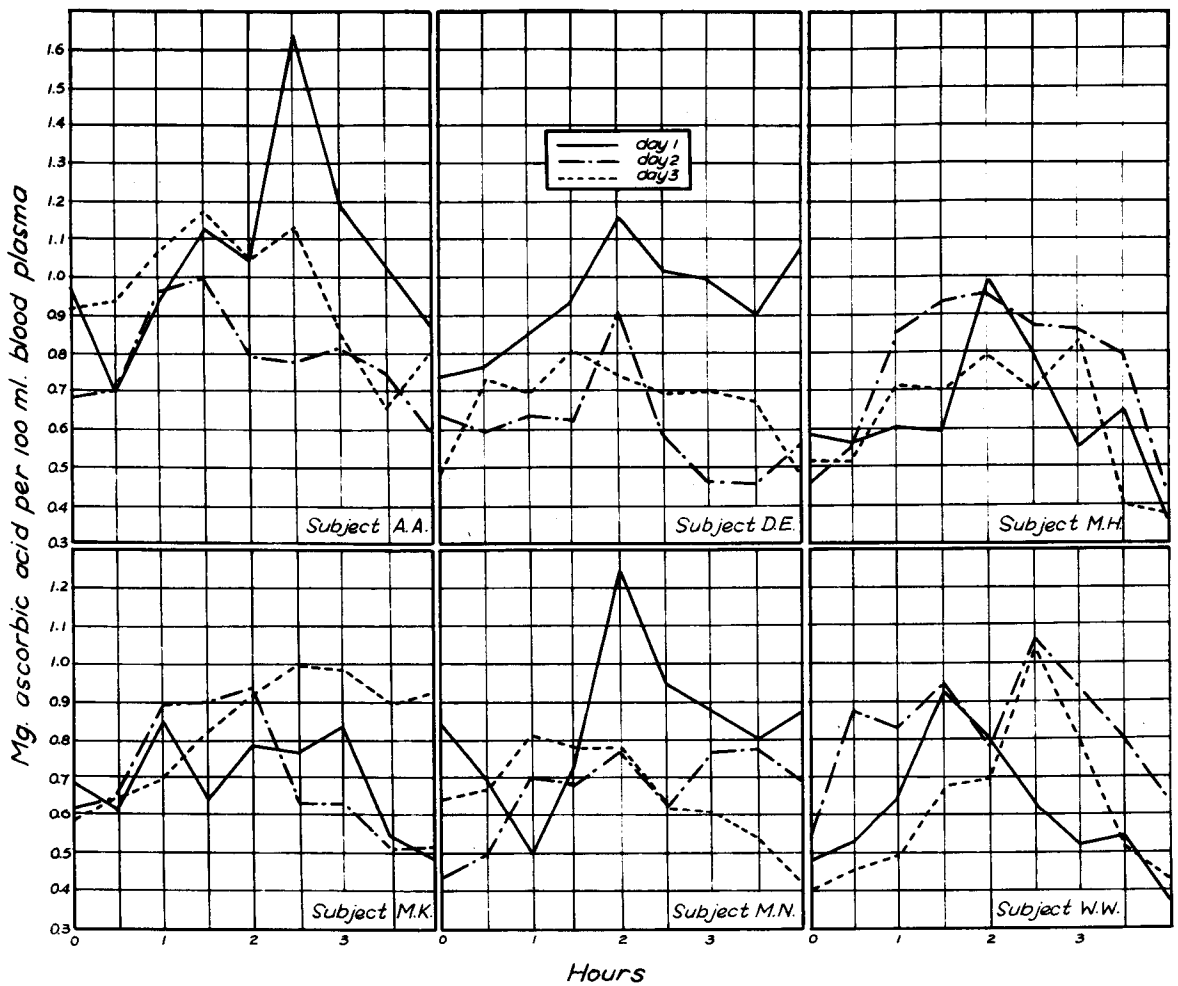

Fig. 10. Concentration of ascorbic acid in plasma in six subjects immediately preceding and at intervals following the ingestion of a standard vitamin C-free meal plus 250 gm. of apple containing approximately 50 mg. of ascorbic acid.

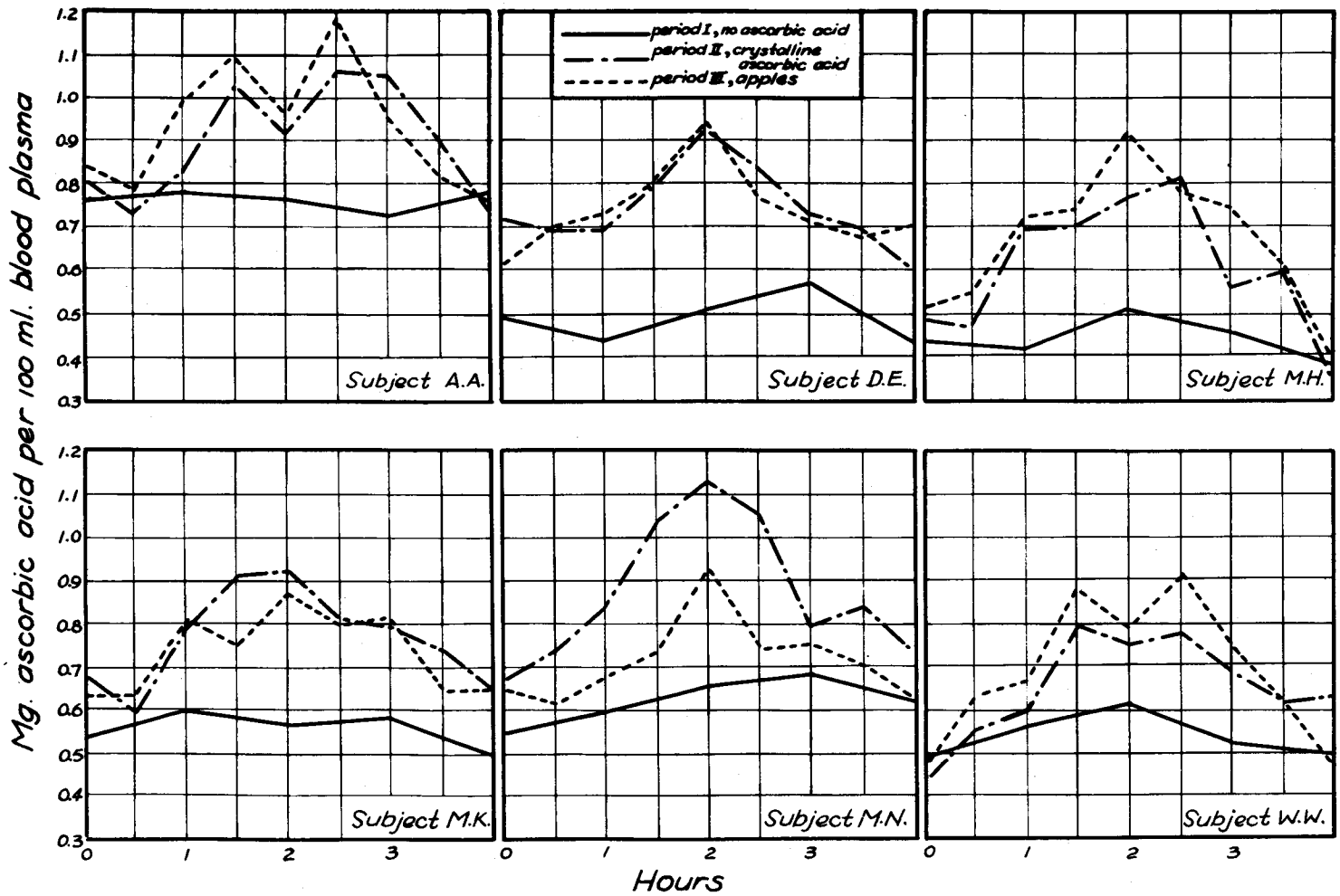

Fig. 11. Average concentration of ascorbic acid in plasma in six subjects immediately preceding and at intervals following the ingestion of a standard meal free of vitamin C or containing 50 mg. of crystalline ascorbic acid or its equivalent in apples.

the blood of D. E. after the ingestion of pure ascorbic acid and of apple are practically identical. Subject M. N. seemed to absorb the crystalline ascorbic acid better than that from the apples. The response of the remaining four subjects, A. A., M. H., M. K., and W. W. was substantially the same to ascorbic acid in apple as to the pure vitamin.

The average response of all subjects in all periods is shown in figure 12. The curves corresponding to the periods in which ascorbic acid was ingested as the crystalline substance and as apple are strikingly similar.

Examination of the residues remaining after thorough washing of the stools indicated that the apple was, in all cases, completely digested. There is reasonable certainty, then, that the increases noted represent the response of the subjects to 50 mg. of food ascorbic acid.

SUMMARY

The data herein presented show that the ascorbic acid in the Willow Twig apple is as readily and effectively absorbed by the human being as is an equivalent amount of crystalline ascorbic acid.

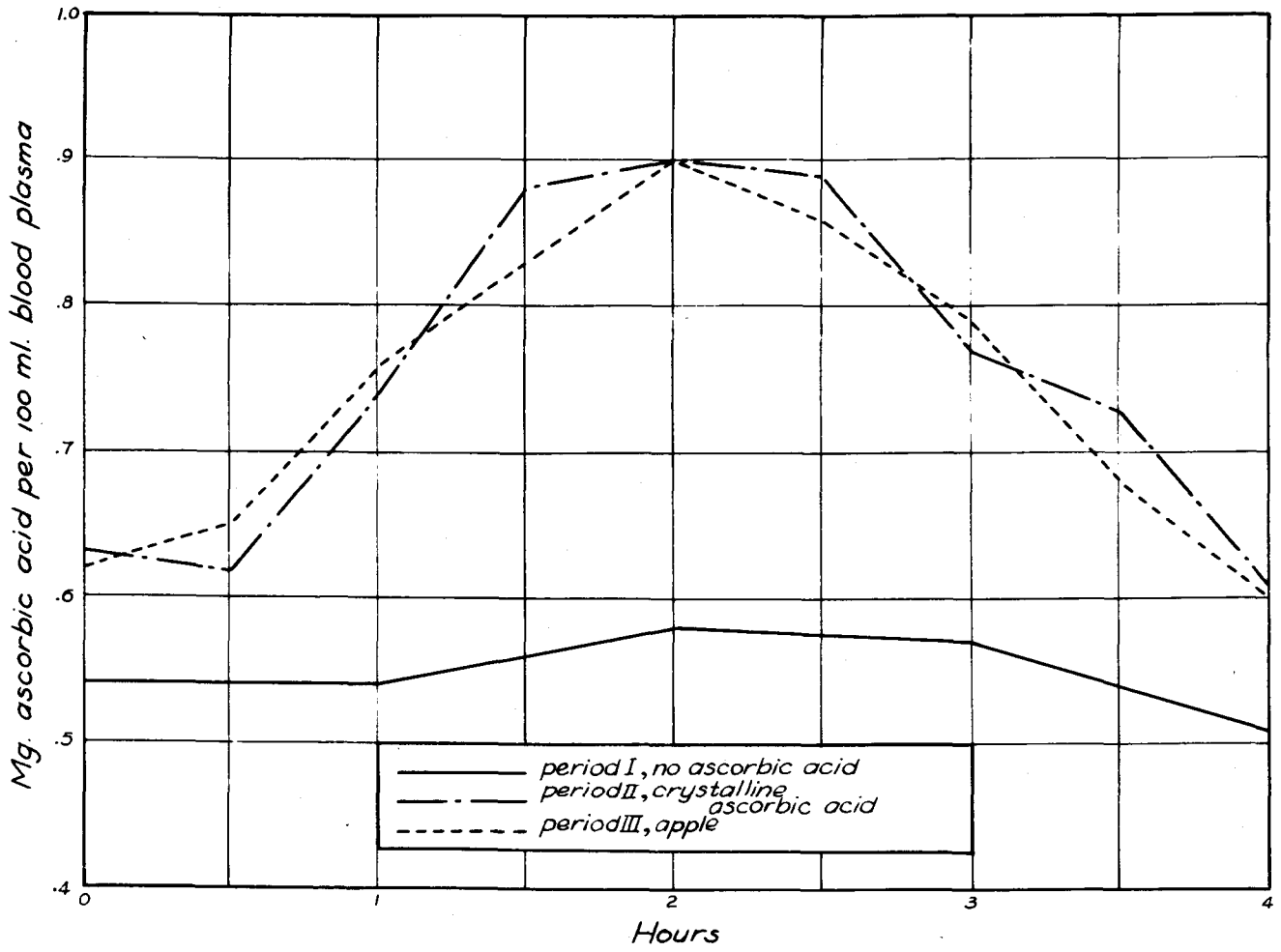

Fig. 12. Average concentration of ascorbic acid in plasma in six subjects immediately preceding and at intervals following the ingestion of a standard meal free of vitamin C or containing 50 mg. of crystalline ascorbic acid or its equivalent in apples.

SUMMARY AND CONCLUSIONS

In the investigation herein reported, and attempt has been made to determine the true nutritive value of the midwestern apple as a dietary source of vitamin C.

Before any of the contemplated studies could be made, it was necessary to arrive at some decision as to what represented an adequate sample of apples for the estimation of mean ascorbic acid content. A fairly comprehensive study of the problem was made, results of which are incorporated in the present report. The great difference in the concentration of vitamin C of individual apples taken from the same tree and the even greater differences in the ascorbic acid content of apples produced by different trees showed the importance of using a large number of apples in a sample and of an equal distribution within the sample of apples derived from different trees. In addition, the apples from any one tree had to represent fruit picked from all sides of the tree. The difference in the concentration of ascorbic acid in apples from the north and the south sides of trees illustrates this point. The vitamin C concentrations of successive samples made up of 20 apples each representing both the north half and the south half

of each of ten trees, were not statistically different from each other. A good base was thus provided for the study of the effect of any processing treatment on the ascorbic acid content of this fruit.

A comprehensive study was made of the concentration of ascorbic acid in different varieties of apples when mature and freshly picked. Analyses of 29 varieties appear in the present report. The quantity of vitamin C present was determined chemically with the use of 2,6-dichlorophenolindophenol, and the amount of dye decolorized when a measured quantity of sample reacted with an excess of dye was determined photocolorimetrically. The results obtained show that the concentration of ascorbic acid in all varieties of apples studied with the exception of the Willow Twig and its sport, the Red Willow, is fairly low, averaging about 6 mg. per cent. The Willow Twig, however, as analyzed over a period of three years, contained quantities of ascorbic acid ranging from 19 to 23 mg. per 100 gm. of tissue. The data seem to indicate that the concentration of ascorbic acid in apples is an inherent varietal characteristic. The importance of the Willow Twig apple as a source of vitamin C in the diet is apparent. Even varieties less rich in ascorbic acid, due to

quantities consumed by many groups of people, may contribute a considerable portion of the daily requirement of this vitamin.

Because apples have good keeping qualities, large quantities are stored for use during the winter months when they become one of the important fruits in the average dietary. Whether or not apples which have been held in storage are a dependable source of vitamin C has been studied only in part heretofore. The relative stability of this vitamin in several varieties, therefore, was tested following different storage treatments. The data indicate that, in general, more of the ascorbic acid present in the freshly picked fruit is retained when the apples are held at 32° F. than when the storage temperature is higher. The stability of the ascorbic acid in apples during storage seems to be determined by the variety of apple, and in some cases by the storage treatment. The vitamin C content of all varieties except the Willow Twig decreases rapidly during the first few weeks of storage. Thereafter the losses are small. In direct contrast to other varieties, a significant synthesis of ascorbic acid occurs in the Willow Twig during a seven month storage period.

Whether analyses made in the chemical laboratory

depict the actual nutritive value of a food has been questioned. In the present instance, the analytical method chosen is not absolutely specific for ascorbic acid inasmuch as certain other substances of no nutritive value also reduce the dye. Furthermore, the method used measures the reduced ascorbic acid present, but not the dehydroascorbic acid, a substance also possessing anti-scorbutic activity. For these reasons, the data obtained were evaluated in terms of dietary significance. It was found that small quantities of dehydroascorbic acid and traces of reductones may be present in freshly picked applés. In general, however, the error introduced by the reductones seems to be offset by the dehydroascorbic acid present, so that actually the determination with the indophenol dye gives a fair approximation of the true vitamin C value of the apple. It is very interesting that reductones could not be detected in the Willow Twig apples even after they had been stored for six months. This finding is in line with the conclusion that the ascorbic acid in this variety of apple is remarkably stable. Determinations of the concentration of dehydroascorbic acid and reductones in other varieties after different intervals of storage are being made in the laboratory at the present time. It will be of

considerable interest to learn if reduced concentrations of ascorbic acid following storage are associated with the development of either dehydroascorbic acid or reductones.

The inherently high concentration of ascorbic acid in the Willow Twig indicates the possibility of producing through a breeding program varieties of apples as valuable as the tomato as contributors of vitamin C to the diet. The importance of such a program to enhance the nutritive value of apples is strengthened by the finding that 100 per cent of the ascorbic acid of the Willow Twig apple is absorbed by the human subject and is, therefore, available for metabolic utilization. Whether the ascorbic acid of the apple withstands the processes of mastication and digestion has been questioned, but data herein presented clearly show that the chemical determination of the vitamin C concentration of this apple variety represents its true nutritive value.

LITERATURE CITED

Becker, E., and diGleria, J.
1937. Potentiometric determination of vitamin C (ascorbic acid).
Z. Vitaminforschung, 6, 86-94.

Bessey, O. A.
1938. A 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.
J. Biol. Chem., 126, 771-784.

Birch, T. W., Harris, L. J., and Ray, S. N.
1933. A micro-chemical method for determining the hexuronic acid (vitamin C) content of foodstuffs, etc.
Biochem. J., 27, 590-594.

Bracewell, M. F., Hoyle, E., and Zilva, S. S.
1930a. The antiscurvy vitamin in apples.
Med. Res. Council, Spec. Rept. Ser., 146.

Bracewell, M. F., Hoyle, E., and Zilva, S. S.
1930b. The antiscorbutic potency of apples.
Biochem. J., 24, 82-90.

Clark, R. M.
1941. Twenty-four years experience with hardy intermediate apple stocks.
Trans. Iowa Hort. Soc., 76, 44-54.

Cohen, B., Gibbs, H. D., and Clark, W. M.
1924. Oxidation-reduction studies VI. Indophenols. (A) Dibromo substitution products of phenolindophenol. (B) Substituted indophenols of the o-type. (C) Miscellaneous.
U. S. Publ. Health Service. Publ. Health Rept., 39, 804-823.

Cohen, B., and Mendel, L. B.
1918. Experimental scurvy of the guinea pig in relation to the diet.
J. Biol. Chem., 35, 425-453.

Curran, K. M., Tressler, D. K., and King, C. G.
1937. Losses of vitamin C during cooking of Northern Spy apples.
Food Res., 2, 549-557.

Dove, W. F., and Murphy, E.
1936. The vitamin C content of apples and its relation to human welfare.
Science, 83, 325-327.

Edgecombe, S. W.
1941. Lessons from demonstration orchards in Iowa.
Trans. Iowa Hort. Soc., 76, 73-91.

Eheart, M. S.
1941. Factors which affect the vitamin C content of apples.
Va. Agr. Exp. Sta. Tech. Bul., 69.

Farmer, C. J., and Abt., A. F.
1936. Determination of reduced ascorbic acid in small amounts of blood.
Proc. Soc. Exp. Biol. and Med., 34, 146-150.

Fellers, C. R., Cleveland, M. M., and Clague, J. A.
1933. Vitamin C in Baldwin apples, juice, cider, and apple sauce.
J. Agr. Res., 46, 1039-1045.

Fellers, C. R., Isham, P. D., and Smith, G. G.
1932. Vitamin C distribution in Baldwin and McIntosh apples.
Proc. Amer. Soc. Hort. Sci., 29, 93-97.

Fish, V. B., Dustman, R. B., and Marsh, R. S.
1944. The ascorbic acid content of several varieties of apples grown in West Virginia.
Proc. Amer. Soc. Hort. Sci., 44, 196-200.

Fujita, A., and Iwatake, D.
1935. Uber die Bestimmung von Vitamin C mittels 2,6-Dichloro-phenol-indophenol.
Biochem. Z., 277, 293-295.

Gould, B. S., and Shwachman, H.
1943. A new method for the bioassay of antiscorbutic substances. Assays of dehydroascorbic acid, 2-ketogulonic acid, iron ascorbate, and the effectiveness of oral and parenteral administration of ascorbic acid.
J. Biol. Chem., 151, 439-453.

Haller, M. H.
1941. Fruit pressure testers and their practical applications.
U. S. Dept. of Agr. Cir., 627.

Harris, L. J.
1933. Chemical test for vitamin C, and the reducing substances present in tumor and other tissues.
Nature, 132, 27-28.

Harris, L. J., Mapson, L. W., and Wang, Y. L.
1942. Vitamin methods IV. A simple potentiometric method for determining ascorbic acid, suitable for use with coloured extracts.
Biochem. J., 36, 183-195.

Harris, L. J., and Ray, S. N.
1933a. Vitamin C and the suprarenal cortex II. Loss of potency of guinea-pig suprarenals in scurvy. With notes on a method for determining anti-scorbutic activity (hexuronic acid) by chemical means.
Biochem. J., 27, 303-310.

Harris, L. J., and Ray, S. N.
1933b. Specificity of hexuronic (ascorbic) acid as antiscorbutic factor.
Biochem. J., 27, 580-589.

Harris, L. J., and Ray, S. N.
1933c. Standardization of the antiscorbutic potency of ascorbic acid.
Biochem. J., 27, 2016-2021.

Hauck, M. M.
1945. Physiological availability of ascorbic acid in foods. I. Cabbage.
Unpublished Thesis, M. S., Library, Iowa State College, Ames, Iowa.

Herrick, R. S.
1941. Report of the Secy-Treas. of the Iowa Fruit Growers Assoc.
Trans. Iowa Hort. Soc., 76, 98-102.

Herrick, R. S.
1942. Report of the Secy-Treas. of the Iowa Fruit Growers Assoc.
Trans. Iowa Hort. Soc., 77, 103-107.

Johansson, E.
1939. Determinations of ascorbic acid content of fruits and fruit products, some vegetables, and other plants (English Summary).
Årsskr. Alnarps Lantbr. Mejeri. o. Trädgårdsinst., 1939, 1-53.

Kessler, W.
1939. Über den Vitamin C-Gehalt deutscher Apfelsorten und seine Abhängigkeit von Herkunft, Lichtgenuss, Düngung, Dichte des Behanges und Lagerung.
Gartenbauwiss. 13, 619-638.

Keys, C. H.
1942. Vitamin C in apples and other materials.
New Zealand J. Sci. and Tech., 24 B, 146-148.

Kidson, E. B.
1944. The vitamin C content of Nelson apples.
New Zealand J. Sci. and Tech., 25B, 134-136.

King, C. G.
1941. Chemical methods for determination of vitamin C.
Indust. and Eng. Chem., Anal. Ed., 13, 225-227.

Kirk, M. M., and Tressler, D. K.
1939. Determination of ascorbic acid. Electrometric titration method.
Indust. and Eng. Chem., Anal. Ed., 11, 322-323.

Leverton, R. M., and Werner, H. O.
1944. Relative importance of various factors in the sampling of potatoes for ascorbic acid content.
Progress Notes. National Coop. Proj. Conservation of Nutr. Value of Foods. State Agr. Exp. Sta. and U. S. Dept. of Agr. cooperating.

Loeffler, H. J., and Ponting, J. D.
1942. Ascorbic acid. Rapid determination in fresh, frozen, or dehydrated fruits and vegetables.
Indust. and Eng. Chem., Anal. Ed., 14, 846-848.

Lugg, J.W.H.
1942. The use of formaldehyde and 2,6-dichlorophenol-indophenol in the estimation of ascorbic acid and dehydroascorbic acid.
Australian J. Exp. Biol. and Med. Sci., 20, 273-285.

Lund, H., Spur, B., and Fridericia, L. S.
1934. The biological and titrimetric determination of vitamin C.
Biochem. J., 28, 1825-1828.

Mapson, L. W.
1942. Vitamin methods V. A note on the determination of ascorbic acid in fruits and vegetables in the presence of SO_2.
Biochem. J., 36, 196-202.

Mapson, L. W.
1943a. Ascorbic acid in dehydrated foods.
Nature, 152, 13-14.

Mapson, L. W.
1943b. Vitamin methods VI. The estimation of ascorbic acid in the presence of reductones and allied substances.
J. Soc. Chem. Indust., 62, 223-232.

Marsh, R. S.
1943. Preliminary report on studies concerning the nutritive value of apples.
The Mountaineer Grower, 14, 4-12.
(Original not seen; cited in U. S. Dept. of Agr. Misc. Pub. 536, 1944.)

Maynard, L. A.
1944. Food production for better health and longer life.
In Conference on Nutrition, Detroit, 1944. Implications of nutrition and public health in the postwar period.
p. 7-19. Children's Fund of Michigan.

Miller, J. C.
1944. Presidential address.
Proc. Amer. Soc. Hort. Sci., 44, 552-556.

Mindlin, R. L., and Butler, A. M.
1937-38. The determination of ascorbic acid in plasma; a macromethod and a micromethod.
J. Biol. Chem., 122, 673-686.

Morell, S. A.
1941. Rapid photometric determination of ascorbic acid in plant materials.
Indust. and Eng. Chem., Anal. Ed., 13, 793-794.

Murneek, A. E.
1943. Changes in ascorbic acid content of apples during storage and when cooked.
Quarterly report, North Central Region, National Coop. Exp. Sta. Proj., Conservation of Nutr. Value of Foods. State Exp. Sta. and U. S. Dept. of Agr. cooperating.

Murneek, A. E.
1945. Environmental factors affecting ascorbic acid content of apples while maturing on the tree.
Progress Notes. National Coop. Proj., Conservation of Nutr. Value of Foods. State Exp. Sta. and U. S. Dept. of Agr. cooperating.

Murphy, E.
1938. Vitamin C and light.
Proc. Amer. Soc. Hort. Sci., 36, 498-499.

Musulin, R. R., and King, C. G.
1936. Metaphosphoric acid in the extraction and titration of vitamin C.
J. Biol. Chem., 116, 409-413.

National Research Council. Food and Nutrition Board.
1945. Recommended dietary allowances, revised 1945.
National Research Council Reprint and Circular Series No. 122.

Olliver, M.
1936. Ascorbic acid content of fruits and vegetables with special reference to the effect of cooking and canning.
J. Soc. Chem. Indust., 55, 153-163T.

Paech, K.
1938. Uber den vitamin C-Gehalt deutscher Apfeln.
Z. Untersuch. Lebensmitt., 76, 234-239.

Plagge, H. H., Maney, A. J., and Gerhardt, F.
1926. Certain physical and chemical changes of Grimes apples during ripening and storage period.
Iowa Agr. Exp. Sta. Res. Bul. 91.

Ponting, J. D.
1943. Extraction of ascorbic acid from plant materials. Relative suitability of various acids.
Indust. and Eng. Chem., Anal. Ed., 15, 389-391.

Potter, M. T.
1933. The vitamin C content of the Winesap apple as influenced by fertilizers.
J. Agr. Res., 46, 367-373.

Ray, S. N.
1934. On the nature of the precursor of the vitamin C in the vegetable kingdom I. Vitamin C in the growing pea seedling.
Biochem. J., 28, 996-1003.

Roe, J. H.
1936. The determination of ascorbic acid as furfural and a comparison of results obtained by this method and by indophenol titration.
J. Biol. Chem., 116, 609-619.

Roe, J. H., and Hall, J. M.
1939. The vitamin C content of human urine and its determination through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid.
J. Biol. Chem., 128, 329-337.

Roe, J. H., and Kuether, C. A.
1943. The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid.
J. Biol. Chem., 147, 399-407.

Roe, J. H., and Oesterling, M. J.
1944. The determination of dehydroascorbic acid and ascorbic acid in plant tissues by the 2,4-dinitrophenylhydrazine method.
J. Biol. Chem., 152, 511-517.

Rosenberg, H. R.
1942. Chemistry and physiology of the vitamins. p. 291.
Interscience Publishers, Inc., New York.

Ruffley, J., Clague, J. A., and Fellers, C. R.
1939. Canned baked apples. Determining suitable varieties for this pack; grading; factors in baking quality; vitamin retention and loss.
Canning Age, 20, 179-181.

Schlaphoff, E., and Leverton, R. M.
1945. Sampling for the determination of ascorbic acid in tomatoes: number of fruits and whole fruit versus sections of fruit.
Progress Notes. National Coop. Proj., Conservation of Nutr. Value of Foods. State Exp. Sta. and U. S. Dept. of Agr. cooperating.

Sherman, H. C., La Mer, V. K., and Campbell, H. L.
1922. The quantitative determination of the anti-scorbutic vitamin (vitamin C).
J. Amer. Chem. Soc., 44, 165-172.

Smith, T.
1897. Swine erysipelas or mouse-septicaemia bacilli from an outbreak of swine disease.
U. S. Bur. An. Indust., Ann. Rept. 12-13, p. 172.

Talk of the Industry.
1942. Astonishing losses of vitamin C.
Food Industries, 14, No. 9, 39.

Tillmans, J.
1930. Das antiskorbutische Vitamin.
Z. Untersuch. Lebensmitt., 60, 34-44.

Tillmans, J., Hirsch, P., and Jackisch, J.
1932a. Das Reduktionsvermogen pflanzlicher Lebensmittel und seine Beziehung zum Vitamin C. III. Der Gehalt der verschiedenen Obst-und Gemüsearten an reduzierendem Stoff.
Z. Untersuch. Lebensmitt., 63, 241-267.

Tillmans, J., Hirsch, P., and Jackisch, J.
1932b. Das Reduktionsvermogen pflanzlicher Lebensmittel und seine Beziehung zum Vitamin C. V. Die antiskorbutische Wirkung verschiedener Auszüge der Gurke.
Z. Untersuch. Lebensmitt., 63, 276-283.

Todhunter, E. N.
1939. Further studies on the vitamin A and C content of Washington grown apples.
Wash. Agr. Exp. Sta. Bul., 375.

Todhunter, E. N.
1943. Some factors affecting the ascorbic acid value of fruits and vegetables of the Pacific Northwest. Proc. Pac. Sci. Congr., 6th, Berkeley, Calif., 6, 389-391.

Todhunter, E. N., Robbins, R. C., and McIntosh, J. A.
1942. The rate of increase of blood plasma ascorbic acid after ingestion of ascorbic acid (vitamin C). J. Nutr., 23, 309-319.

United States Department of Agriculture.
1943. Agricultural statistics 1943. U. S. Gov't. Printing Office, Washington, D. C.

West, C., and Zilva, S. S.
1944. Synthesis of vitamin C in stored apples. Biochem. J., 38, 105-108.

Zilva, S. S.
1927. The antiscorbutic fraction of lemon juice. Biochem. J., 21, 689-697.

Zilva, S. S.
1932. The non-specificity of the phenolindophenol reducing capacity and its fractions as a measure of their antiscorbutic activity. Biochem. J., 26, 1624-1627.

Zilva, S. S., Kidd, F., and West, C.
1930. Vitamin content of apples. Food Invest. Bd. Rept., Gt. Brit. Sci. and Indust. Res. Dept. 1930, p. 65-66.

Zilva, S. S., Kidd, F., West, C., and Perry, E. O. V.
1934. Vitamin C content of apples. Food Invest. Bd. Rept., Gt. Brit. Sci. and Indust. Res. Dept. 1934, p. 164-165.

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APPENDIX

APPENDIX TABLE OF CONTENTS

Table 1

Concentration of ascorbic acid in various dilutions of standard solutions and photoelectric colorimeter scale divisions

Standard solution number	Dilution	Concentration of ascorbic acid in sample	Scale divisions
		mg. per ml.	
1	a	.005200	13.0
	b	.005200	13.6
2	a	.005416	13.0
	b	.005416	11.9
3	a	.004323	7.7
	b	.004323	8.0
4	a	.007478	16.4
	b	.007478	13.6
5	a	.003716	6.8
	b	.003716	8.0
6	a	.007153	15.8
	b	.007153	16.0
7	a	.003224	7.7
	b	.003224	8.5
8	a	.006282	14.8
	b	.006282	14.8
9	a	.004254	6.3
	b	.004254	8.3
10	a	.006921	11.8
	b	.006921	14.6
11	a	.005884	13.9
	b	.005884	14.0
12	a	.004473	8.2
	b	.004473	8.3
13	a	.007581	17.4
	b	.007581	18.7
	c	.007581	18.9
14	a	.003219	9.2
	b	.003219	8.7
	c	.003219	9.0
15	a	.008352	21.1
	b	.008352	20.5
	c	.008352	19.0

Continued on next page

Table 1 (Continued)

Standard solution number	Dilution	Concentration of ascorbic acid in sample	Scale divisions
		mg. per ml.	
16	a	.001968	3.5
	b	.001968	4.7
	c	.001968	3.6
17	a	.004541	8.5
	b	.004541	7.2
	c	.004541	8.5
18	a	.005298	9.3
	b	.005298	9.1
	c	.005298	10.9
19	a	.004155	9.5
	b	.004155	11.0
	c	.004155	10.0
20	a	.005959	13.2
	b	.005959	13.2
	c	.005959	14.7
21	a	.006967	17.9
	b	.006967	16.8
	c	.006967	16.0
22	a	.004241	10.2
	b	.004241	8.8
	c	.004241	10.3
23	a	.005082	13.5
	b	.005082	12.3
	c	.005082	12.8
24	a	.004158	9.9
	b	.004158	11.3
	c	.004158	11.1
25	a	.005034	11.9
	b	.005034	10.8
	c	.005034	8.7
26	a	.005050	9.2
	b	.005050	10.4
	c	.005050	9.6
27	a	.004824	10.8
	b	.004824	11.1
	c	.004824	10.9

Continued on next page

Table 1 (Continued)

Standard solution number	Dilution	Concentration of ascorbic acid in sample	Scale divisions
		mg. per ml.	
28	a	.003136	3.5
	b	.003136	5.2
	c	.003136	7.6
29	a	.002877	5.8
	b	.002877	11.7
	c	.002877	7.5
30	a	.006625	14.9
	b	.006625	13.7
	c	.006625	15.5
31	a	.003522	10.1
	b	.003522	7.4
	c	.003522	7.1
32	a	.006040	15.2
	b	.006040	13.9
	c	.006040	16.2
33	a	.003418	10.0
	b	.003418	9.7
	c	.003418	8.8
34	a	.005455	12.5
	b	.005455	12.7
35	a	.004026	9.9
	b	.004026	9.5
	c	.004026	9.0
36	a	.006425	14.7
	b	.006425	15.6
	c	.006425	14.8
37	a	.01059	27.3
	b	.02118	54.1
	c	.03177	81.4
	d	.04237	108.4
	e	.05296	135.0
38	a	.008632	21.7
	b	.01726	43.7
	c	.02589	67.8
	d	.03453	88.6
	e	.04316	112.3
39	a	.003072	9.9
	b	.003072	6.4
	c	.003072	7.3

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Table 1 (Continued)

Standard solution number	Dilution	Concentration of ascorbic acid in sample	Scale divisions
		mg. per ml.	
40	a	.006338	16.5
	b	.006338	17.2
	c	.006338	16.5
41	a	.003336	6.9
	b	.003336	7.4
	c	.003336	7.2
42	a	.004530	11.7
	b	.004530	11.5
	c	.004530	11.0
43	a	.007287	18.8
	b	.007287	19.8
	c	.007287	19.1
44	a	.006440	15.9
	b	.006440	15.2
	c	.006440	15.6
45	a	.005071	12.7
	b	.010143	25.0
	c	.02029	50.0
	d	.03043	76.4
46	a	.005118	12.4
	b	.01024	24.9
	c	.04095	101.4
47	a	.009455	21.8
	b	.009455	22.0
	c	.009455	20.8
	d	.03782	94.0
48	a	.009334	23.0
	b	.009344	23.0
	c	.009344	22.3
	d	.03734	94.3
49	a	.01218	30.9
	b	.02436	62.6
	c	.03654	91.6
	d	.04872	124.7
50	a	.008767	21.2
	b	.008767	21.7
	c	.008767	21.2
51	a	.01031	24.9
	b	.01031	24.9
	c	.01031	25.5

Table 2

Concentration of dehydroascorbic acid in various dilutions of standard solutions and photoelectric colorimeter scale divisions

Standard solution number	Conc. of dehydroascorbic acid in sample	Scale divisions
	mcgm. per ml.	
1	0.398	5.5
	1.195	27.5
	1.593	35.5
	1.992	48.0
2	0.623	14.0
	1.039	26.3
3	0.785	19.0
	1.570	37.5
4	1.254	28.5
	1.505	35.0
	1.755	39.5
	2.006	45.2
5	0.242	1.7
	0.484	11.0
	0.727	19.0
	0.969	24.7
	1.211	30.7
	1.453	36.0
	1.695	41.0
	1.938	46.0

Table 3

Concentration (mg. per cent) of ascorbic acid in plasma day by day in experimental period I (no ascorbic acid) as observed in six subjects

Time in hours*	Subject A.A.			Subject D.E.		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
0	0.75	0.61	0.93	0.51	0.56	0.41
1	0.85	0.60	0.88	0.44	0.47	0.39
2	0.74	0.67	0.88	0.53	0.47	0.51
3	0.65	0.71	0.80	0.53	0.69	0.45
4	0.77	0.71	0.84	0.53	0.39	0.36
	Subject M.H.			Subject M.K.		
0	0.53	0.42	0.35	0.50	0.50	0.60
1	0.48	0.36	0.38	0.60	0.46	0.71
2	0.61	0.57	0.34	0.47	0.45	0.75
3	0.51	0.54	0.34	0.51	0.49	0.70
4	0.45	0.37	0.33	0.50	0.36	0.60
	Subject M.N.			Subject W.W.		
0	0.62	0.53	0.45	0.55	0.45	0.45
1	0.64	0.57	0.57	0.62	0.46	0.31
2	0.82	0.57	0.56	0.54	0.49	0.50
3	0.69	0.65	0.68	0.44	0.37	0.44
4	0.73	0.57	0.52	0.46	0.47	0.26

*0 time represents fasting level

Table 4

Concentration (mg. per cent) of ascorbic acid in plasma day by day in experimental period II (50 mg. crystalline ascorbic acid) as observed in six subjects

Time in hours*	Subject A.A.			Subject D.E.		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
0	0.90	0.66	0.86	0.58	0.82	0.72
½	0.65	0.67	0.86	0.70	0.77	0.60
1	0.85	0.73	0.91	0.71	0.75	0.60
1½	1.07	0.96	1.04	0.87	0.72	0.78
2	0.97	0.85	0.91	0.83	1.08	0.86
2½	1.25	0.91	1.02	0.65	0.99	0.88
3	1.23	0.91	1.01	0.63	0.95	0.61
3½	0.77	0.85	1.07	0.71	0.71	0.66
4	0.71	0.56	0.92	0.39	0.84	0.56
	Subject M.H.			Subject M.K.		
0	0.46	0.42	0.55	0.69	0.75	0.56
½	0.39	0.48	0.50	0.64	0.53	0.60
1	0.67	0.78	0.62	0.61	0.98	0.80
1½	0.70	0.78	0.63	0.94	0.84	0.95
2	0.60	0.96	0.76	0.87	0.92	0.98
2½	0.82	1.00	0.62	0.73	0.87	0.87
3	0.71	0.50	0.47	0.62	1.11	0.66
3½	0.73	0.54	0.53	0.69	0.88	0.64
4	0.27	0.48	0.26	0.60	0.66	0.66
	Subject M.N.			Subject W.W.		
0	0.62	0.52	0.83	0.41	0.42	0.49
½	0.63	0.60	0.97	0.50	0.60	0.55
1	0.75	0.91	0.86	0.52	0.39	0.89
1½	1.07	0.95	1.10	0.80	0.80	0.80
2	0.66	1.21	1.48	0.68	0.78	0.78
2½	0.74	1.10	1.31	0.78	0.80	0.75
3	0.73	0.76	0.89	0.38	0.81	0.87
3½	1.03	0.60	0.90	0.40	0.65	0.81
4	0.62	0.80	0.76	0.46	0.63	0.81

*0 time represents fasting level

Table 5

Concentration (mg. per cent) of ascorbic acid in plasma day by day in experimental period III (50 mg. ascorbic acid as apple) as observed in six subjects

Time in hours*	Subject A.A.			Subject D.E.		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
0	0.97	0.69	0.92	0.73	0.63	0.47
½	0.70	0.70	0.94	0.76	0.60	0.73
1	0.94	0.96	1.07	0.84	0.63	0.69
1½	1.12	0.99	1.18	0.94	0.62	0.81
2	1.04	0.79	1.04	1.15	0.91	0.73
2½	1.65	0.77	1.14	1.01	0.58	0.69
3	1.19	0.81	0.85	0.98	0.46	0.70
3½	1.03	0.75	0.65	0.90	0.46	0.67
4	0.87	0.59	0.81	1.07	0.56	0.47
	Subject M.H.			Subject M.K.		
0	0.58	0.45	0.51	0.68	0.61	0.59
½	0.56	0.55	0.51	0.61	0.64	0.64
1	0.60	0.85	0.71	0.84	0.89	0.70
1½	0.59	0.94	0.70	0.54	0.90	0.82
2	1.00	0.96	0.79	0.77	0.93	0.92
2½	0.79	0.88	0.70	0.76	0.63	0.99
3	0.55	0.86	0.83	0.83	0.63	0.98
3½	0.65	0.80	0.40	0.53	0.50	0.90
4	0.36	0.42	0.38	0.47	0.52	0.93
	Subject M.N.			Subject W.W.		
0	0.84	0.43	0.64	0.47	0.54	0.40
½	0.69	0.49	0.66	0.52	0.88	0.45
1	0.50	0.70	0.81	0.65	0.83	0.49
1½	0.75	0.67	0.77	0.92	0.95	0.77
2	1.24	0.77	0.77	0.80	0.78	0.79
2½	0.95	0.63	0.63	0.63	1.06	1.05
3	0.87	0.76	0.61	0.52	0.93	0.81
3½	0.80	0.77	0.54	0.55	0.79	0.52
4	0.87	0.59	0.41	0.38	0.63	0.43

*0 time represents fasting level

AN ANNOTATED LIST OF REFERENCES

METHODOLOGY

Cohen, B., and Mendel, L. B.
1918. Experimental scurvy of the guinea pig in relation to the diet.
J. Biol. Chem., 35, 425-453.

Scurvy may be produced experimentally in guinea pigs with suitably chosen diets. The nature and sequence of symptoms is described.

Sherman, H. C., LaMer, V. K., and Campbell, H. L.
1922. The quantitative determination of the antiscorbutic vitamin (vitamin C).
J. Amer. Chem. Soc., 44, 165-172.

Relative amounts of vitamin C in different foods may be measured by determining how much of each food is required to prevent scurvy in guinea pigs 6 to 8 weeks old and weighing 300-350 gm. When less than the "minimum protective dose" is fed, the severity of the scurvy produced is given a quantitative rating based on the weight curve, duration of life, symptoms, and autopsy findings. The basal diet is designed to furnish optimum quantities of all known essential nutrients other than vitamin C.

Bracewell, M. F., Hoyle, E., and Zilva, S. S.
1930. The antiscurvy vitamin in apples.
Med. Res. Council, Spec. Rept. Ser., 146.

The method of estimation of vitamin C depends on the degree of protection from scurvy which the substance tested affords to young guinea pigs subsisting on a scorbutic diet. Guinea pigs weighing 250-300 gm. were used. The scorbutic diet was made up of:

Bran	6	parts	by	volume
Barleymeal	2	"	"	"
Middlings	3	"	"	"
Fishmeal	1	"	"	"
Crushed oats	4	"	"	"

It was offered ad libitum in addition to 40-60 cc. of autoclaved milk made up from a dried powder. Fed this diet, guinea pigs succumbed to scurvy within 4-5 weeks.

Weight curves of guinea pigs used to test the antiscorbutic potency of a number of varieties of apples are shown.

Bessey, O. A., and King, C. G.
1933. The distribution of vitamin C in plant and animal tissues, and its determination.
J. Biol. Chem., 103, 687-698.

The method presented is a modification of one proposed by Tillmans, Hirsch, and Jackisch (Z. Untersuch. Lebensmitt., 63, 241-246, 1932) for the direct titration of vitamin C. Experiments are reported which demonstrate its value in physiological studies.

Birch, T. W., Harris, L. J., and Ray, S. N.
1933. A micro-chemical method for determining the hexuronic acid (vitamin C) content of foodstuffs, etc.
Biochem. J., 27, 590-594.

A detailed description of an adaptation of the dye titration method (Harris and Ray, Biochem. J., 27, 303-310, 1933) to a micro-chemical scale is given. Results are shown for a large number of foodstuffs analyzed by this method and by biological test.

Harris, L. J.
1933. Chemical test for vitamin C, and the reducing substances present in tumor and other tissues.
Nature, 132, 27-28.

The method for estimating the ascorbic acid content of foodstuffs, based on titration in acid solution with 2,6-dichloro-phenol-indophenol was applied to forty common sources of the vitamin and gave results in excellent agreement with values determined by the biological method.

Certain materials other than ascorbic acid also reduce the indicator. Some materials which react appreciably with the dye are free cysteine, products obtained by heating solutions of certain sugars (especially in alkaline media), yeast, whole oats, and incubated pea mush.

Harris, L. J., and Ray, S. N.
1933. Vitamin C and the suprarenal cortex II. Loss of potency of guinea-pig suprarenals in scurvy. With notes on a method for determining anti-scorbutic activity (hexuronic acid) by chemical means.
Biochm. J., 27, 303-310.

A method is described in which an acid extract of the test material is titrated with 2,6-dichlorophenolindophenol. The dye is standardized against iodine.

Emmerie, A.
1934. Separation of cysteine from ascorbic acid by mercuric acetate.
Biochem. J., 28, 268-269.

Cysteine, an interfering substance in the determination of ascorbic acid by means of 2,6-dichlorophenolindophenol solution, may be precipitated by mercuric acetate.

Tauber, H., and Kleiner, I. S.
1935. A method for the quantitative determination of ascorbic acid (vitamin C). The vitamin C content of various plant and animal tissues.
J. Biol. Chem., 108, 563-570.

A method is described in which an acid ferricyanide solution is reduced by ascorbic acid. The amount of reduced ferricyanide is measured by treating the solution with ferric gum ghatti reagent. The Prussian blue formed is determined colorimetrically.

Results obtained using this method check with those obtained by titration with indophenol to a reasonable degree.

Farmer, C. J., and Abt, A. F.
1936. Determination of reduced ascorbic acid in small amounts of blood.
Proc. Soc. Exp. Biol. and Med., 34, 146-150.

A micro-method is described which permits the estimation of ascorbic acid in as little as 0.3 ml. of blood. The ability of deproteinized plasma to reduce indophenol dye is the basic principle involved.

Roe, J. H.
1936. The determination of ascorbic acid as furfural and a comparison of results obtained by this method and by indophenol titration.
J. Biol. Chem., 116, 609-619.

When boiled with HCl, ascorbic acid (but not dehydroascorbic acid) forms furfural which is determined by the color formed with aniline acetate. A method is described consisting of the determination of the furfural formed by boiling an acid extract of a tissue in which the ascorbic acid has been oxidized by passage through norit, under non-reducing (with HCl alone) and reducing (with HCl containing stannous chloride) conditions. The value obtained with the HCl-$SnCl_2$ mixture minus that given with HCl alone is the amount of furfural from ascorbic acid.

Comparison of results of this method and the indophenol titration method upon 16 plant tissues agreed within limits of experimental error.

Lyman, C. M., Schultze, M. O., and King, C. G.
1937. The effect of metaphosphoric acid and some other inorganic acids on the catalytic oxidation of ascorbic acid.
J. Biol. Chem., 118, 757-764.

Metaphosphoric acid inhibits the copper-catalyzed oxidation of ascorbic acid by decreasing the amount of copper effective in the catalysis, in addition to the effect that is due to the pH of the solution. The rate of oxidation of ascorbic acid in solutions of metaphosphoric acid depends upon the ratio of metaphosphoric acid to copper, and not the ratio of metaphosphoric acid to ascorbic acid.

Mack, G. L., and Tressler, D. K.
1937. Vitamin C in vegetables VI. A critical investigation of the Tillmans method for the determination of ascorbic acid.
J. Biol. Chem., 118, 735-742.

A strongly ionized acid is recommended for use in the extraction procedure in the Tillmans method for the determination of ascorbic acid to insure a pH low enough to prevent enzymic oxidation of ascorbic acid. The catalytic action of copper is inhibited by the addition of 2 per cent metaphosphoric acid to the extracting medium. By preventing the oxidation of ascorbic acid throughout the determination, the hydrogen sulfide treatment may be eliminated.

During prolonged hydrogen sulfide treatment in weak acid solutions, substances other than dehydroascorbic acid are reduced. The use of a strongly ionized acid prevents the interfering materials from reacting with the dye.

Bessey, O. A.
1938. A 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.
J. Biol. Chem., 126, 771-784.

Evidence for the validity and precision of the method is presented. Several extractants were tested, 3 per cent metaphosphoric acid being found most satisfactory. Ascorbic acid and dehydroascorbic acid analyses are reported for several vegetables.

Evelyn, K. A., Malloy, H. T., and Rosen, C.
1938. The determination of ascorbic acid in urine with the photoelectric colorimeter.
J. Biol. Chem., 126, 645-654.

A method is described in which visual titration is replaced by an objective photoelectric measurement of the amount of dye decolorized when a measured quantity of sample reacts with an excess

of dye. Standardization of the dye solution is not necessary. Errors due to interfering colored substances are eliminated. The measurement may be completed within 5 seconds after the addition of dye, reducing errors due to non-ascorbic acid reducing substances.

Menaker, M. H., and Guerrant, N. B.
1938. Standardization of 2,6-dichlorophenolindophenol. An improved method.
Indust. and Eng. Chem., Anal. Ed., 10, 25-26.

Fifteen ml. of dye solution are pipetted into a 50 ml. Erlenmeyer flask and 0.5-1.0 gm. of potassium iodide and 0.5-1.0 ml. of dilute sulfuric acid are added. The mixture is shaken to facilitate the oxidation of the potassium iodide, and the liberated iodine is titrated with 0.01 N sodium thiosulfate, using the usual starch indicator.

The chief advantages of the method are that the sodium thiosulfate solution remains stable after it reaches equilibrium and that the end point of the titration is sharp.

Scudi, J. V., and Ratish, H. D.
1938. A colorimetric method for the determination of ascorbic acid.
Indust. and Eng. Chem., Anal. Ed., 10, 420-423.

The reduction of diazotized sulfanilamide by ascorbic acid was studied and a quantitative method for the estimation of the vitamin devised.

Esselen, W. B., and Fuller, J. E.
1939. The oxidation of ascorbic acid as influenced by intestinal bacteria.
J. Bact., 37, 501-521.

Certain bacteria, particularly members of the coliform group, inhibit the oxidation of ascorbic acid in culture media.

Two strains of E. coli were found which reduced approximately 90 per cent of the dehydroascorbic acid present to its equivalent of ascorbic acid in five hours.

Kirk, M. M., and Tressler, D. K.
1939. Determination of ascorbic acid. Electrometric titration method.
Indust. and Eng. Chem., Anal. Ed., 11, 322-323.

An electrometric titrimeter was used in two ways: (1) the qualitative unit alone, and (2) the quantitative unit for plotting a titration curve. Both methods were used in titrations with 2,6-dichlorophenolindophenol and with iodine. Reducing substances other than ascorbic acid did not interfere with the end point.

Roe, J. H., and Hall, J. M.
1939. The vitamin C content of human urine and its determination through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid.
J. Biol. Chem., 128, 329-337.

Ascorbic acid is oxidized to dehydroascorbic acid and the 2,4-dinitrophenylhydrazine derivative formed which is dissolved in HCl containing $SnCl_2$ and boiled under pressure to reduce the nitro groups. The furfural formed is then determined colorimetrically by the aniline acetate method.

Gunsalus, J. C., and Hand, D. B.
1941. The use of bacteria in the chemical determination of total vitamin C.
J. Biol. Chem., 141, 853-858.

A method is described for the quantitative determination of total vitamin C in biological materials by reduction of dehydroascorbic acid to ascorbic acid with a resting suspension of B. coli, followed by direct titration with 2,6-dichlorophenolindophenol.

The method is not applicable to all biological materials. It can be applied successfully to milk, fruit, juices, and urine.

King, C. G.
1941. Chemical methods for determination of vitamin C.
Indust. and Eng. Chem., Anal. Ed., 13, 225-227.

Sources of error in methods involving the use of 2,6-dichlorophenolindophenol as the oxidizing agent are discussed. Compounds are listed which interfere seriously with the determination of dehydroascorbic acid. The error introduced by hydrogen sulfide treatment is pointed out. Methods for detecting and avoiding interference are mentioned.

Kirk, M. M.
1941. Polarigraphic determination of ascorbic acid. Indust. and Eng. Chem., Anal. Ed., 13, 625-626.

A preliminary report is presented of vitamin C determinations made with a Fisher Elecdropode or polarigraph. No quantitative determinations were made. Results obtained, however, indicate the possibility of adapting the method for accurate quantitative analysis. The determination is not hindered by the presence of pigments, is specific, and is sensitive to small amounts of electrolyte.

Kuether, C. A., and Roe, J. H.
1941. Determination of ascorbic acid in whole blood. Proc. Soc. Exp. Biol. and Med., 47, 487-489.

A method is described for preventing the oxidation of ascorbic acid by oxyhemoglobin. The oxyhemoglobin may be reduced by alternate evacuation and treatment with carbon dioxide under pressure before deproteinization with metaphosphoric acid.

Morell, S. A.
1941. Rapid photometric determination of ascorbic acid in plant materials. Indust. and Eng. Chem., Anal. Ed., 13, 793-794.

An adaptation and modification of the photometric determination of ascorbic acid in blood serum as reported by Mindlin and Butler (J. Biol. Chem., 122, 673, 1937-38) and modified by Bessey (J. Biol. Chem., 126, 771, 1938) to include colored or turbid solutions and plant tissue extracts is described. The procedure for the preparation of

the standard curve in the calibration of the Evelyn photoelectric colorimeter is given. The application of the method to the analysis of beans and cabbage is described.

Stotz, E.
1941. A clinical method for the determination of ascorbic acid in blood plasma and urine.
J. Lab. and Clin. Med., 26, 1542-1545.

The method described is based on the one developed by Mindlin and Butler for the determination of plasma ascorbic acid. It involves the quantitative extraction of oxidized 2,6-dichlorophenolindophenol from acid solution with xylene. The dye is allowed contact with the ascorbic acid for the short time necessary for the reaction, then extracted into xylene, where it is no longer subjected to an acid medium or to slowly reducing substances, and is, therefore, stable for hours.

Carruthers, C.
1942. An improved photometric method for ascorbic acid.
Indust. and Eng. Chem., Anal. Ed., 14, 826-828.

Directions are given for a microphotometric method for the estimation of ascorbic acid based upon the difference in transmission of buffered 2,6-dichlorophenolindophenol before and after reduction. Interference due to other reducing substances is inhibited by the addition of mercuric chloride.

Dunker, C. F., Fellers, C. R., and Esselen, W. B.
1942. A comparison of four methods for determining vitamin C with a 25-day, weight-response bioassay.
Food Res., 7, 260-266.

Of the methods compared in this investigation, the 2,6-dichlorophenolindophenol titration method and the 25-day, weight-response bioassay method gave good checks with the standard Sherman bioassay method.

The weight-response bioassay method provides a simple and accurate method of checking the results of chemical determinations for vitamin C in food products. It should prove useful, particularly in the evaluation of the antiscorbutic properties of a foodstuff containing the biologically inactive d-gluco-ascorbic acid and d-iso-ascorbic acid which are being used in the food industry as antioxidants.

Harris, L. J., Mapson, L. W., and Wang, Y. L.
1942. Vitamin methods IV. A simple potentiometric method for determining ascorbic acid, suitable for use with coloured extracts.
Biochem. J., 36, 183-195.

The theory of the electrochemical reaction is discussed. A potentiometric procedure is described and the advantages of the method indicated. Results obtained when various fruits and vegetables were analyzed for ascorbic acid by visual and by potentiometric methods agree to within 1-2 per cent.

Harris, L. J., and Olliver, M.
1942. Vitamin methods III. The reliability of the method for estimating vitamin C by titration against 2,6-dichlorophenolindophenol. 1. Control tests with plant tissues.
Biochem. J., 36, 155-182.

Evidence is given that if specified precautions are taken, direct titration of the acid extract against 2,6-dichlorophenolindophenol can be recommended for all ordinary routine analyses of plant materials as giving the total antiscorbutic activity.
Interfering substances were not found in measurable quantities in any fresh fruits or vegetables.

Highet, D. M., and West, E. S.
1942. A procedure for the determination of ascorbic acid based upon the use of a standardized solution of 2,6-dichlorophenolindophenol in xylene.
J. Biol. Chem., 146, 655-662.

A method for the estimation of ascorbic acid in biological fluids is described. It is applicable to

the determination of ascorbic acid in various fruit juices, urine, and blood plasma or serum. The analysis of plasma and serum ascorbic acid may be made directly upon the material without preparation of a filtrate.

Loeffler, H. J., and Ponting, J. D.
1942. Ascorbic acid. Rapid determination in fresh, frozen or dehydrated fruits and vegetables. Indust. and Eng. Chem., Anal. Ed., 14, 846-848.

A modification of the method described by Morell (Indust. and Eng. Chem., Anal. Ed., 13, 793-794, 1941) is presented. The use of a solution of 1 per cent metaphosphoric acid eliminates the need of buffering.

Lugg, J.W.H.
1942. The use of formaldehyde and 2,6-dichlorophenol-indophenol in the estimation of ascorbic acid and dehydroascorbic acid.
Australian J. Exp. Biol. and Med. Sci., 20, 273-285.

Methods for estimating the concentration of ascorbic acid and dehydroascorbic acid from metaphosphoric acid extracts of biological materials are discussed.

Ascorbic acid condenses with formaldehyde readily at pH 3.5 but only very slowly at pH 1.5. On the basis of this observation, a method is described which is believed to be specific for ascorbic acid.

Mapson, L. W.
1942. Vitamin methods V. A note on the determination of ascorbic acid in fruits and vegetables in the presence of SO_2.
Biochem. J., 36, 196-202.

Sulfur dioxide is used extensively for preserving fruit, fruit juices, and fruit pulps. Since it reduces 2,6-dichlorophenolindophenol almost instantaneously, it is impossible to determine

ascorbic acid in products so treated unless special precautions are taken. Two procedures for overcoming the difficulty are described: (1) an extract of the material in 5 per cent metaphosphoric acid is subjected to exhaustion *in vacuo* to remove the SO_2, and (2) acetone is added to form the acetone-bisulfite complex which neither reduces the dye itself nor interferes with the reduction of the dye by ascorbic acid.

Ramsey, J. B., and Colichman, E. L.
1942. Potentiometric determination of vitamin C. Combined use of 2,6-dichlorophenolindophenol and iodate.
Indust. and Eng. Chem., Anal. Ed., 14, 319-321.

This method was developed to make use of the specific oxidation of ascorbic acid by 2,6-dichlorophenolin, but at the same time, to depend upon a stable potassium iodate solution as the only standard oxidant, thereby eliminating the necessity of standardizing the unstable dye solution.

Reid, M. E.
1942. Protection of ascorbic acid during its extraction from plant tissues.
Food Res., 7, 288-294.

The vitamin C content of test materials was determined by titration against 2,6-dichlorophenolindophenol.

The preservation of vitamin C during its extraction with metaphosphoric acid from tissues of high oxidative activity depends upon the amount of acid used in the early phases of grinding and upon sufficient volume to cover all parts of the sample when the first crushing of cells takes place.

Cooking has a protective effect on vitamin C in tissues of high oxidative activity.

Gould, B. S., and Shwachman, H.
1943. A new method for the bioassay of antiscorbutic substances. Assays of dehydroascorbic acid, 2-ketogulonic acid, iron ascorbate, and the effectiveness of oral and parenteral administration of ascorbic acid.
J. Biol. Chem., 151, 439-453.

A new bioassay is described, based upon the increase in serum "alkaline" phosphatase of scorbutic guinea pigs observed after the administration of a critical dose of ascorbic acid.

Dehydroascorbic acid was found to be 80 per cent as active as ascorbic acid.

Hochberg, M., Melnick, D., and Oser, B. L.
1943. Photometric determination of reduced and total ascorbic acid.
Indust. and Eng. Chem., Anal. Ed., 15, 182-188.

Evidence is presented of the greater specificity of the improved photometric method, as compared with the visual titration and the various titrimetric and photometric procedures. The importance of determining dehydroascorbic acid, initially present in some materials and produced in others when proper analytical precautions are not taken, is stressed.

Koenig, R. A., Schiefelbusch, T. L., and Johnson, C. R.
1943. Chromogenic reagent for vitamin C determinations.
Indust. and Eng. Chem., Anal. Ed., 15, 181-182.

Ferridipyridyl sulfate is suggested as a reagent for the spectrophotometric determination of vitamin C. Ferridipyridyl ion is fairly stable, and on reduction with vitamin C forms the extremely stable pink or deep red ferrodipyridyl ion.

The new reagent has been used mainly in assaying commercial ascorbic acid, citrus fruit juices, and dried foods. It is being compared with other methods for ascorbic acid in analyzing more complex systems.

Mapson, L. W.
1943. Ascorbic acid in dehydrated foods.
Nature, 152, 13-14.

Reductones closely resemble ascorbic acid in many ways. They are found in certain dehydrated foods which have been subjected to heat treatment. A method is described in which the initial titration measures ascorbic acid and reductones. The ascorbic

acid present is then removed by condensation with formaldehyde. The remaining reducing power is ascribed to reductones.

Mapson, L. W.
1943. Vitamin methods VI. The estimation of ascorbic acid in the presence of reductones and allied substances.
J. Soc. Chem. Indust., 62, 223-232.

A method is described, and critically evaluated, involving titration against 2,6-dichlorophenolindophenol in the presence of formaldehyde, making possible the differentiation of ascorbic acid and reductones. Reductones and similar substances, if present, interfere in the analysis of ascorbic acid in foods.

Evidence is given showing that reductones are produced when dehydrated vegetables are dried at too high a temperature, or when stored at temperatures above 25° C.

Pepkowitz, L. P.
1943. The rapid determination of ascorbic acid by the adaptation of Stotz's method to plant materials.
J. Biol. Chem., 151, 405-412.

An adaptation of Stotz's method for the determination of ascorbic acid in blood and urine is described for the rapid estimation of the vitamin in plant materials. The method depends on the selective solubility in xylene of non-reduced 2,6-dichlorophenolindophenol from acid solutions. The method is especially useful for highly colored or turbid extracts and is applicable to fresh, frozen, or dehydrated plant materials.

Ponting, J. D.
1943. Extraction of ascorbic acid from plant materials. Relative suitability of various acids.
Indust. and Eng. Chem., Anal. Ed., 15, 389-391.

Thirteen acids were compared as to their stabilizing effect upon ascorbic acid solutions under

conditions favorable to oxidation. Only metaphosphoric and oxalic acids appeared suitable. They were far superior to any of the others and about equally satisfactory.

Roe, J. H., and Kuether, C. A.
1943. The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid.
J. Biol. Chem., 147, 399-407.

The 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid is treated with 85 per cent sulfuric acid to produce a colored product, and the intensity of the color is measured in a photoelectric colorimeter. The general procedure is described and a calculation chart for use with the Evelyn photoelectric colorimeter presented. The specificity of the method is clearly demonstrated.

Snow, G. A., and Zilva, S. S.
1943. A critical examination of Lugg's method for the determination of l-ascorbic acid.
Biochem. J., 37, 630-640.

The kinetics of the reaction between formaldehyde and l-ascorbic acid or chemically related substances were studied.

The possible structure of the formaldehyde-ascorbic acid complex is given.

The studies show that the use of Lugg's method greatly reduced the error in the titration method for the determination of ascorbic acid in the presence of small quantities of reductone or reductic acid. Additional corrections are necessary when large quantities of reductone are present.

An evaluation of the chemical assay of ascorbic acid.
1944. Nutrition Reviews, 2, 58-59.

The importance is stressed of accounting for reductones present when analyzing such substances as malt extracts, heated cereal grains, and fermented juices for ascorbic acid by the indophenol method.

The possibility of using the electrolytic method in determining dehydroascorbic acid is suggested.

Gawron, O., and Berg, R.
1944. Estimation of vitamin C in presence of iron salts. Stepwise determination of vitamin C and ferrous iron with dichlorophenolindophenol.
Indust. and Eng. Chem., Anal. Ed., 16, 757.

With the use of isolated vitamin C-iron salt systems, a method was developed for the estimation of vitamin C in which ferrous and ferric iron did not interfere. Whether or not the method may be applied to food products was not determined.

Heinze, P. H., Kanapaux, M. S., Wade, B. L., Brimball, P. C., and Foster, R. L.
1944. Ascorbic acid content of 39 varieties of snap beans.
Food Res., 9, 19-26.

A modification of the Morell method (Indust. and Eng. Chem., Anal. Ed., 13, 793-794, 1941) is described in which an unbuffered one per cent solution of metaphosphoric acid is used for the extraction.

Kawerau, E., and Fearon, W. R.
1944. Ascorbic acid. Part 2. Factors determining stability in aqueous solution.
Sci. Proc. Royal Dublin Soc., 23 (N.S.), 171-180.

Methods available for protecting the vitamin from destruction following oxidation are described. Four classes of protectors are of special importance: (1) substances capable of stabilizing the vitamin by combining with it either in its reduced or oxidized form, (2) reducing agents capable of maintaining the vitamin in the stable reduced form, (3) buffers that keep the solvent from becoming alkaline, and (4) substances that act as anticatalysts, either by depressing the ionization of copper present in the solution or by combining with "ascorbic oxidases".

Lucas, E. H.
1944. Determining ascorbic acid in large numbers of plant samples.
Indust. and Eng. Chem., Anal. Ed., 16, 649-652.

A procedure is described for the determination of ascorbic acid in plant material found useful in plant breeding and other plant research. A grinding machine was designed making possible the simultaneous disintegration of ten samples.

The concentration of ascorbic acid in samples of various plant materials was determined on a large scale.

An extensive survey was made to determine whether losses of any significance might occur if the extract to be tested was filtered through paper instead of being centrifuged. The results justified the abandonment of centrifugation in favor of the timesaving filtration procedure.

Studies showed that titrations as used in the procedure presented could be accomplished safely and reliably with unbuffered solutions.

Roe, J. H., and Oesterling, M. J.
1944. The determination of dehydroascorbic acid and ascorbic acid in plant tissues by the 2,4-dinitrophenylhydrazine method.
J. Biol. Chem., 152, 511-517.

A description of a modification of the original method (Roe and Kuether, J. Biol. Chem., 147, 399-407, 1943) for the determination of the total ascorbic acid in blood and urine is given. The reduced ascorbic acid present is stabilized with thiourea to allow measurement of dehydroascorbic acid only.

Advances in ascorbic acid methodology.
1945. Nutrition Reviews, 3, 16-17.

A discussion is presented of recently reported methods for the determination of ascorbic acid both in plant and animal tissues, which complement some of the older methods.

Stewart, A. P., and Sharp, P. F.
1945. Determination of vitamin C in the presence of interfering reducing substances. Selective oxidation-reduction method.
Indust. and Eng. Chem., Anal. Ed., 17, 373-376.

Ascorbic acid and interfering substances are catalytically oxidized by ascorbic acid oxidase, followed by the specific reduction of dehydroascorbic acid to ascorbic acid by a suspension of Escherichia coli or Staphylococcus albus. The ascorbic acid formed is then determined by indophenol titration.

ASCORBIC ACID IN APPLES

Kohman, E. F., Eddy, W. H., and Carlsson, V.
1924. Vitamins in canned foods II. The vitamin C destructive factor in apples.
Indust. and Eng. Chem., 16, 1261-1263.

This paper is the second in a series of researches to establish to what extent vitamin C is destroyed in the canning process, the causes underlying its destruction, and ways and means of ensuring its preservation.

Albermarle Pippin and Stayman Winesap apples were studied. The method of Sherman, LaMer, and Campbell (J. Amer. Chem. Soc., 44, 165-172, 1922) was used in the assay of their vitamin C content.

When the apples were canned after being covered with a salt solution, there was no apparent loss of vitamin C, irrespective of the time of processing within the limits of commercial practice. The procedure is common practice in the commercial canning of apples. Storing of the canned apples for 8 months gave no evidence of losses of vitamin C. There was a marked deterioration in vitamin C content in raw apples held in cold storage from October to March.

Bracewell, M. F., Hoyle, E., and Zilva, S. S.
1930. The antiscorbutic potency of apples.
Biochem. J., 24, 82-90.

The following English varieties were tested: Bramley's Seedling, Worcester Pearmain, Cox's Orange Pippin, Woodbine, Dabinett, and King Edward. The following imported varieties also were tested: Cleopatra, Jonathan, Strawberry Pearmain, Kings, and Cox's Orange Pippin. Bramley's Seedling was markedly more active antiscorbutically than any of the other varieties.

Losses were determined in the vitamin C content of apples stored at 1° C. in air, or at 10° C. in an atmosphere of mixed gases containing 10 per cent CO_2, 11 per cent O_2, and 79 per cent N_2 for

about three months. The gas-stored apples showed a definitely greater deterioration than those stored in air.

The method of analysis used is described in another article by the same authors (Med. Res. Council, Spec. Rept. Series, 146, 1930). The flesh only of the apples was tested.

Bracewell, M. F., Kidd, F., West, C., and Zilva, S. S. 1931. The antiscorbutic potency of apples II. Biochem. J., 31, 138-143.

Newton Wonder and Lane's Prince Albert apples were studied. The antiscorbutic potency of Newton Wonder was similar to that of Cox's Orange Pippin. Lane's Prince Albert was intermediate between Cox's Orange Pippin and Bramley's Seedling.

Bramley's Seedling apples frozen and stored at -20° C. for four months did not lose an appreciable amount of their antiscorbutic activity. Bramley's Seedling apples stored in air at 3° C. for five months did not lose any of their antiscorbutic activity.

The concentration of vitamin C increases as the skin is approached from the core and is more than six times as great in the peel as in the flesh near the core.

The method of analysis used is described in another article (Bracewell, Hoyle, and Zilva, Med. Res. Council, Spec. Rept. Ser., 146, 1930).

Bracewell, M. F., Wallace, T., and Zilva, S. S. 1931. The antiscorbutic potency of apples III. Biochem. J., 25, 144-146.

King Edward apples containing about 0.0307 per cent nitrogen were about 1.5 times as potent antiscorbutically as apples of the same variety containing about 0.0387 per cent nitrogen. No significant difference in vitamin C content was found between Bramley's Seedling containing high and low quantities of nitrogen.

Crane, M. B., and Zilva, S. S.
1931. The antiscorbutic vitamin of apples IV.
J. Pom. and Hort. Sci., 9, 228-231.

Cultivated apples are of two fundamentally different kinds, the so-called "diploids" with 34 and "triploids" with 51 chromosomes, respectively. Bramley's Seedling is the only one of the "triploid" varieties tested so farm Since the "diploid" and "triploid" apples differ widely in various characteristics, other "triploid" varieties (Belle de Boskoop and Blenheim) were tested in order to ascertain whether a relationship exists between the vitamin C content and chromosome number of the apple. The Belle de Boskoop was equal in antiscorbutic activity to Bramley's Seedling. The Blenheim, however, was not of high antiscorbutic potency.

Zilva, S. S., Kidd, F., and West, C.
1931. Vitamin content of apples.
Food Invest. Bd. Rept., Gt. Brit. Sci. and Indust. Res. Dept. 1931, 128-129.

The effect of the temperature of storage upon the stability of vitamin C in apples was studied. There appeared to be a greater loss of the vitamin in Cox's Orange Pippin apples stored at 10° C. than in those stored at 1° C., but the difference was not statistically significant.

Crane, M. B., and Zilva, S. S.
1932. The antiscorbutic potency of apples V.
Biochem. J., 26, 2177-2181.

A number of triploid varieties were tested for vitamin C and the results compared with those previously obtained with diploid and triploid apples.

As in the case of the diploid, the triploid apples showed a variation in antiscorbutic activity. Of the nine triploid varieties tested, Bramley's Seedling, Belle de Boskoop, and Gennet Moyle stood out as exceptionally active, and Reinette du Canada, Blenheim Orange, Warmer's King, and Ribston Pippin were of approximately the same potency as

Lane's Prince Albert, an outstandingly active variety of the diploid group. The Gravenstein variety, although a triploid, was the least active apple tested in this investigation.

The results suggest that some connection may exist between the chromosome number and the antiscorbutic potency of the apple, but do not supply conclusive evidence.

Fellers, C. R., Isham, P. D., and Smith, G. G.
1932. Vitamin C distribution in Baldwin and McIntosh apples.
Proc. Amer. Soc. Hort. Sci., 29, 93-97.

The method of Sherman, LaMer, and Campbell (J. Amer. Chem. Soc., 44, 165-172, 1922) was used to determine the vitamin C content of the apples.

Baldwin apples held in storage for four months contained 20 per cent less vitamin C than they had when freshly picked. The skin of the apples was four times as rich in the vitamin as the flesh immediately beneath it, and from six to ten times as rich as the flesh near the core.

McIntosh apples contained only 10 to 16 per cent as much vitamin C as Baldwin apples.

Fresh centrifuge-extracted juice of Baldwin apples was only slightly inferior in vitamin content to the fresh fruit.

Canned strained apple sauce appeared to contain little or no vitamin C. Freshly prepared unstrained apple sauce, however, retained approximately 20 to 30 per cent of the fruit's original vitamin C content.

Zilva, S. S., Kidd, F., and West, C.
1932. The effect of freezing upon vitamin C of apples.
Food Invest. Bd. Rept., Gt. Brit. Sci. and Indust. Res. Dept. 1932, 89.

There was no appreciable loss in ascorbic acid content of Bramley's Seedling apples tested immediately after freezing at -20° C. Additional samples of the frozen apples were stored at -5, -10, -15, and -20° C. for about seven months and then tested for antiscorbutic activity. In the apples stored at

-5° C. the loss in vitamin C was great; in those stored at -10°, somewhat less, but almost 50 per cent; in the apples stored at -15° C. the loss was just appreciable; while in those held at -20° C., no loss was detectable.

Zilva, S. S., Kidd, F., and West, C.
1932. The effect of stock upon vitamin C of apples. Food Invest. Bd. Rept., Gt. Brit. Sci. and Indust. Res. Dept. 1932, 91.

Two varieties of apples, Bramley's Seedling and Cox's Orange Pippin, were grown on three different stocks, broadleaved English, Doucin amelliora, and Jaune de Metz, and tested for their antiscorbutic potency. The results of the investigation indicate that the concentration of vitamin C in a given variety of apple is not influenced by the stock on which it is grown.

Fellers, C. R., Cleveland, M. M., and Clague, J. A.
1933. Vitamin C in Baldwin apples, juice, cider and apple sauce.
J. Agr. Res., 46, 1039-1045.

The Baldwin apple is a fairly good source of vitamin C. Storage caused a gradual loss in antiscorbutic potency. Spraying the trees had no effect on the vitamin content.

Freshly expressed juice retained almost all of the vitamin C. Ciders preserved by heat pasteurization or sodium benzoate, and canned strained apple sauce retained very little vitamin C.

The method of Sherman, LaMer, and Campbell (J. Amer. Chem. Soc., 44, 165-172, 1922) was used in determining the antiscorbutic activity.

Potter, M. T.
1933. The vitamin C content of the Winesap apple as influenced by fertilizers.
J. Agr. Res., 46, 367-373.

The influence of the application of a complete fertilizer (N, P, and K) upon the vitamin C content

of the apple, as contrasted with apples from trees receiving no fertilizer was studied. The method of Sherman, LaMer, and Campbell (J. Amer. Chem. Soc. 44, 165-172, 1922) was used.

Sixty per cent of the animals receiving apples from fertilized trees were protected or developed only mild scurvy, while not one of the animals fed apples from non-fertilized trees was protected, and 80 per cent developed moderate to very severe scurvy. Winesap apples from trees receiving applications of a complete fertilizer appeared to be a better source of vitamin C than apples from trees not so fertilized, when fed at the five-gram level.

Potter, M. T.
1933 The Winesap apple as a source of vitamin C. J. Home Ec., 25, 52-56.

The technique of Sherman, LaMer, and Campbell (J. Amer. Chem. Soc. 44, 165-172, 1922) with several modifications was used to measure the vitamin C content of the apples.

Winesap apples from trees not fertilized and from trees receiving application of complete fertilizer were selected for the study. The daily dose needed for protection was near 10 gm. At this level, differences were not apparent between apples from fertilized and unfertilized trees.

Wallace, T., and Zilva, S. S.
1933. The antiscorbutic potency of apples VI. Biochem. J., 27, 693-698.

Bramley's Seedling and King Edward VII apples were used in the study. The concentration of ascorbic acid was determined by the biological assay method used in previous studies of this series (Bracewell, Hoyle, and Zilva, Med. Res. Council, Spec. Rept. Ser., , 146, 1930).

The nitrogen content of the fruit was lowered by substituting "grass" for arable culture and by bark-ringing vigorous trees. Apples in which the concentration of nitrogen was lowered showed a consistently high antiscorbutic activity. Although the nitrogen content and the vitamin C content of

the apples were always inversely related, there was no strict proportionality between them.

Acidity, sucrose, and ash constituents showed no correlation with the vitamin activity.

The decreases in nitrogen content in all cases were associated with increases in the ratio, total sugars/nitrogen, the increases being due to lowered values for nitrogen and increased high values for total sugars.

Zilva, S. S., Kidd, F., and West, C.
1933. The effect on the vitamin C content of apples of storage in the frozen state in the presence and absence of molecular oxygen.
Food Invest. Bd. Rept., Gt. Brit. Sci. and Indust. Res. Dept. 1933, 80.

Bramley's Seedling apples were frozen and stored in air at -5, -10, and -20° C. and in a vacuum at -20° C. The samples were analyzed for ascorbic acid content by the biological method. Considerable quantities of the vitamin in the apples stored in air were destroyed, while very little was lost in the apples stored in a vacuum.

Batchelder, E. L.
1934. Vitamin C in Delicious apples before and after storage.
J. Nutr., 7, 647-654.

The biological method of Sherman and Smith (J. Amer. Chem. Soc., 44, 165-172, 1930) for the determination of ascorbic acid was followed with a few changes.

The concentration of vitamin C in Delicious apples grown in Washington was determined when freshly picked and after storage. Three sets of determinations were made. In the first set, the apples were fed from October to December; in the second, from January to March; and in the third, from March to May. The apples fed in the fall were stored at 32° F. The winter and spring tests included apples stored at 45° F. as well as those held at 32° F.

The vitamin C content of the apples was .04 to .05 units per gram. No loss of vitamin C was evident after storage for 6 months at 32° F. About one-sixth of the ascorbic acid in the apples was lost after storage at 45° F. for three months, and about one-fourth after six months at this temperature.

Manville, I. A., McMinis, A. S., and Chuinard, F. G.
1934. Vitamin studies on apples I. The vitamins A, B, and C content of the Rome Beauty, Delicious, Stayman, Yellow Newton, and Winesap.
J. Amer. Diet. Assoc., 10, 135-152.

The concentration of vitamin C was determined by the biological method. The vitamin C content of the Delicious, Rome Beauty, Stayman, and Winesap was 3 units, while that of the Yellow Newtown was 6.

Smith, G. G., and Fellers, C. R.
1934. Vitamin C content of twenty-one Massachusetts grown varieties of apples.
Proc. Amer. Soc. Hort. Sci., 31, 89-95.

The vitamin C content of 21 varieties of apples was determined by the method of Sherman, LaMer, and Campbell (J. Amer. Chem. Soc., 44, 165-172, 1922). Seasonal or other variation, except storage, caused little change in the vitamin C content in any one variety. The vitamin C content of an apple variety seems to be unrelated to the chromosome number.

Zilva, S. S., Kidd, F., West, C., and Perry, E.O.V.
1934. Vitamin C content of apples.
Food Invest. Bd. Rept., Gt. Brit. Sci. and Indust. Res. Dept. 1934, 164-165.

The red peel of Bramley's Seedling apples was found to be much more potent in vitamin C than the green peel.

Apples picked in mid-September were stored in pure oxygen and in pure nitrogen at 1° C. Up to the middle of December, biological tests carried out (using peeled fruit) by the prophylactic method

did not reveal any difference in ascorbic acid content of the stored apples. Both samples seemed as potent antiscorbutically as the freshly picked fruit.

Batchelder, E. L, and Overholser, E. L.
1936. Factors affecting the vitamin C content of apples.
J. Agr. Res., 53, 547-551.

A modification of the method of Sherman, LaMer, and Campbell (J. Amer. Chem. Soc., 44, 165-172, 1922) was used in the determination of the concentration of ascorbic acid in the apples. Delicious and Winesap apples were used in the tests.

The ratio of leaf area to fruit affected the vitamin C content of the apples only as it affected the size of fruit produced. The smaller apples appeared to be higher in vitamin C content than the larger ones, but when the ratio of skin to pulp was taken into account, the same concentration of vitamin C was present in the tissues of apples grown under widely different leaf-fruit ratios.

Storage at 40° F. resulted in a greater loss of vitamin C than did storage at 32° F.

Dove, W. F., and Murphy, E.
1936. The vitamin C content of apples and its relation to human welfare.
Science, 83, 325-327.

In regions adapted to apple production, varieties of apples high in ascorbic acid content can be depended upon as the principal source of vitamin C. Difficulties have been encountered in attempts to consider vitamin enrichment in fruit-breeding programs. One problem is that of educating the consumer to like new varieties.

The second difficulty is that the generation process in the fruit tree is slow and expensive.

Preliminary tests were made on the leaves of Northern Spy and McIntosh apple trees. The indophenol titration method was used to determine the ascorbic acid content. Northern Spy apples were five or six times as potent in vitamin C as

McIntosh apples. The leaves of McIntosh apple trees contained only two-thirds as much of the vitamin as the leaves of Northern Spy.

It is suggested that in breeding new varieties of apples for high vitamin C content the leaves of seedlings may be tested long before the trees will bear fruit, and thus save time.

Manville, I. A., McMinis, A. S., and Chuinard, F. G.
1936. Vitamin studies on apples.
Food Res., 1, 121-140.

The vitamin C content of apples, as analyzed by the biological method, does not appear to be related to chromosome number.

The vitamin C values of apples studied were:

Arkansas Black	2.5 units per ounce
Baldwin	2.5-3.0 units per ounce
Delicious	2.5 units per ounce
Gravenstein	3.7 units per ounce
Jonathan	1.5-2.0 units per ounce
Spitzenberg	7.0 units per ounce
Winesap	3.0 units per ounce

Rudra, M. N.
1936. Distribution of vitamin C in different parts of common Indian foodstuffs.
Biochem. J., 30, 701-703.

The indophenol titration method was used to determine the ascorbic acid content of foods.

In fresh and young fruits and vegetables the ascorbic acid in the skin was invariably more concentrated than in the flesh, but in samples stored for some time the ascorbic acid in the skin was sometimes less concentrated than that in the flesh.

Curran, K. M., Tressler, D. K., and King, C. G.
1937. Losses of vitamin C during cooking of Northern Spy apples.
Food Res., 2, 549-557.

The ascorbic acid content of the apples was determined by the method of Bessey and King (J. Biol.

Chem., 103, 687-698, 1933) as modified by Mack and Tressler (J. Biol. Chem., 118, 735-742, 1937). Both the reduced and oxidized forms of the vitamin were measured. Biological assays also were made.

The vitamin C content of the raw Northern Spy apples was approximately 11 mg. per 100 gm. Apple sauce made from peeled or unpeeled fruit contained 7 mg. ascorbic acid per 100 gm. Unstrained sauce from peeled apples retained 75 per cent of the total original ascorbic acid, while the strained sauce made from unpeeled apples retained 68 per cent.

Approximately 80 per cent of the ascorbic acid was lost when apples were baked. The same amount was lost during the baking of apple pie. After the pie had stood for 48 hours, the loss was increased to 88 per cent.

Thornton, N. C.
1937. Carbon dioxide storage XI. The effect of CO_2 on the ascorbic acid content of some fruits and vegetables.
Proc. Amer. Soc. Hort. Sci., 35, 200-201.

The ascorbic acid content of the fruits and vegetables was determined by the indophenol titration method.

Northern Spy, Baldwin, and Russet apples were tested after exposure to various concentrations of CO_2 for as long as 10 days at various temperatures. No changes in ascorbic acid content were detected.

Todhunter, E. N.
1937. The nutritive value of apples.
Wash. Agr. Exp. Sta. Pop. Bul., 152.

The chemical composition and vitamin value of apples are discussed. Factors affecting the vitamin C content of apples are reviewed. A table showing the vitamin units in different varieties of apples is given.

Murphy, E.
1938. Vitamin C and light.
Proc. Amer. Soc. Hort. Sci., 36, 498-499.

The observation that larger amounts of vitamin C are present in the periphery than nearer the center of certain fruits and vegetables indicates that light is probably one of the factors influencing production of vitamin C.

The "sunny" side of the apple contained larger amounts of vitamin C than the "shady" side. Fifty pairs of tests were completed, using nine varieties of apples.

Eighteen pairs of determinations were made on comparable peeled samples of apples. The vitamin C content of the "sunny" side exceeded that of the "shady" side in 15 of the 18 pairs of tests.

Eleven pairs of determinations on unpeeled comparable samples were made. The ascorbic acid content of the "sunny" side was highest in 10 of the tests.

Twenty-one pairs of tests were made comparing the "sunny" and "shady" sides of the same apple. Again the "sunny" side was highest in 17 cases.

The titrimetric method as modified by Bessey and King (J. Biol. Chem., 103, 687-698, 1933) was used.

Paech, K.
1938. Uber den Vitamin C-Gehalt deutscher Apfel.
Z. Untersuch. Lebensmitt., 76, 234-239.

The indophenol titration method was used. Twelve varieties of apples were analyzed. The concentration of vitamin C was again determined after storage of the fruit at 2.5° C. There were no losses of the vitamin in apples stored at this temperature as long as the fruit remained sound.

Zilva, S. S., Kidd, F., and West, C.
1938. Ascorbic acid in the metabolism of the apple fruit.
New Phytologist, 37, 345-357.

Bramley's Seedling apples were used for the tests. The concentration of ascorbic acid was determined by titration with indophenol, as well as by biological tests with guinea pigs.

It was found that vitamin C was present in apples in both the reduced and oxidized forms (ascorbic acid and dehydroascorbic acid). Although

the total quantity of vitamin C present in the two forms remained constant, per unit of weight, throughout the growth of the apple, there was a change in the relative proportions of the two forms. As the apple approached maturity the proportion of ascorbic acid increased, and that of dehydro-ascorbic acid decreased.

Johansson, E.
1939. Determinations of ascorbic acid content of fruits and fruit products, some vegetables, and other plants (English Summary).
Arsskr. Alnarps Lantbr., Mejeri. o. Tradgardsinst. 1939, 1-53.

The concentration of ascorbic acid, as determined by titration with 2,6-dichlorophenolindophenol, in 150 varieties of apples is reported.

In general, the early varieties had a lower ascorbic acid content than the late ones. One variety, the White Winter Calville, which contained about 50 mg. per cent ascorbic acid, was much superior to all other varieties.

The ascorbic acid content was greater in the more highly colored half of the fruit than in the other.

When apples were stored at a low temperature (2-4° C.), the loss of ascorbic acid was small.

Kessler, W.
1939. Über den Vitamin C-Gehalt deutscher Apfelsorten und seine Abhangegkeit von Herkunft, Lichtgenuss, Dungung, Dichte des Behanges und Lagerung.
Gartenbauwiss., 13, 619-638.

The ascorbic acid content of a number of varieties of apples, exclusive of peel, was determined by titration against indophenol dye. The Rote Stern Rennette contained the least (0.3 mg. per 100 gm.) and the Ontario the most (23.4 mg. per 100 gm.) ascorbic acid of the varieties tested. For a given variety, samples from southern Germany were generally a richer source of vitamin C than those from the north.

The concentration of ascorbic acid was 30 to 50 per cent higher on the side of the fruit exposed

to the sun than on the side which ripened in the shade.

Apples from trees with a heavy crop load contained less vitamin C than those from trees with fewer apples.

Apples stored at temperatures below 5° C. for 5 months lost very little vitamin C.

Rudolph, W.
1939. Über den Vitamin C-Gehalt der Apfel.
Ernahrung, 4, 161-171.

A review of the investigations made in many countries concerning the vitamin C content of apples is given.

Ruffley, J., Clague, J. A., and Fellers, C. R.
1939. Canned baked apples. Determining suitable varieties for this pack; grading; factors in baking quality; vitamin retention and loss.
Canning Age, 20, 179-181.

The concentration of ascorbic acid in Baldwin, Northern Spy, York, Delicious, and Rhode Island Greening apples was determined. Comparable samples of each variety were glazed or baked, and then canned. The vitamin C retention of canned baked apples was compared with that of canned glazed apples.

The dye titration method was used.

Todhunter, E. N.
1939. Further studies on the vitamin A and C content of Washington grown apples.
Wash. Agr. Exp. Sta. Bul. 375.

Winesap apples from fertilized plots had no higher vitamin C content than fruit from unfertilized plots.

As analyzed by biological assay, apples of the Jonathan and Delicious varieties from the same trees but differing in amount of red color were not markedly different in vitamin C content.

The concentration of ascorbic acid in Esopus (Spitzenberg), Stayman Winesap, and White Winter

Pearmain apples was determined biologically also. The ascorbic content of Winter Banana apples was determined by the dye titration method.

No difference in vitamin C content was found between samples of Rome Beauty apples from two irrigation plots, one receiving 30, and the other, 60 acre-inches of water per season. Winesap apples from plots receiving 60 acre-inches of water appeared to be higher in vitamin C than apples of the same variety from plots receiving only 30 acre-inches of water.

Eheart, M. S.
1941. Factors which affect the vitamin C content of apples.
Va. Agr. Exp. Sta. Tech. Bul. 69.

A modification of the indophenol titration method was used for the determination of the vitamin C content of apples.

The average ratio between the vitamin C concentration in the peel and that in the flesh was 4.7.

Three of the varieties studied showed a significant correlation, and ten showed no significant correlation between size and vitamin C content.

Twenty-one varieties of apples were analyzed when sufficiently ripe for a dessert apple. The vitamin C content varied from 1.5 to 8.4 mg. per 100 gm.

In 19 varieties of apples, the concentration of dehydroascorbic acid averaged 18.2 per cent of the reduced form. The concentration of the dehydro form decreased during storage.

The effect of cooking was studied in York and Albemarle Pippin apples. Over 83 per cent of the vitamin C was lost when the apples were baked or made into pie or sauce. Soaking in dilute salt solution before cooking preserved the vitamin C content in apple sauce to a great extent.

Storage of apples at 38° F. caused a progressive loss of vitamin C until only two-thirds of the original amount remained after 24 weeks.

Keys, O. H.
1942. Vitamin C in apples and other materials. New Zealand J. Sci. and Tech., 24B, 146-148.

A modification of the indophenol titration method was used to determine the vitamin C content of the foods tested.

The vitamin C content of apples varied chiefly with variety. Sturmer apples were higher in vitamin C (25 mg. per 100 gm.) than any other variety tested, with the peel containing five or six times as much as the flesh.

Apples held in cold storage retained their vitamin C over an 8-month-period, but those held in gas storage showed marked losses.

Fish, V. B.
1943. The effect of storage upon the ascorbic acid content of some West Virginia apples. Proc. Amer. Soc. Hort. Sci., 43, 73-78.

The method used for determining the ascorbic acid content was based on that reported by Clegg and Satterfield (J. Amer. Diet. Assoc., 16, 39-42, 1940). For colored extracts the method of Harris, Mapson, and Wang (Biochem. J., 36, 183-195, 1942) was used.

The ascorbic acid content of six varieties of apples (Grimes, Delicious, Starking, York, Stayman, and Rome) was determined when they were received in September and October and after storage at 0-2° C. for 2 and 4 months. There was considerable loss of the vitamin during the first two months of storage. The ascorbic acid was lost more rapidly from the skin than from the flesh.

Todhunter, E. N.
1943. Some factors affecting the ascorbic acid value of fruits and vegetables of the Pacific Northwest. Proc. Pac. Sci. Cong., 6th, Berkeley, Calif., 6, 389-391.

Ascorbic acid is well conserved in fruits and vegetables stored at low temperatures or preserved by freezing. Much of the vitamin was lost, however, when frozen fruits and vegetables were allowed to stand after thawing.

The skin of the apples and the seed coat of green peas were several times as rich in ascorbic acid as the rest of the tissues.

Fish, V. B., Dustman, R. B., and Marsh, R. S.
1944. The ascorbic acid content of several varieties of apples grown in West Virginia.
Proc. Amer. Soc. Hort. Sci., 44, 196-200.

The method of Morell (Indust. and Eng. Chem., Anal. Ed., 13, 793-794, 1941) was used in the analysis of the apples for vitamin C. Colored extracts were analyzed by a modification of the method proposed by Stotz (J. Lab. and Clin. Med., 26, 1542-1545, 1941).

Several varieties, analyzed at approximately the same stage of maturity, differed considerably in ascorbic acid content.

Wealthy and McIntosh apples harvested two weeks before maturity lost ascorbic acid rapidly when stored at 20° C. Wealthy and McIntosh apples remaining on the trees continued to gain slightly in ascorbic acid content for as long as 42 days after the normal picking time.

All varieties studied except Rome Beauty and York Imperial lost ascorbic acid rapidly when stored at 3° C. for short periods of time.

Kidson, E. B.
1944. The vitamin C content of Nelson apples.
New Zealand J. Sci. and Tech., 25B, 134-136.

The method of Harris and Olliver (Biochem. J., 36, 155-182, 1942) was used in estimating the vitamin C content of the fruit.

A number of varieties of apples grown commercially in the Nelson district were tested. The Sturmer variety was unusually rich in the vitamin, one sample containing as much as 36 mg. per cent. The skin was approximately four times as rich as the flesh.

When samples of Sturmer and Granny Smith apples were held at room temperature in the dark for three months, about 90 per cent of the ascorbic acid originally present was retained.

West, C., and Zilva, S. S.
1944. Synthesis of vitamin C in stored apples.
Biochem. J., 38, 105-108.

Samples of Bramley's Seedling apples were picked on July 2, July 20, August 10, and October 12 and analyzed for vitamin C. Duplicate samples were stored at 3° C. until February 11, February 25, March 10, and April 6, respectively, and upon removal from storage were analyzed for vitamin C. The concentration of ascorbic acid was significantly higher in the stored than in the freshly picked fruit in all samples except the one picked on October 12. The data indicate that synthesis of vitamin C in apples takes place on storage and that the capacity for this synthesis diminishes with the age of the fruit.