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A Study of Ascorbic Acid Metabolism of Adolescent Children

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A generous supply of ascorbic acid was furnished by Merck and Company.

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SUMMARY

A study of the requirement for ascorbic acid of children during adolescence has been made. Using as subjects four adolescent girls and four early-adolescent boys, three levels of ascorbic acid intake were studied. All of the food eaten by the children for a period of three weeks was weighed, and the amounts recorded. Samples of all foods which were known or presumed to contain ascorbic acid were analyzed by the method of Loeffler and Ponting (1942). Daily fasting plasma levels of ascorbic acid, using blood samples obtained by fingerprick, were determined for each child by the micro-method of Farmer and Abt (1936).

With each of the two groups of subjects, three intake levels were tested for one week each. During the first week, the children ate their usual diet. The second week served as a saturation period. During the third week the total ascorbic acid intake of each child equalled the amount recommended for his age and sex by the National Research Council.

The four girls, aged 14, 13, 13, and 12, maintained mean plasma ascorbic acid values of 0.44, 0.47, 0.23, and 0.20 mg per cent respectively, during the first week. At this time they were consuming their usual diet which contained a mean of 44 mg of ascorbic acid per day. The boys, 13, 13, 12, and 12 years of age, on a mean daily intake of 61 mg in their usual diet maintained higher mean plasma levels: 0.78, 0.73, 0.75, and 0.75 mg per cent.

In order to insure saturation during the second week, each child received 200 mg of crystalline ascorbic acid in addition to his diet from which high sources of ascorbic acid had been eliminated. The girls, from oldest to youngest, reached peaks of ascorbic acid in the plasma at 1.04, 1.13, 1.35, and 1.03 mg per cent; and the boys, 1.37 (Raymond's value before illness), 1.17, 1.10, and 1.25 mg per cent. All of the subjects showed more variation on the saturation level than during either of the other periods.

During the third week the ascorbic acid in the diet was limited to 20 mg per day, and a supplement was given to bring the total intake to the amount recommended by the National Research Council (1945). Each of the girls received a total of 80 mg per day. The two boys aged 13 years received a total of 90 mg and the two aged 12 received a total of 75 mg per day. After two days of adjustment to the new level, daily variation lessened and the means for the plasma values which were obtained the last five days were, for the girls: 0.89, 0.86, 1.21, and 0.91; for the boys: 0.91, 0.84, 0.88, and 0.98 mg per cent. These values are lower than their saturation values for all subjects, but considerably higher than the 0.60 mg per cent considered by Butler (1940) to be adequate.

In addition to the experimental study, a survey was made of the ascorbic acid in plasma of children living at the Children's Farm Home. Eighty-one children, 43 boys and 38 girls, between the ages of 11 and 18 years were tested. The mean plasma ascorbic acid value for the girls was 0.49 mg per cent and the mean age was 13 years 10 months, which is well within the age range of adolescence for girls. The mean plasma value for the boys was 0.65 mg per cent, and the mean age 13 years 6 months. The boys were younger, physiologically, than the girls. Except for the girls aged 12 to 13 years, the values for children of comparable ages were higher at the Children's Farm Home than those of rural youth in Oregon tested by Hoppe and Fincke (1943-44). Almost all of the values found in both studies, however, were either below or only slightly above the level considered to be adequate by Butler (1940).

A Study of Ascorbic Acid Metabolism of Adolescent Children

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INTRODUCTION

Investigators have used various approaches to the problem of determining the ascorbic acid requirement of man. Some of the studies are based on plasma ascorbic acid levels, others on urinary excretion levels, and still others combine and compare the two. In order to study this requirement, the daily intake of ascorbic acid must be known. Studies in which the intake is controlled and the food weighed, sampled, and analyzed for ascorbic acid are most accurate, but in some studies food intake records are kept and the vitamin content calculated by using tables of nutritive value.

Previous studies made with adults

Belser, Hauck, and Storvick (1939) comparing plasma and urinary ascorbic acid for seven adults found that two of their subjects required from 70 to 85 mg of ascorbic acid per day to maintain tissue saturation, three subjects required from 85 to 100 mg, and two required more than 100 mg. This was a range from about 1.0 to 1.6 mg per kg per day.

Storvick and Hauck (1942) found a range from 1.07 to 1.58 mg per cent in the plasma saturation values of six adults. Intakes of 65 to 150 mg of ascorbic acid plus 10 mg in the diet were required to maintain tissue saturation. These figures were established from data collected by daily determination of urinary excretion and fasting plasma ascorbic acid. A definite correlation was observed between the daily urinary excretion of ascorbic acid and the concentration of ascorbic acid in the plasma on intakes of 75 mg or less, but no consistently significant correlation was found between the two variables at intakes above 75 mg.

Ralli, Friedman, and Sherry (1939), studying daily urinary excretion levels and occasional plasma ascorbic acid levels of three adults, found 100 mg of ascorbic acid the optimum daily intake as reflected by maximum retention and low excretion. They believed the plasma ascorbic acid level was a more accurate index of vitamin C nutrition when the intake was below 100 mg daily, but responses to intakes higher than 100 mg daily were shown more accurately by urinary excretion. They suggested that plasma ascorbic acid values of 1.0 mg per cent or higher indicated "complete" saturation of the tissues by vitamin C.

Three adult subjects studied by Todhunter and Robbins (1940) required 1.6 to 1.7 mg per kg per day to maintain the tissues in a state of saturation as indicated by the ascorbic acid in urine and plasma. A total daily intake of 60

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mg of ascorbic acid resulted in plasma ascorbic acid values above 1.0 mg per cent. A daily ingestion of more than 120 mg of ascorbic acid was required to raise the blood plasma to 1.4 mg per cent.

Fincke and Landquist (1942), using college students, studied the ascorbic acid intakes required to maintain plasma levels of 0.8 mg per cent and also to maintain saturation. Daily intakes of approximately 1.1, 0.8, 1.1, and 1.2 mg ascorbic acid per kg per day were necessary to maintain an average plasma ascorbic acid concentration of 0.8 mg per cent. To maintain tissue saturation three subjects needed 1.7, 1.8, and 2.0 mg per kg of body weight per day.

Kline and Eheart (1944) measured the variation among nine women in the amount of ascorbic acid necessary for saturation as indicated by urinary response to a 400 mg test dose. The subjects were considered saturated if 50 per cent or more of the test dose was excreted in 24 hours. Six of the women required 1.4 to 1.8 mg per kg, one required 0.6 mg or less per kg, and the other two required 2.2 mg or more per kg.

The twelve subjects of Dodds and MacLeod (1944) showed increased plasma ascorbic acid values on intakes of 1.0 mg per kg of body weight which had been shown to be the approximate utilization value. Three of the subjects reached saturation on such an intake.

Plasma ascorbic acid determinations were made at the beginning and conclusion of a 10 to 14 day experimental period in which the 56 subjects of Bryan et al. (1941) followed their usual diets. Weights of all foods consumed were recorded as a basis for estimation of ascorbic acid intake from tables of food composition. Sixty-five correlations on 56 subjects showed that plasma ascorbic acid concentration increased in a more or less linear fashion with increasing dietary intake up to about 1.7 to 1.9 mg per kg per day, at which time the plasma values approximated 1.0 mg per cent. This was considered the saturation level since a further increase of intake had little effect on plasma ascorbic acid concentration.

Previous studies made with children

Among younger age groups we find that studies have been conducted with preschool and school age children. Everson and Daniels (1936) studied the retention of ascorbic acid by following the urinary excretion of three boys 39, 57, and 59 months of age. Retentions of ascorbic acid paralleled the ingestions only up to 7.5 mg per kg, but higher ingestions were without influence on the retentions of the children studied. The highest retentions were found in the youngest child, suggesting that possibly there is a greater demand for vitamin C by the younger tissue.

Meyer (1943) also studying preschool children found that a daily intake of 31 mg of ascorbic acid was the marginal level for tissue saturation of the four subjects studied in 1939-40. A daily intake of 23 to 25 mg of ascorbic acid was not sufficient for tissue saturation in the eight children of the 1940-41 and 1941-42 studies. Meyer concluded that the requirement of these children was not related to sex, age, or body weight.

Roberts and Roberts (1942) studied five children 7 to 12 years of age, determining daily urinary excretion and occasional plasma values. A 300 mg test dose was given following each level of intake. The two youngest children required 65 mg of ascorbic acid, and the three oldest, 75 mg for saturation as determined by 50 per cent excretion of the test dose in 24 hours.

Dietary histories and blood plasma levels of 93 healthy city children were studied by Bessey and White (1942). They found that 3 oz or more of orange juice or an equivalent amount of ascorbic acid (45 mg) in citrus fruits or

tomatoes maintained a blood plasma level of 0.7 mg per cent, which they considered indicative of a liberal intake.

Thirty preadolescent girls between 6 and 12 years of age were studied by Roberts et al. (1943). Estimates of the dietary intakes for all subjects were based on ascorbic acid analyses of food eaten by six of the subjects over a one-week period. Using plasma levels of 0.7 mg per cent and excretion in 24 hours of 50 per cent of a 300 mg test dose as the criteria indicating the satisfactory state of ascorbic acid nutrition, these workers found that intakes of 32 or 42 mg were not adequate. Only one subject on the 52 mg intake met the standard, while four-fifths of those receiving 62 mg and all of those receiving 72 mg of ascorbic acid were saturated. It was concluded that 62 to 72 mg of ascorbic acid were adequate for preadolescent girls.

As far as the authors have been able to determine in reviewing the literature, the ascorbic acid requirements of adolescent children have not been studied. The present study was conducted with young adolescents. Ascorbic acid analyses were made of their food and of their fasting blood plasma. Although it would have been desirable to analyze the urine, it was felt that collections could not be supervised closely enough to give reliable data. Even without values for urinary excretion, the fasting plasma values obtained on known levels of ascorbic acid intake compared with those values attained during saturation, may be used to determine the ascorbic acid requirement.

BRIEF PLAN OF EXPERIMENTAL STUDY AND SURVEY

In order to determine the requirement for ascorbic acid of children during early adolescence, an experimental study and a survey of adolescents living at the Children's Farm Home were undertaken.

About 160 children, ranging in age from 5 to 18 years, are housed in eight cottages at the Children's Farm Home, Corvallis, Oregon. The farm home is sponsored by the Women's Christian Temperance Union. It cares for state-committed orphans and children from broken homes. Financial support is also given through State aid and the Community Chest. Boys and girls live in separate cottages, except at the "health cottage," where a registered nurse resides, and where convalescents and children requiring special care are housed. In order to simulate family life, children of different ages live in each cottage. Each cottage has its own cook and housemother. All food is supplied through a central commissary, but its use depends upon the choices of the cooks.

Three levels of ascorbic acid intake were studied, using as subjects four adolescent girls and four early-adolescent boys. All of the food eaten by the children for a period of three weeks was weighed, and the amounts recorded. Samples of all foods which were known or presumed to contain some ascorbic acid were analyzed by the method of Loeffler and Ponting (1942). Daily fasting plasma levels of ascorbic acid were determined for each child by the micromethod of Farmer and Abt (1936).

With each of the two groups of subjects, three intake levels were tested for one week each. During the first week, the children ate their usual diet. The second week served as a saturation period. During the third week the total ascorbic acid intake of each child equalled the amount recommended for his age and sex by the National Research Council (1945).

A survey of the plasma ascorbic acid levels of 81 children between the ages of 11 and 18 years was made. The results of this survey will be compared

with standards accepted to indicate adequate previous ascorbic acid intake, and with results of a survey of the nutritional status of rural children in the state of Oregon (Hoppe and Fincke, 1943-44).

ANALYTICAL PROCEDURES

Sampling and analysis of foods

The children's food was weighed before serving. Standard portions were given if enough of the food was provided. Weights of all foods actually consumed were obtained by subtracting the weight of each food left on the plate from the weight of each food served. From the analyses of the foods, the total day's intake of ascorbic acid was calculated.

Unless the food was homogeneous (*e.g.*, mashed potatoes, of which only one sample was taken), duplicate samples of all foods which might contain ascorbic acid were weighed on waxed paper, using a Chatillon balance. These samples were taken at the same time that the portions of food were weighed for the children. The foods analyzed included all fruits, vegetables, milk and cream, as well as mixtures containing them. The method of Loeffler and Ponting (1942) was used for all food analyses.

Equipment and solutions used in Loeffler and Ponting's method of food analysis for ascorbic acid were:

1. Metaphosphoric acid—6 per cent solution, made fresh daily and diluted to 1 per cent for extractant as needed.
2. Dye solution—2, 6-dichlorobenzenoneindophenol: 120 mg per liter, made up once each week, and kept in a dark bottle under refrigeration. This was diluted 1:10 each day as needed.
3. Evelyn photoelectric colorimeter, with filter 520.
4. Stop watch.
5. 9 ml wide-tip calibrated pipette.
6. 1.0 ml quantitative pipette.
7. Funnels, 10 ml pipettes, beakers, one-ounce glasses[1], trip balance, graduated cylinders.

Liquids to be assayed were taken immediately after the meal to be weighed on a trip balance in the laboratory. Ten-gram portions of liquids were then mixed with 70 ml of 1 per cent metaphosphoric acid.

Either 25 gm or 50 gm samples were taken from the other foods; the smaller amount was taken from citrus fruits, tomatoes, or from foods which filter slowly, such as starchy vegetables. Each sample of food to be assayed was placed immediately in a Mason jar containing 90 ml of 1 per cent metaphosphoric acid, and 10 ml of 1 per cent metaphosphoric acid were used to wash the waxed paper used for weighing the foods. The contents of the jars were transferred to Waring Blendor units, and the jars washed with 250 ml of 1 per cent metaphosphoric acid, making a total of 350 ml of 1 per cent metaphosphoric acid. At no time did the proportion of food to acid exceed 1:7. This conforms with the recommendation of Loeffler and Ponting.

The foods were blended with the acid for five minutes, and allowed to settle in the Blendor if necessary. Then the liquid portion, containing the extracted ascorbic acid, was filtered through ordinary filter paper into a dry one-ounce glass. Filtrates were analyzed colorimetrically as follows:

1. The colorimeter was set at 100 with a tube containing distilled water. One milliliter of 1 per cent metaphosphoric acid was placed in one

[1]These were lipless beverage glasses, which served many purposes in food analyses, and were also convenient to hold solutions for the plasma analyses.

colorimeter tube, and 9 ml of the dye solution were added rapidly. The tube was agitated, placed in the instrument, and the galvanometer reading taken 15 seconds after the beginning of the addition of dye. This value was recorded as the G_1, or blank value.

2. One-milliliter portions of the filtrate to be tested were placed in each of four matched colorimeter tubes. Nine milliliters of distilled water were then added to one of these tubes, and the instrument set at 100 with this tube to correct for turbidity and color in the sample.

3. Nine milliliters of dye were then added to the first of the remaining three tubes containing the filtrate, the tube agitated and placed in the instrument.

a. The fifteen-second reading was recorded as $G_{2.15}$.

b. The thirty-second reading was recorded as $G_{2.30}$.

The difference, if any, between the two readings was subtracted from the $G_{2.15}$, resulting in the G_2 value, so that the observed reduction of the dye would represent only that caused by the action of ascorbic acid, which is practically instantaneous.

This procedure was repeated for each of the tubes containing the sample, resulting in 6 sample readings for each food, when foods were assayed in duplicate), and 2 blank readings.

The observed G_1 values and the final G_2 values were corrected using the correction table provided with the instrument. Then the L_1 ($L_1 = 2 - \log G_1$) and L_2 ($L_2 = 2 - \log G_2$) values were recorded, using the table of L values provided with the Evelyn colorimeter. The average L_2 value for each sample was subtracted from the average of all L_1 values obtained in the same series of determinations.

Ascorbic acid content of food, in milligrams per 100 grams of food, was calculated using the following equation:

$$\text{Milligrams of AA/100 g of food} = \frac{K\,(L_1 - L_2)\,(\text{Wt of acid} + \text{wt of sample})}{\text{weight of sample}}$$

where K was the constant 8.6, which had been determined in preliminary work with standard ascorbic acid solutions. The weight of the sample was taken to represent the weight of the water it contained, since most fruits and vegetables contain more than 90 per cent water.

Determination of reduced ascorbic acid in plasma

REFERENCE: 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.

EQUIPMENT:

1. Oxalated blood vials.
2. Clean vials for centrifuging deprotenized plasma.
3. 0.2 ml pipettes.
4. Microburette (special design of Farmer and Abt, obtained from Sargent & Co., Chicago).
5. Titration tile.
6. Lancet.
7. Toothpicks.
8. Small corks for vials.
9. Glass points for titrating.
10. Clinical centrifuge.

REAGENTS:

1. Lithium oxalate, 2 per cent solution.
2. Mercury.
3. 5 per cent metaphosphoric acid (fresh daily).
4. 2.5 per cent metaphosphoric acid (fresh daily).
5. Dye: 0.2 g of sodium 2,6-dichlorobenzenoneindophenol and 50 ml of pH 6.8 phosphate buffer, made up to 500 ml with redistilled water. Diluted 1:10 for use.
6. Standard ascorbic acid solutions: 40 mg ascorbic acid plus 20 ml 2 per cent metaphosphoric acid in 2 per cent sulphuric acid, made up to 100 ml with 2 per cent metaphosphoric acid. This was diluted for use by taking 2 ml of the above solution and making up to 100 ml with 2 per cent metaphosphoric acid. The standard ascorbic acid solutions which were titrated contained 0.008 mg ascorbic acid per milliliter.

   Dilutions were made from two standard solutions for standardizing the dye. A third standard was prepared and diluted if good checks were not obtained with the first two.

Standardization of the dye

A. A rubber tube with glass stopper at one end was filled with clean mercury and put on the large end of the microburette. The microburette was filled with the dye by holding the curved tip in the dye solution and turning the screw of the microburette holder clockwise until a small drop of mercury was expelled into the dye solution, and then filled to the desired point by turning the screw in the reverse direction.

B. Three 0.2 ml aliquots of each of the standard ascorbic acid solutions were transferred to depressions of the titration tile and were titrated with 2,6-dichlorobenzenoneindophenol, using the Farmer and Abt microburette. The dye was added until a faint pink color appeared. 0.2 ml of 2.5 per cent metaphosphoric acid which had been pipetted into an adjacent depression of the tile was titrated with the dye until the faint pink color matched the ascorbic acid sample.

C. Calculation:

$$\frac{AA \text{ in aliquot}}{\text{ml dye used for aliquot} - \text{ml dye used for blank}} = \text{dye equivalent}$$

Example:

Standard solution	Ml	Titration		Aliquot — blank	Dye equivalent	
		Aliquot	Blank			
1	0.2	0.0840	0.0035	0.0805	0.0199	$\frac{0.0016}{0.0840 - 0.0035} = 0.0199$

Determination of ascorbic acid in blood plasma

Blood was collected by finger prick into an oxalated vial, stirred with a toothpick, stoppered and centrifuged for 3 to 5 minutes.

Then 0.1 ml of plasma and 0.1 ml of redistilled water were pipetted with the same pipette into a conical tip vial. 0.2 ml of 5 per cent metaphosphoric acid was added and mixed thoroughly by tapping the vial against the palm of the hand. The coagulated protein was centrifuged down (about 5 minutes).

Two 0.2 ml aliquots of deproteinized plasma were transferred to each of two depressions in the titration tile and titrated with a solution of sodium 2,6-dichlorobenzenoneindophenol until a faint pink color appeared. 0.2 ml of 2.5 per cent metaphosphoric acid in an adjacent depression of the tile was titrated with the dye until the faint pink color matched the color of the plasma sample.

Calculation:

(ml dye used for *AA* in plasma — ml dye for blank)
(dye equivalent) (2,000) = mg *AA* per 100 ml blood plasma

Example:

Subject	Ml plasma	Titration		Plasma — blank	Dye equivalent	AA
		Plasma	Blank			
						Mg per cent
Mary Smith	0.2	0.0145	0.0045	0.0100	0.0204	0.4080*

*(0.0145 — 0.0045) (0.0204) (2,000) = 0.4080.

SURVEY OF THE PLASMA ASCORBIC ACID VALUES OF ADOLESCENTS

During the first week of the experimental study for both boys and girls, an effort was made to relate plasma ascorbic acid to the daily intake on the diet usually served at the Children's Farm Home. A survey was made of the plasma levels of the adolescent children living at the Farm Home to get a more complete picture of their state of nutrition with respect to ascorbic acid.

Eighty-one of the 104 children between the ages of 11 and 18 were tested —43 boys and 38 girls. Two determinations were made on each of 10 of the girls of Frances Elizabeth cottage, and an average of the two values is reported for those children. One successful determination of plasma ascorbic acid was made for each of the other children. Blood samples were obtained by finger-prick, either before breakfast or before lunch when breakfast contained *no fruit*. All samples which showed any hemolysis were discarded, and the child was either tested again or omitted from the reported data.

All of the cottages at the Children's Farm Home except one, which housed only a few children of the ages to be tested, cooperated in this survey. Our original intention had been to test all of the 104 children above 11 years of age, but only 5 boys and 5 girls under 12 years of age were finally tested. The mean age of the boys tested was 13 years 6 months, and of the girls, 13 years 10 months.

On the scatter-diagram, Figure 1, the values obtained by the determination of plasma ascorbic acid are plotted against the ages in years. Difference in sex is indicated. The children tested in this survey varied in age, sex, cottage, and access to outside sources of food. Although their ascorbic acid levels varied greatly, some groupings may be observed.

Most of the low values for ascorbic acid in plasma for both boys and girls were observed in the adolescent children. The boys showed many high values before the age of 14, with an abrupt drop in values from 14 until 17, the years

when boys are growing rapidly (Roberts, 1935). Except for those of two girls who had access to outside sources of food, most of the plasma ascorbic acid values for girls were concentrated below 0.60 mg per cent during the period of accelerated growth, that is, between 12 and 15 years. Even during definite

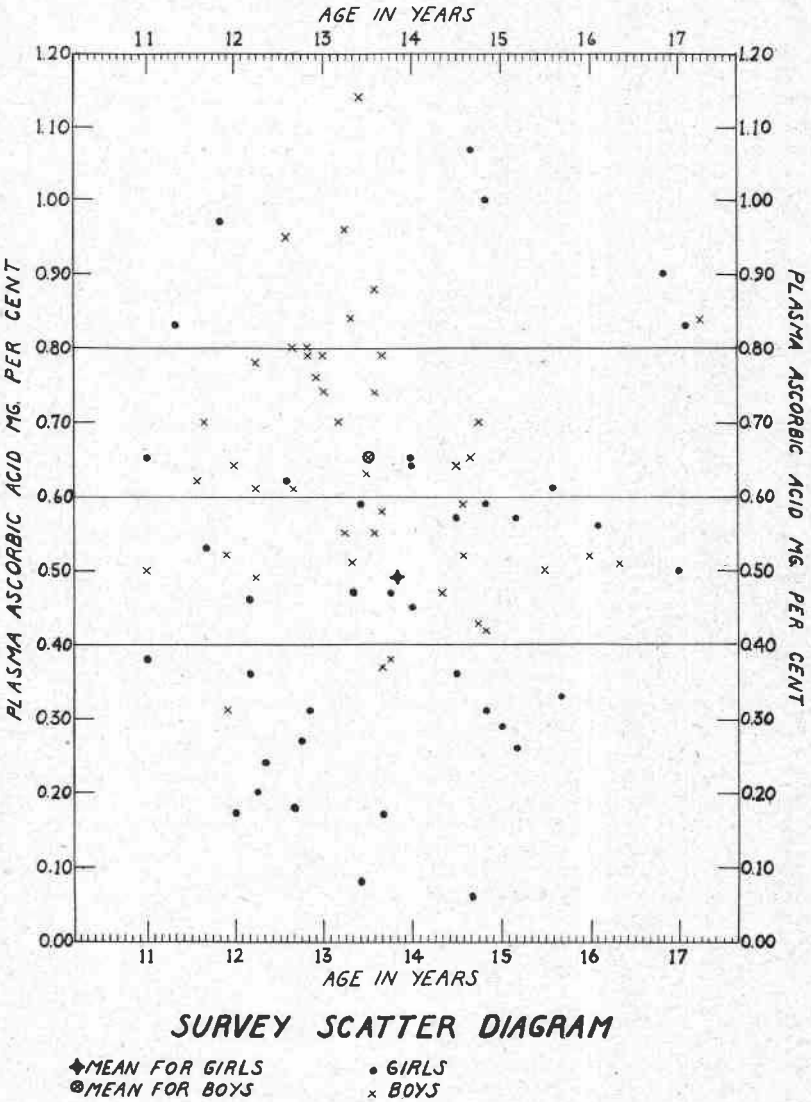


Figure 1.

adolescence, the values for boys were higher than those for girls, and the girls' mean was only 75 per cent of the boys' mean. Probably some of the differences between individuals were caused by the amounts of food eaten and perhaps by the quality of cooking and choice of food served in the individual cottages.

The Committee on Vitamins of the American Academy of Pediatrics, headed by Allan M. Butler (1940), reports that a plasma ascorbic acid value of 0.60 mg per cent can be regarded as adequate. Seventy-one per cent (27) of the plasma levels of the girls and 42 per cent (18) of those of the boys of the Children's Farm Home fell below this level. The mean plasma value for the entire group tested, however, was 0.56 mg per cent, which very nearly meets this standard. The mean plasma level for 233 children between the ages of 12 and 16 years living in five counties tested by Hoppe and Fincke (1943-44) in a survey of rural children in Oregon, was 0.48 mg per cent. A comparison of the data obtained in their survey, including those from Marion County, which is adjacent to Benton, in which the Children's Farm Home is located, is included in Table 1. Comparable age groups have been selected. It will be observed that all groups of rural children showed values lower than comparable groups at the Children's Farm Home, except for girls of 12 and 13 years of age. Many children in all of the age groups tested in both studies evidently needed more ascorbic acid in their daily diets.

Names of children tested at the Children's Farm Home, and their individual plasma ascorbic acid values appear in Table 2.

RESULTS AND DISCUSSION OF THE FOOD ANALYSES

Table 3 presents the results of the analyses of foods served during the experimental study. The total number of times each food was served, the mean ascorbic acid content of each food, and the results of the individual analyses according to cottage, are included in this table. The number of analyses reported for each food equals the number of times the food was served to the subjects at the cottage designated, during the three weeks of the experimental study.

In order to increase accuracy, the ascorbic acid content of the diet was kept low during the controlled periods, and the greater part of the daily allowance of ascorbic acid, given as a supplement, was weighed on the analytical balance. For example, during the second and third weeks of the study, all citrus fruits and other foods high in ascorbic acid were eliminated from the diet of the subjects in order to control more closely the ascorbic acid intake. Furthermore, during the third week, even potatoes were eliminated, and it is for these reasons that the foods low in ascorbic acid are reported so much more frequently than the good sources of this vitamin. Some of the canned foods, such as peaches and string beans, were substituted in the diets of the subjects for citrus fruits and tomatoes served to the other children in the cottage.

The dietary changes made at each of the cottages were similar to each other, but the experimenters noted a striking difference in the palatability of foods served at the two cottages. There was also a marked difference in the daily intake of ascorbic acid between the boys and the girls during their first weeks. Therefore the results of the food analyses for ascorbic acid were inspected and the diets at the two cottages were compared.

All foods were obtained from a central commissary. Differences between the food values of the meals served were caused chiefly by the choices of the

Table 1. Comparison of Ascorbic Acid in Plasma of Children of Marion County, Five Oregon Counties Including Marion, and the Children's Farm Home

Location	12-13 years				14-15 years				Entire group	
	Boys		Girls		Boys		Girls			
	Number of subjects	Ascorbic acid in plasma	Number of subjects	Ascorbic acid in plasma	Number of subjects	Ascorbic acid in plasma	Number of subjects	Ascorbic acid in plasma	Number of subjects	Mean plasma ascorbic acid
		Mg per cent		*Mg per cent*		*Mg per cent*		*Mg per cent*		*Mg per cent*
Marion County[1]	23	0.52	32	0.50	15	0.42	10	0.47	60	0.49
Five counties[1]	65	0.54	73	0.50	45	0.36	50	0.48	233	0.48
Children's Farm Home	26	0.71	14	0.33	9	0.55	15	0.52	64	0.56

[1]Data obtained by Hoppe and Fincke (1943-1944).

Table 2. Results of Survey of Plasma Ascorbic Acid Values

Plasma ascorbic acid values grouped according to age February 11, from birthday to birthday. Children tested at the Children's Farm Home, 1946

Age 11

Kenneth Earl	0.70
Jeanne Granite	0.53
Ann Grimm	0.97
Iris Hoxsworth	0.38
Wilma Loghry	0.83
David Roscoe	0.62
Evelyn Straub	0.65
Robert Willard	0.52
Clifford Anderson	0.31
Thomas Robertson	0.50
10 children, mean	0.601
5 boys, mean	0.530
5 girls, mean	0.672

Age 12

Richard Allen	0.80
Gliva Baketel	0.18
Benny Hess	0.76
James Hill	0.80
Jack Hively	0.61
Jim Hively	0.49
Louetta Kenney	0.46
Charles Lantto	0.95
Mary Meek	0.31
Leroy Mizner	0.78
William Mulkey	0.61
Juanita Sheler	0.24
Sharon Vandervort	0.20
Billy Wells	0.64
Betty Jean White	0.27
Dorothy Knapp	0.17
Leona Stutz	0.62
Robert Thomaston	0.79
Florence Wooley	0.36
19 children, mean	0.528
10 boys, mean	0.723
9 girls, mean	0.312

Age 13

Rodney Bennett	0.79
James Fitzwater	0.74
Joseph Granite	0.74
Bobby Griggs	0.88
Raymond Haertel	0.84
Billy Hays	0.79
Virgil Hively	0.37
Iola Hoxsworth	0.59
Richard Hunt	0.63
Betty Lou Jackson	0.17
Melvin Johnson	1.14
Stasia Kenney	0.47
Robert Lantto	0.58
Jean Loghry	0.47
Harold Ridenour	0.38
Donald Roseman	0.70
Harry Straub, Jr.	0.51
Thomas Stubblefield	0.55
Gerald Vandervort	0.55
Jimmy Wells	0.96
Marjorie White	0.08
21 children, mean	0.616
16 boys, mean	0.697
5 girls, mean	0.356

Age 14

Barbara Ahl	0.64
Donald Allen	0.42
Billie Baketel	0.45
Shirley Bennett	0.06
Jackie Bowers	0.59
Jackie Clapp	0.59
Clarence Edwards	0.65
Jack Emerald	0.64
Frank Granite	0.43
Gerald Hoard	0.47
Wyma Hoxsworth	0.31
Wayne Lewis	0.70
Lenora Maynard	1.00
Charlotte Mizner	0.65
Carol Seeley	1.07
Jo Stalker	0.57
Laura Straub	0.36
George Stubblefield	0.52
18 children, mean	0.562
8 boys, mean	0.554
10 girls, mean	0.570

Age 15

Kenneth Hill	0.50
Marie McDuffy	0.26
Betty Jo Robertson	0.29
Goldie Sheets	0.33
Janette Sheler	0.57
Betty Wooley	0.61
6 children, mean	0.427
1 boy	0.50
5 girls, mean	0.412

Age 16

Vida Hoxsworth	0.56
Raymond Lenhart	0.52
Vera Straub	0.90
Walter White	0.51
4 children, mean	0.623

Age 17

Ruth Archer	0.83
Herbert Hart	0.84
Patty Sheets	0.50
3 children, mean	0.723

cooks. In the Powers (boys') cottage, potatoes contained less mean ascorbic acid per 100 g than in the Frances Elizabeth, but they were served oftener, and in a variety of ways which increased the children's interest in eating them. Canned vegetables and fruits were served more generously at the boys' than at the girls' cottage.

Another difference which affected the total intake of ascorbic acid was the frequency with which salads were served. Salads appeared 4 times at the girls' cottage, twice with gelatin, and were usually prepared well in advance of the meal. Their mean ascorbic acid content was 0.50 mg per 100. Salads were served 8 times at the boys' cottage, and were 10 times as high in ascorbic acid (mean 5.01 mg per 100 gm). It was interesting to observe that the salads in this cottage were prepared just before serving, and often seasoned with lemon juice instead of vinegar. Both cottages served raw carrot strips often, and sometimes raw turnip strips.

All of the desserts except citrus fruits served at both cottages contained very little ascorbic acid. As a result, the differences in the ascorbic acid content of the diets were caused almost entirely by the choices and methods of preparation of foods served during the main course.

After the superintendent of the Children's Farm Home had seen the preliminary charts of plasma levels for this study, he doubled the order of citrus fruits for each cottage.

Figure 2. The four girls and two research workers seated at the table at Frances Elizabeth Cottage, Children's Farm Home. One research worker weighed each portion served, while the other recorded the weights.

Figure 3. Weighing milk for the experimental subjects at Powers Cottage. First servings of foods were usually weighed before the children came to the table, in order to shorten their meal time.

Figure 4. The four adolescent girls who served as subjects during the experimental study. According to decreasing age, they are, left to right: Jo, Stasia, Betty Lou, and Sharon.

Table 3. Ascorbic Acid Content of Foods Served at Two Children's Farm Home Cottages

Food	Ascorbic acid content per 100 grams		Mean ascorbic acid content per 100 grams of food served at both cottages
	Frances Elizabeth Cottage	Powers Cottage	
	Milligrams	*Milligrams*	*Milligrams*
Apples, raw (6)*	0.96 0.21 2.20 2.89 3.72 0.00	....	
Average	1.66		1.66
Apple, cooked			
Apple Betty (1)	0.00	.....	0.00
Apple juice† (2)	0.00	0.00	0.00
Apple roll (1)	0.28	.....	0.28
Apple sauce (9)	0.00 0.00 0.00	0.07 1.42 0.00 0.00 0.00 0.00	
Average	0.00	0.42	0.17
Banana, sliced† (1)	.....	12.26	12.26
Beans, navy (2)	3.61	.....	
Baked with tomato sauce	3.99	.....	
Average	3.80		3.80
Beans, soy; baked	.....	2.97	2.97
Beans, soy; left-over mashed	.....	5.03	5.03
Beans, string-canned (21)	0.69 2.89 2.96 3.58 1.38 0.76 0.00 2.27 2.20 1.68	2.00 0.00 1.79 2.71 3.44 4.77 4.26 4.00 3.48 2.06 2.06	
Average	1.84	2.78	2.33
Beets, cooked (8)	1.53 0.41 2.97 1.42 3.48	0.13 1.42 2.97	
Average	1.96	1.51	1.79
Cabbage, raw (1)	.....	64.68	64.68
Carrots, buttered (4)	2.75 0.00 3.44	1.24	
Average	2.06	1.24	1.86
Carrots, creamed (1)	0.89	.....	0.89

* Numbers in parentheses indicate total times served in the two cottages.
† Supplementary food provided by experimenters.

Table 3 (Continued). Ascorbic Acid Content of Foods Served at Two Children's Farm Home Cottages

Food	Ascorbic acid content per 100 grams		Mean ascorbic acid content per 100 grams of food served at both cottages
	Frances Elizabeth Cottage	Powers Cottage	
	Milligrams	*Milligrams*	*Milligrams*
Carrot strips, raw (15)*	3.58	4.47	
	3.65	4.47	
	2.06	3.35	
	2.84	4.77	
	4.26	4.20	
	2.32	2.27	
	2.97	3.74	
	.....	3.61	
Average	3.10	3.86	3.50
Celery, raw† (3)	.....	5.55	
	.....	8.13	
	.....	6.06	
Average	.....	6.58	6.58
Chard, canned (1)	.....	1.38	1.38
Cheese sauce (2)	0.00	.....	
	0.00	.....	
Average	0.00		0.00
Cherries, canned (1)	.....	5.02	5.02
Cherry juice (1)	.....	5.37	5.37
Cocoa (6)	1.86	.....	
	0.55	.....	
	1.10	.....	
	0.96	.....	
	1.45	.....	
	1.65	.....	
Average	1.26		1.26
Corn cake (1)	0.00	.....	0.00
Corn, creamed (3)	6.95	6.45	
	7.19	.....	
Average	7.07	6.45	6.86
Corn relish (1)	4.77	.....	4.77
Corn soup, creamed (1)	2.61	.....	2.61
Cream (41)	0.55	1.03	
	0.62	0.34	
	0.34	0.48	
	0.09	0.21	
	0.55	0.69	
	0.62	0.89	
	1.10	0.96	
	0.28	0.28	
Cream (continued)	0.00	1.17	
	0.21	1.10	
	0.55	1.17	
	0.34	0.34	
	0.34	0.83	
	0.83	0.55	
	0.89	0.48	
	0.41	1.31	
	0.28	1.03	
	1.03	0.76	
	0.48	0.89	
	0.83	0.55	
	.....	0.34	
Average	0.51	0.73	0.62

* Numbers in parenthesis indicate total times served in two cottages.
† Supplementary food provided by experimenters.

Table 3 (Continued). Ascorbic Acid Content of Foods Served at Two Children's Farm Home Cottages

Food	Ascorbic acid content per 100 grams		Mean ascorbic acid content per 100 grams of food served at both cottages
	Frances Elizabeth Cottage	Powers Cottage	
	Milligrams	*Milligrams*	*Milligrams*
Cream, whipped (8)*	0.00	0.65	
	0.00	0.00	
	0.48	0.00	
	.....	0.52§	
	.....	1.16§	
Average	0.16	0.47	0.35
Eggs, scrambled (3)	0.00	0.13	
	0.83	.....	
Average	0.42	0.13	0.32
Figs† (2)	.....	1.81	
	.....	0.39	
Average	.....	1.10	1.10
Fig juice† (1)	.....	2.84	2.84
Fruit juice mixture (1)	.....	20.62	20.62
Grapefruit juice, canned (1)	.....	20.34	20.34
Grapefruit, pink (1)	.....	37.16	37.16
Grape juice (2)	0.00	.....	
	0.52	.....	
Average	0.26	.....	0.26
Gravy (4)	0.52	0.00‡	
	.....	3.61‡	
	.....	3.74‡	
Average	0.52	2.45	1.97
Ice Cream (8)			
Chocolate	0.07	1.29	
	.....	1.29	
Maple	0.00	.....	
	0.00	.....	
Vanilla	0.00	.....	
Blackberry	.....	1.29	
Maple nut	.....	0.00	
Average	0.02	0.97	0.49
Jam (8)			
Apricot	0.28	.....	
Boysenberry	2.58	.....	
Peach	0.00	.....	
	0.48	.....	
Grape	.....	0.00	
	.....	0.90	
	.....	1.94	
Peach and persimmon	.....	3.10	.....
Jello (5)	0.00	2.39	
	.....	0.90	
	.....	2.89	
	.....	2.06	
Average	0.00	2.06	1.65

* Numbers in parenthesis indicate total times served in two cottages.
† Supplementary food provided by experimenters.
§ With jelly.
‡ With meat.

Table 3 (Continued). Ascorbic Acid Content of Foods Served at Two Children's Farm Home Cottages

Food	Ascorbic acid content per 100 grams		Mean ascorbic acid content per 100 grams of food served at both cottages
	Frances Elizabeth Cottage	Powers Cottage	
	Milligrams	*Milligrams*	*Milligrams*
Jelly (5)*			
Apricot	.....	0.52	
	.....	0.90	
Cranberry	.....	7.10	
	.....	2.19	
Unknown	.....	0.00	
Average	.....	2.14	2.14
Lemon sauce (2)	1.88	3.61	2.75
Lettuce (4)	2.06	1.42	
	2.58	2.32	
Average	2.32	1.87	2.10
Macaroni (3)			
With meat, egg, and tomato	6.81	.....	
With meat, egg, and tomato, left over	3.30	.....	
With meat and tomato	.....	3.35	
Average	5.02	3.35	4.49
Milk (47)	0.83	1.24	
	1.24	1.24	
	1.10	1.58	
	1.65	1.03	
	1.72	1.51	
	1.10	1.17	
	1.10	1.17	
	0.55	0.28	
	1.31	0.41	
	1.58	0.76	
	1.65	1.31	
	0.83	1.24	
	0.83	1.86	
	1.65	1.10	
	0.89	1.79	
	0.83	2.55§	
	2.48	1.03	
	1.51	0.96	
	1.24	1.44	
	1.10	1.38	
	1.31	1.24	
	1.03	0.34	
	1.44	0.48	
	.....	0.41	
Average	1.26	1.15	1.20
Onions, boiled (2)	5.02	4.52	4.77
Orange (2)	42.81	.....	
	54.05	.....	
Average	48.43		48.43
Orange and pear mixture (1)	29.87	.....	29.87
Peach juice (13)	1.93	1.93	
	1.45	2.75	
	4.68	2.20	
	4.06	1.86	
	2.27	1.17	
	1.10	.....	
	3.51	.....	
	1.10	.....	
Average	2.51	1.98	2.31

* Numbers in parenthesis indicate total times served in two cottages.
§ Heated.

Table 3 (Continued). Ascorbic Acid Content of Foods Served at Two Children's Farm Home Cottages

Food	Ascorbic acid content per 100 grams		Mean ascorbic acid content per 100 grams of food served at both cottages
	Frances Elizabeth Cottage	Powers Cottage	
	Milligrams	*Milligrams*	*Milligrams*
Peach pulp, canned (13)*	2.96	1.65	
	1.72	3.44	
	3.52	2.00	
	4.06	2.00	
	2.34	1.65	
	1.93	.....	
	1.24	.....	
	3.30	.....	
Average	2.63	2.15	2.44
Pear juice (18)	0.55	0.55	
	1.03	0.55	
	0.21	0.21	
	0.00	0.62	
	0.00	0.48	
	0.07	0.96	
	0.00	.....	
	0.28	.....	
	0.07	.....	
	0.69	.....	
	0.28	.....	
	0.69	.....	
Average	0.32	0.56	0.40
Pear pulp, canned (18)	0.28	0.62	
	0.76	0.76	
	0.89	0.48	
	0.28	0.21	
	0.00	1.10	
	0.07	0.69	
	0.07	.....	
	0.00	.....	
	0.21	.....	
	0.00	.....	
	0.55	.....	
	0.76	.....	
Average	0.32	0.61	0.42
Peas, canned (3)	.....	8.26	
	.....	9.29	
	.....	8.13	
Average	.....	8.56	8.56
Pea soup (2)	0.00	1.79	0.90
Pickles (2)			
Dill	.....	3.92	
Green tomato	1.91	.....	.....
Pie (5)			
Pumpkin filling	2.55	.....	
Squash filling	1.31	.....	
Caramel	0.00	.....	
Custard	.....	0.00	
Prune filling	.....	3.35	
Potatoes, baked (2)	.....	9.80	
	.....	11.74	
Average	.....	10.77	10.77
Potatoes, boiled (8)	15.48	10.97	
	15.09	13.67	
	9.98	.....	

* Numbers in parenthesis indicate total times served in two cottages.

Table 3 (Continued). Ascorbic Acid Content of Foods Served at Two Children's Farm Home Cottages

Food	Ascorbic acid content per 100 grams		Mean ascorbic acid content per 100 grams of food served at both cottages
	Frances Elizabeth Cottage	Powers Cottage	
	Milligrams	*Milligrams*	*Milligrams*
	12.26	.....	
	11.48	.....	
	10.71	.....	
Average	12.50	12.32	12.46
Potatoes, escalloped (1)*	.....	7.35	7.35
Potatoes, hash brown or fried (3)	.....	2.32	
	.....	5.16	
	.....	10.06	
Average	.....	5.85	5.85
Potatoes, mashed (6)	5.93	6.32	
	7.87	6.58	
	5.93	2.71	
Average	6.58	5.20	5.89
Prunes, canned (8)	0.96	1.31	
	0.00	3.03	
	0.96	2.55	
	1.10	.....	
	0.89	.....	
Average	0.78	2.30	1.35
Prunes, canned, juice (8)	0.96	0.34	
	0.55	2.20	
	0.41	1.79	
	0.83	.....	
	0.69	.....	
Average	0.69	1.44	0.97
Prunes, stewed (2)	.....	0.00§	
	.....	0.00‖	
Average	.....	0.00	0.00
Pudding (9)			
Chocolate	.....	1.81	
	.....	0.00	
Bread	.....	0.07	
Butterscotch	.....	0.00	
Custard	.....	0.00	
Floating Island	.....	1.29	
Lemon	.....	0.00	
Rice	.....	0.00	
		0.00	
Raspberry juice (1)	.....	6.74	6.74
Raspberry pulp (2)	.....	5.68	
	.....	3.10	
Average	.....	4.39	4.39
Salad (12)			
Apple carrot, raisin	0.34	.....	
	1.16	.....	
Apple, carrot, raisin with gelatin	0.00	.....	
Apple, carrot, raisin, cabbage	1.65	25.28	
	.....	2.55	
	.....	9.29	
	.....	1.29	

* Numbers in parenthesis indicate total times served in two cottages.
§ Pulp.
‖ Juice.

Table 3 (Continued). Ascorbic Acid Content of Foods Served at Two Children's Farm Home Cottages

Food	Ascorbic acid content per 100 grams		Mean ascorbic acid content per 100 grams of food served at both cottages
	Frances Elizabeth Cottage	Powers Cottage	
	Milligrams	*Milligrams*	*Milligrams*
Potato	.....	0.77	
	.....	0.39	
Macaroni	.....	0.52	
Macaroni (left-over)	.....	0.00	
Sandwich filling (1)*	.....	0.90	0.90
Squash (2)	10.87	0.83§	5.85
Stew, veal (2)	.....	2.34	
		0.00‖	
Average	.....	1.17	1.17
Tomato and bread, cooked (2)	.....	14.38	
	.....	15.07	
Average	.....	14.73	14.73
Tomato catsup (3)	.....	7.22	
	.....	6.06	
	.....	5.92	
Average	.....	6.40	6.40
Tomato, cooked (1)	.....	14.38	14.38
Tomato relish (1)	1.91	.....	1.91
Tomato soup, creamed (1)	6.64	.....	6.64
Turnip, raw (3)	16.02	29.92	.....
	.....	38.71	.....
Average	16.02	34.31	28.22
Vegetable soup (6)	5.44	0.41	
	0.00	1.20	
	0.00	0.00	
Average	1.81	0.54	1.18

* Numbers in parenthesis indicate total times served in two cottages.
§ Mashed.
‖ Left over.

Figure 5. The four young adolescent boys who served as subjects. Standing in order of decreasing age, left to right, they are: Raymond, James, Jim, and Jack. Jack and Jim are fraternal twins.

THE EXPERIMENTAL STUDY

PLAN

Four adolescent girls and four early-adolescent boys served as subjects. All of the children were apparently healthy, and examination of their medical records showed no evidences of abnormality. Figures 2, 3, 4, and 5 are photographs of the subjects and of meal service arrangements at the Children's Farm Home.

Table 4. DESCRIPTION OF SUBJECTS

	Age		Mean weight during study	Height
	Years	*Months*	*Pounds*	*Inches*
Girls				
Jo	14	6	134	64.0
Stasia	13	9	101	65.5
Betty Lou	13	8	110	64.5
Sharon	12	3	107	62.0
Boys				
Raymond	13	5	98	62.0
James (Hill)	12	11	93	64.0
Jack	12	4	86	60.0
Jim (Hively)	12	4	82	61.5

All of the foods which the subjects ate were weighed and recorded during a period of three weeks for each group. Foods containing ascorbic acid, or which might be presumed to contain some, were analyzed after each meal by the method of Loeffler and Ponting (1942). Details of this method, and the procedures used for sampling foods during the study, are reported on pages 8-9. Daily blood samples were taken by fingerprick, before breakfast, and analyzed for ascorbic acid using the micromethod of Farmer and Abt (1936). Details of the procedures will be found on pages 9-11 of this report.

Each experimental period of three weeks was divided into three parts. During the first week, the children ate the regular diet which was served in their cottage. The second week served as a saturation period. During the third week each child received the total amount of ascorbic acid recommended for his age and sex by the National Research Council.

During the week on their regular diet, the portion of food which was allotted in the kitchen for the 4 children and 2 experimenters was divided into approximately 6 parts, in addition to the sample, and the first serving was usually the same for each child. If quantity permitted, the first serving was a "standard" serving. During this week, the children were allowed to have second servings of any food which they requested, as long as the supply allowed. Ascorbic acid intake varied, as did all other constituents, during this week, according to the inclination of each child. In this way the effect of the diet as served and as eaten, upon the ascorbic acid levels in the blood plasma, could be observed.

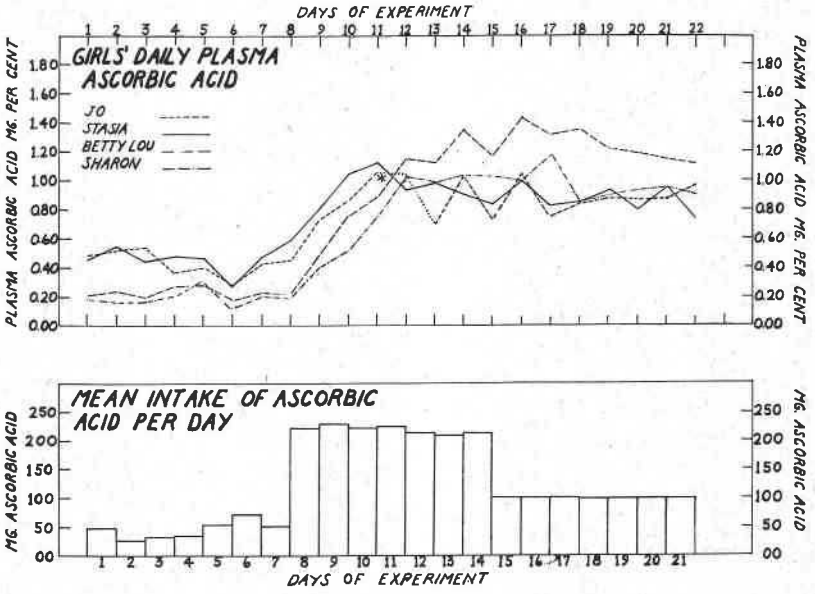
The second week served as a saturation period. In addition to the regular diet, from which significant sources of ascorbic acid were removed, the children received a supplement of 200 mg of pure ascorbic acid. The supplements had been weighed on the quantitative balance in the college laboratory, and were dissolved in redistilled water just before being administered. As soon as his morning blood sample was found to be satisfactory, each child took his supplement, rinsed the glass or beaker three times with tap water, and drank these rinsings. Shortly afterward the children ate breakfast.

During the third week, the ascorbic acid allowed from food was limited to 20 mg per day. If after analysis and calculation, the intake from food was found to be less than 20 mg, supplementary pure ascorbic acid was given after supper. Pure ascorbic acid was also given before breakfast, as the saturation supplement had been, in order to bring the total intake to the amount recommened by the National Research Council (1945). The girls received a total of 80 mg of ascorbic acid daily: 20 mg from food and 60 mg as crystalline ascorbic acid dissolved in redistilled water. From oldest to youngest, the girls received 1.3, 1.7, 1.6, and 1.6 mg of ascorbic acid per kg of body weight. Using the age groupings of the National Research Council (1945), the boys fell into two groups. The fraternal twins, Jack and Jim, who were recently 12, received a total of 75 mg: 55 mg of crystalline ascorbic acid plus 20 mg in food. The older boys, James, 12 years 11 months, and Raymond, 13 years 5 months, received 70 mg of crystalline ascorbic acid plus 20 mg in food, making their total daily intake 90 mg. From oldest to youngest, the boys received 2.0, 2.1, 1.9, and 2.0 mg of ascorbic acid per kg during this period. Since all of the boys were considerably under normal weight, they received more ascorbic acid per kg of body weight than did the girls.

The intake of ascorbic acid from food had to be controlled during the last two periods. During the saturation period, the children were served approximately equal amounts of all foods containing ascorbic acid. During the third week, their servings of these foods were identical, so that within the limits of

variation in the food, each child received the same amount of ascorbic acid from food. The children were allowed to eat *ad libitum* bread, butter, meat, and other foods which contained no ascorbic acid. All foods, however, were weighed. At least one of the three research workers concerned in making this study was present whenever the children ate.

Between-meal eating presented some problems. The girls were given extra food when they returned from school; usually graham crackers with grape or apple juice purchased in town for the experiment. The boys ate their main meal at noon, and the cottage supplied them with no food after school. None of the boys in this cottage was supposed to eat between meals. In order to abide by the house rules, and prevent the possibility of accustoming the subjects to between-meal feedings which they could not have after the study ended, no food was given at such times. It was impressed upon both the girls and the boys that all foods which they ate between meals must be brought to the laboratory to be weighed. The investigators felt that they had the children's cooperation, and the only foods available to them were candies and sweets which contained no ascorbic acid.



COMPARISON OF GIRLS' DAILY PLASMA ASCORBIC ACID WITH THEIR MEAN INTAKE OF ASCORBIC ACID PER DAY

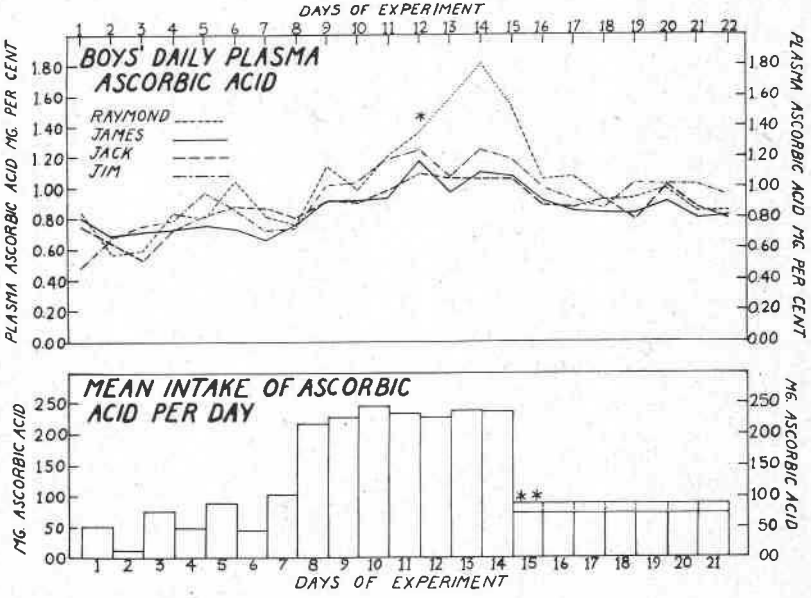
*..... PERIOD OF ILLNESS

Figure 6.

RESULTS AND DISCUSSION

Tables 5 and 6 present the results of daily fasting plasma ascorbic acid determinations, and the corresponding daily intakes of each child during the study. Each new period began in the morning after the last blood sample for the preceding period had been taken, and the study ended before breakfast on the 22nd day. Since no effort was made to change the diet of the children during the first period, the means for the entire period are reported, with the average deviations from the mean, as well as the range of values obtained during the period. The first two days were omitted from the calculation of means for the saturation periods to eliminate the effect of the previous level of intake.

The first two days after saturation, at the beginning of the period on the National Research Council recommended allowance, were eliminated for the same reason. Average deviations from their means for the last five days of these two periods, and the range of values obtained during these days, are reported with the means for each subject. The data from the study are also plotted on Figures 6 and 7.



COMPARISON OF BOYS' DAILY PLASMA ASCORBIC ACID WITH THEIR MEAN INTAKE OF ASCORBIC ACID PER DAY

* ASPIRIN AND CITROCARBONATE WERE TAKEN

** RAYMOND AND JAMES 90 MG. ASCORBIC ACID PER DAY
JACK AND JIM 75 MG. ASCORBIC ACID PER DAY

Figure 7.

Date	Jo		Stasia		Betty Lou		Sharon	
1946	Intake	Plasma	Intake	Plasma	Intake	Plasma	Intake	Plasma
	Mg	*Mg per cent*	*Mg*	*Mg per cent*	*Mg*	*Mg per cent*	*Mg*	*Mg per cent*
Usual diet								
1-12	49	0.49	48	0.46	49	0.21	49	0.18
1-13	21	0.52	24	0.54	29	0.23	25	0.16
1-14	38	0.54	29	0.45	32	0.20	30	0.17
1-15	37	0.38	34	0.49	34	0.27	34	0.21
1-16	56	0.40	53	0.47	51	0.29	55	0.31
1-17	88	0.29	62	0.28	61	0.18	76	0.13
1-18	62	0.43	41	0.46	44	0.23	60	0.21
1-19	----	0.45	----	0.59	----	0.22	----	0.20
For entire first period								
Mean	50.1	0.44	41.6	0.47	42.9	0.23	47.0	0.20
Average deviation from the mean	±15.9	±0.06	±10.9	±0.06	±9.6	±0.03	±14.9	±0.04
Range	21–88	0.29–0.54	24–62	0.28–0.59	29–61	0.18–0.29	25–76	0.13–0.31
	Mg	*Mg per cent*	*Mg*	*Mg per cent*	*Mg*	*Mg per cent*	*Mg*	*Mg per cent*
*Saturation period**								
1-19	227	-----	219	-----	219	-----	216	-----
1-20	230†	0.73	228†	0.81	229†	0.50	226†	0.41
1-21	221	0.87	221	1.05	221	0.75	224	0.52
1-22	223	1.06	223	1.13	223	0.89	223	0.76
1-23	212	1.04‡	214	0.93	214	1.15	214	1.03
1-24	207	0.71‡	210	0.98	210	1.13	210	0.99
1-25	214	1.03	214	0.90	214	1.35	214	1.02
1-26	-----	0.74	-----	0.84	-----	1.17	-----	1.03
For last five days of second period								
Mean	215.4	0.92	216.4	0.96	216.4	1.14	217.0	0.97
Average deviation from the mean	±5.3	±0.15	±4.5	±0.08	±4.5	±0.10	±5.2	±0.08
Range	207–223	0.71–1.06	210–223	0.84–1.13	210–223	0.89–1.35	210–224	0.76–1.03
	Mg	*Mg per cent*	*Mg*	*Mg per cent*	*Mg*	*Mg per cent*	*Mg*	*Mg per cent*
N.R.C. recommended allowances§								
1-26	79	-----	79	-----	82	-----	82	-----
1-27	80	1.05	80	1.00	80	1.43	80	1.01
1-28	80	0.75	80	0.83	80	1.32	80	1.17
1-29	79	0.84	79	0.85	79	1.35	79	0.86
1-30	80	0.88	80	0.94	80	1.22	80	0.90
1-31	81	0.87	81	0.81	81	1.19	81	0.93
2- 1	80	0.89	80	0.94	80	1.15	80	0.95
2- 2	----	0.96	-----	0.74	----	1.13	----	0.91
For last five days of third period								
Mean	80.0	0.89	80.0	0.86	80.0	1.21	80.0	0.91
Average deviation from the mean	±0.4	±0.03	±0.4	±0.07	±0.4	±0.06	±0.4	±0.02
Range	79–81	0.84–0.96	79–81	0.74–0.94	79–81	1.13–1.35	79–81	0.86–0.95

* Daily supplement 200 mg.
† Without value for corn.
‡ Jo was home in bed "ill."
§ Daily supplement 60 mg.

Girls

Individual daily plasma ascorbic acid levels with mean daily intakes are shown in Table 5 and plotted on Figure 6. It may be observed that the girls started the study with a mean dietary intake of 44 mg of ascorbic acid per day. They seemed to be grouped into two pairs by their plasma levels; Jo and Stasia showed plasma levels markedly above those of Betty Lou and Sharon. The variations in intake and in plasma ascorbic acid were small from day to day for all the girls. After the beginning of the saturation doses, the increases in plasma ascorbic acid of all the girls were abrupt and of about the same degree, making parallel paths on the graph. When apparent saturation levels had been attained, daily variations for all of the subjects were marked. This confirms the observations of Storvick and Hauck (1942), who observed wider variations in daily plasma values on high intakes than on low intakes of ascorbic acid.

Variations were especially great for Jo, who claimed to be ill* during the period marked with dotted lines on Figure 6, that is, on the eleventh and twelfth days of the study.

Levels for the first two days of the 80 mg period show the same variation and heights as those during the saturation period, but for the last five determinations variations lessened markedly. Sharon "leveled off" at a point (about 0.9 mg per cent) which seems to be about 0.1 mg per cent lower than her saturation level. Stasia showed the greatest daily variation during the third period, with values ranging from 0.94 to 0.74 mg per cent during the last five days. Betty Lou dropped steadily from a near-peak of 1.35 to 1.13, a value lower than any since the beginning of her saturation values. Jo was the only one of the four girls who showed a definite upward climb after the first drop on the 80 mg level. It may be significant to note that Jo was the oldest, and was physically much more mature than the other girls. The mean plasma ascorbic acid values for the girls, when they received the National Research Council recommended allowance for ascorbic acid, did not show a statistically significant difference from the mean plasma values attained during the saturation period.

Probably an intake of 80 mg of ascorbic acid per day would maintain these girls somewhat below saturation, but the values for the last five days of the study indicate that their plasma ascorbic acid values would probably remain above those which indicate an adequate state of ascorbic acid nutrition.

Boys

The first period of observation with the boys showed them to be receiving a mean daily ascorbic acid intake of 61 mg and maintaining mean blood plasma levels well within the range of adequacy. Daily variations were rather marked throughout the study for most of the boys. James, however, varied little from day to day except during the saturation week. In spite of the daily variation, some general trends are apparent upon inspection of Figure 7. There was a slight but definite increase in blood plasma ascorbic acid during the saturation period for each boy without exception. The plasma levels of all of the boys dropped immediately with the lowered intake of ascorbic acid on the fifteenth day, and except for the daily variations they remained at levels lower than their saturation levels, but varied around 0.9 mg per cent, which is appreciably above the level considered to indicate adequate ascorbic acid nutrition. It was found that the differences between the mean plasma ascorbic acid values for

* This subject was somewhat unstable. The nurse reported no evidence of illness.

Date	Raymond		James		Jack		Jim	
1946	Intake	Plasma	Intake	Plasma	Intake	Plasma	Intake	Plasma
Usual diet	*Mg*	*Mg per cent*	*Mg*	*Mg per cent*	*Mg*	*Mg per cent*	*Mg*	*Mg per cent*
2-18	45	0.84	51	0.80	53	0.75	56	0.49
2-19	13	0.57	13	0.69	13	0.64	13	0.67
2-20	64	0.59	74	0.71	79	0.53	86	0.75
2-21	45	0.83	50	0.73	46	0.73	50	0.79
2-22	97	0.80	88	0.75	84	0.82	92	0.97
2-23	46	1.04	45	0.73	43	0.88	43	0.86
2-24	104	0.81	114	0.66	104	0.87	94	0.72
2-25	.....	0.77	.....	0.77	.....	0.81	....	0.74
For entire first period								
Mean	59.1	0.78	62.1	0.73	60.3	0.75	62.0	0.75
Average deviation from the mean	±25.0	±0.10	±25.6	±0.03	±24.6	±0.09	±24.6	±0.09
Range	13–104	0.57–1.04	13–114	0.66–0.80	13–104	0.53–0.88	13–94	0.49–0.97
*Saturation period**	*Mg*	*Mg per cent*	*Mg*	*Mg per cent*	*Mg*	*Mg per cent*	*Mg*	*Mg per cent*
2-25	217	.....	217	.....	217	.....	217	.....
2-26	227	1.14	227	0.91	227	0.92	227	1.02
2-27	243	0.98	243	0.92	243	0.90	243	1.04
2-28	232	1.21	232	0.94	232	0.98	232	1.19
3- 1	227	1.37	227	1.17	227	1.10	227	1.25
3- 2	234†	.....	239	0.97	239	1.06	239	1.07
3- 3	233	1.81‡	240	1.10	240	1.06	233	1.25
3- 4	.....	1.54	.....	1.08	.....	1.06	.....	1.19
For last five days of second period								
Mean	233.8	1.48	236.2	1.05	236.2	1.05	234.8	1.19
Average deviation from the mean	±3.8	±0.19	±5.4	±0.08	±5.4	±0.03	±5.0	±0.05
Range	227–243	1.21–1.81	227–243	0.94–1.17	227–243	0.98–1.10	227–243	1.07–1.25
N.R.C. recommended allowance	*Mg*	*Mg per cent*	*Mg*	*Mg per cent*	*Mg*	*Mg per cent*	*Mg*	*Mg per cent*
Daily supplement	(70)	.....	(70)	.....	(55)	.....	(55)	.....
3- 4	90	.....	90	.....	75	.....	75	.....
3- 5	90	1.06	90	0.92	75	0.89	75	1.00
3- 6	90	1.07	90	0.85	75	0.87	75	0.93
3- 7	90	0.92	90	0.83	75	0.93	75	0.86
3- 8	90	0.94	90	0.83	75	0.78	75	1.03
3- 9	90	0.99	90	0.91	75	1.01	75	1.02
3-10	90	0.85	90	0.80	75	0.87	75	1.02
3-11	....	0.85	....	0.82	....	0.81	.....	0.95
For last five days of third period								
Mean	90.0	0.91	90.0	0.84	75.0	0.88	75.0	0.98
Average deviation from the mean	±0.0	±0.05	±0.0	±0.03	±0.0	±0.07	±0.0	±0.06
Range	....	0.85–0.99	....	0.80–0.91	....	0.78–1.01	....	0.86–1.03

* Daily supplement 200 mg.
† In bed with slight fever.
‡ Aspirin given evening of March 1.

the period of the National Research Council recommended allowance and those for the saturation period were statistically significant.

During the third period no difference could be observed between the levels for the boys receiving 90 mg per day and those receiving 75 mg per day.

The outstanding feature of Figure 7 is the peak reached by Raymond on the fourteenth day. He had been found to have a fever of 102½° F. after supper on the twelfth day. He was given two tablets of aspirin and one tablespoon of citro-carbonate by the housemother, and he did not come for a blood sample the next morning. He remained in bed with a slight fever during that day, although he was kept on as a subject, and all of his food was weighed. He received most of his 234 mg of ascorbic acid as diluted grapefruit juice, with a supplement of 60 mg of pure ascorbic acid at night, to bring his intake near to that of the other boys. The next morning his plasma level had reached 1.81 mg per cent. Raymond had no fever during that day, and the following morning his plasma level was still much higher than that of the other children, 1.54 mg per cent, although this level is only 0.10 mg per cent higher than Betty Lou's peak of 1.43 per cent (see Figure 6).

It was thought that perhaps the ingested aspirin could have caused the rise in Raymond's plasma value, by mobilizing the stores of ascorbic acid, by interfering with the titration value, or by interfering with excretion. A preliminary study conducted with three normal, healthy women in our laboratory failed to show any increase in plasma ascorbic acid at any time after a test-dose of aspirin (10 grains), although blood samples were taken at 4, 24, 28, and 48 hours after the aspirin had been ingested.

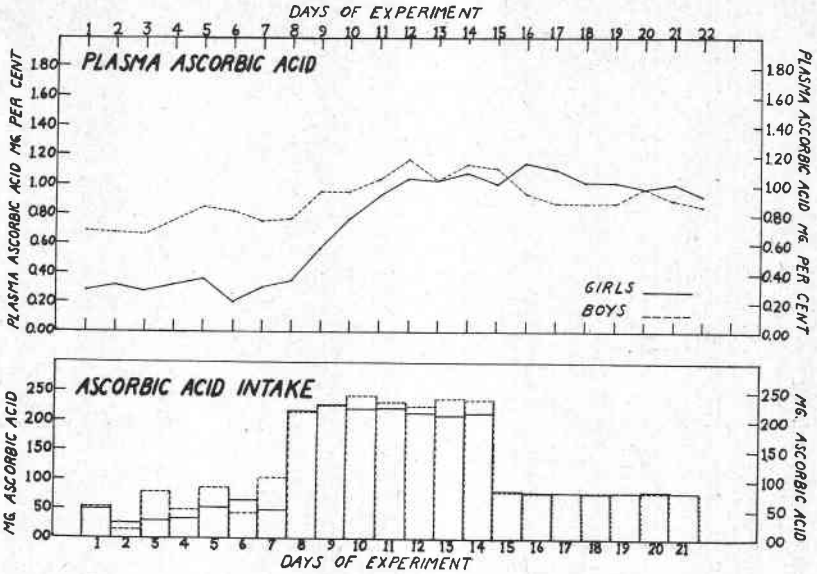
A similar study was made to test the effect of citro-carbonate. The same three women failed to show a significant increase in plasma ascorbic acid at any time after ingesting 6 grams (1 tablespoonful) of citro-carbonate dissolved in water. Samples of plasma were tested at 4, 24, 28, and 48 hours after the dose of citro-carbonate. All values (range: 0.76 to 1.07 mg per cent) obtained in both studies were well within the variations expected in normal subjects.

For comparison between boys' and girls' plasma ascorbic acid levels, means for each day were calculated excluding those of the two children who were ill, Raymond and Jo. Means of the daily plasma values and the intakes for the two groups are shown on Figure 8. Combined means for the entire first period, and for the last five days of the second and third periods, with the range of individual values, are shown in Table 7.

It will be readily observed that while the boys' intake for the first period is higher than the girls', there is a much greater difference in their plasma levels. This may be due in part to the developmental age of the two groups: the girls were definitely adolescents, while the boys had not yet begun their pubertal growth spurt.

Mean levels for saturation were about the same, but the boys attained the height of their saturation values somewhat earlier than the girls, and dropped more abruptly when the intake was reduced. Mean levels attained on the last five days of the third period were 0.89, 0.86, 1.21, and 0.91 for the girls, and 0.91, 0.84, 0.88, and 0.98 for the boys. Table 7 shows the combined mean for the three boys to be 0.09 mg per cent lower than the girls' mean, but this difference is caused by Betty Lou's high value.

While it would have been interesting to know whether the children, and especially Betty Lou, could maintain these high plasma levels for prolonged periods on the intake recommended by the National Research Council, the results for this week suggest that their plasma values would be maintained above the level of adequacy according to Butler (1940).



COMPARISON OF MEANS FOR DAILY PLASMA ASCORBIC ACID LEVELS OF THREE GIRLS AND THREE BOYS WITH THEIR MEANS OF DAILY ASCORBIC ACID INTAKE

Figure 8.

Table 7. Comparison of the Combined Means of Intake and Plasms Ascorbic Acid, of Three Boys and Three Girls

Period	Girls		Boys	
	Intake	Plasma	Intake	Plasma
	Mg	*Mg per cent*	*Mg*	*Mg per cent*
Mean of entire first period	43.8	0.30	61.5	0.74
Range of individual values	24–76	0.13–0.59	13–114	0.49–0.97
Last five days of saturation				
Mean	216.6	1.02	235.7	1.10
Range	210–224	0.76–1.35	227–243	0.94–1.25
Last five days of N.R.C. allowance				
Mean	80.0	0.99	80.0	0.90
Range	79–81	0.74–1.35	75–90	0.78–1.03
Intake, mg kg	1.6	.....	2.0	.....

The studies reviewed in the introduction include the requirements of many adults for saturation with ascorbic acid. Although some investigators have questioned the existence of any relationship between ascorbic acid requirement and body weight (Hathaway and Meyer 1941; Ralli et al. 1939), many workers have reported their results in mg per kg of body weight. Of 30 adults whose requirements for tissue saturation were studied by Belser, Hauck, and Storvick (1939); Ralli et al. (1939); Todhunter and Robbins (1940); Fincke and Landquist (1942); Storvick and Hauck (1942); and Kline and Eheart (1944), the estimated requirements of 24 fell between 1.3 and 1.8 mg/kg.

The 31 mg intake found by Hathaway and Meyer (1941) to maintain tissue saturation in four preschool children was equivalent to 1.5 to 1.8 mg/kg for these children. The five children from 7 to 12 years of age studied by Roberts and Roberts (1942) required from 1.7 to 2.4 mg/kg to maintain tissue saturation. Thus, on the basis of body weight, requirements for tissue saturation of preschool children and of adults do not appear to differ greatly. The requirements for older children (7 to 12 year group) fell within a relatively narrow range but were significantly higher than those for preschool children or adults.

In the present study, it was found that these eight young adolescents, on the recommended allowances of the National Research Council (1945), which corresponded to 1.3 to 1.7 mg/kg for the girls and 1.9 to 2.1 mg/kg for the boys, did not attain as high plasma values as they did during the saturation period when they received 200 mg per day. For the one week period, however, these allowances were adequate to maintain plasma values which are associated with "good" stores of ascorbic acid. This first study of the ascorbic acid requirement of young adolescent boys and girls suggests that they may require somewhat more ascorbic acid per kg of body weight to maintain tissue saturation than do younger children or adults.

CONCLUSIONS AND RECOMMENDATIONS

1. The daily allowance of ascorbic acid recommended for adolescents by the National Research Council (1945), as tested during this study, resulted in plasma values below the highest attainable by the individuals but well above those considered adequate. It would probably be advisable during the strain of adolescent growth to maintain all children near saturation, and since the recommended allowances accomplish this in these subjects diets may well be planned to include this amount (about 80 mg per day) for every child.

2. The results of the survey of plasma ascorbic acid of 81 children between the ages of 11 and 18 years, living at the Children's Farm Home, seem to indicate that the diets of children living in this well-regulated institution are somewhat better than the average of children of comparable ages in rural Oregon (Hoppe and Fincke 1943-44). Means for the plasma ascorbic acid of adolescents tested both at the Children's Farm Home and in rural areas in Oregon were below the level considered adequate (0.60 mg per cent). Obviously, efforts need to be made to improve the quality of the diets of adolescents.

3. The data obtained in this study point the way to further investigation: the requirements of older adolescent children for ascorbic acid need to be studied. Requirement studies have previously been made with adults and with children up to twelve years of age. The data found with these 12- to 14-year-old subjects add to our knowledge of the human requirement for ascorbic acid. It is hoped that later studies will complete the range of requirements in adolescents for both sexes.

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