DETERMINATION OF THE ASCORBIC ACID INTAKE
NECESSARY TO MAINTAIN AN ADEQUATE LEVEL
OF THE VITAMIN IN THE BLOOD PLASMA

by

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DETERMINATION OF THE ASCORBIC ACID INTAKE NECESSARY TO MAINTAIN AN ADEQUATE LEVEL OF THE VITAMIN IN THE BLOOD PLASMA

INTRODUCTION

HISTORY. Descriptions of scurvy have appeared in literature since the time of Hippocrates. For centuries it was a constant menace to soldiers, sailors, explorers and others who were obliged to live for considerable periods of time on a diet which contained neither fruits nor vegetables. It is known to have afflicted the Crusaders, and Vasco da Gama reported the death of 100 of his crew of 160 on the historical voyage around the Cape of Good Hope. There are also many accounts of suffering and loss of life caused by scurvy during the long sea voyages following the discovery of America.

Jacques Cartier, on his second voyage to Newfoundland in 1535, spent the winter near an Indian village in Quebec. He found that the Indians, too, suffered from scurvy but apparently healed themselves by drinking a concoction which they made from the leaves and twigs of certain evergreen trees. Cartier and his men tried the remedy and found it successful.

In 1747, Lind, a surgeon in the British navy, demonstrated the antiscorbutic value of oranges and lemons by experiments conducted among the sailors. Lind believed
that this scurvy-preventing property was due to "something contained in the natural juices of the plant".

As time went on many and various theories were advanced as to just what this "something" might be but it was not until the early part of the twentieth century that definite progress was made toward a satisfactory explanation.

The discovery by Holst and Frölich in 1907 that scurvy could be produced in guinea pigs by feeding a diet of rice, oats or other grains led to many studies concerning the distribution of the antiscorbutic substance and its stability under various conditions. The work of Holst and Frölich was confirmed by such investigators as Hess and Hendol, and in 1921 Zilva began the long series of experiments which finally resulted in the isolation of the vitamin.

In 1927 King and his coworkers prepared a concentrate from lemon juice according to Zilva's method and were able to remove more and more of the contaminating material until, in the spring of 1932, King and Waugh announced the isolation of the vitamin in crystalline form. They found that its chemical and physical properties corresponded to those of the hemuronic acid which Szent-Györgyi had isolated from cabbage and oranges in 1928 while working with factors involved in tissue respiration, and almost simultaneously Szent-Györgyi showed that the hemuronic acid which he had prepared would protect guinea pigs from scurvy.
Szent-Györgyi was able to obtain fairly large quantities of the compound from Hungarian red pepper so the study of its chemical nature proceeded rapidly from then on. Synthesis of the compound was accomplished by Reichstein and coworkers and by Haworth and associates in 1938.

After its chemical identity had been established Szent-Györgyi and Haworth suggested for the substance, then known merely as vitamin C, the name ascorbic acid which is now generally accepted.

PHYSIOLOGY. The work of Wolbach and his associates has clearly established the relationship of vitamin C to the physical state of the intercellular material. In a review of the pathology of vitamin C deficiency Dalldorf (11) describes the phenomenon thus, "Under normal conditions the type cell, the fibroblast, lies in an amorphous ground substance within which fibrils (reticulum) are formed which may in turn become gathered into wavy bands of collagen. In this transformation the fibrils seem to become cemented together by a translucent matrix, the formation suggesting the setting of a gel". In vitamin C deficiency this gelatinous substance ceases to form affecting, according to Wolbach (57), "the collagen of all fibrous tissue structures, the matrices of bone, dentin and cartilage, and all non-epithelial cement substance including that of vascular endothelium". The chemical mechanism of this relationship is, however, still unknown.
It is known that ascorbic acid enters readily into oxidation-reduction reactions, and many papers have reported the mechanism of such reactions in vitro. The heavier metallic ions, particularly copper, seem to catalyze many of these reactions (3)(2)(56)(41) and it is thought that copper, even in the minute concentrations in which it occurs in the body fluids, may be an important factor in the reactions in vivo. This catalytic action of copper appears to be controlled by the formation of unionized copper complexes with proteins, certain of the amine acids and glutathione (2)(56)(41). The homochromogens have also been shown to have a catalytic influence (3).

Ascorbic acid seems to be of considerable importance in the processes of tissue respiration where it apparently acts as a hydrogen-transport agent or respiratory catalyst. Evidence has indicated (7)(50) that glutathione may play an important part in these reactions.

There is also evidence that vitamin C has an essential role in the growth processes of both plants and animals. It seems to be concentrated in the regions of most rapid growth. Dry seeds contain no ascorbic acid but as soon as they begin to sprout the vitamin develops. Unripe seeds may contain considerable amounts of the vitamin but the concentration approaches zero as ripening advances.

In the animal organism ascorbic acid is found in largest amounts in the more vital regions, these tissues
characterized by a high metabolic activity, such as the pituitary body, corpus luteum, adrenal cortex, thymus, liver, brain, sex organs, spleen, kidney and heart. The tissues of young animals are consistently richer in the vitamin than those of older animals.

Although the human body, at any stage of its development, is apparently unable to synthesize ascorbic acid, the distribution of the vitamin in the tissues corresponds to that in the bodies of animals which can (61).

The ascorbic acid requirement of the human fetus and new born infant appears to be taken care of by the transfer of the vitamin through the umbilical cord (1) and by the high ascorbic acid content of human milk (33).

Under normal dietary conditions there is a constant excretion of ascorbic acid by the human kidney. This excretion seems to be dependent not only upon the immediate intake of ascorbic acid but also upon the amount stored in the tissues. Even when there is no dietary intake excretion in the urine takes place, decreasing in rate, until the tissue stores presumably are depleted, at which stage scurvy sets in. When ascorbic acid is added to the diet, there is little excretion until the tissue stores are replenished at which time excretion again occurs. When the maximum storage capacity of the tissues, or saturation, has been reached the urinary excretion rises rapidly.
METHODS OF DETERMINING STATE OF NUTRITION. Severe vitamin C deficiency is accompanied by widespread degenerative changes in all parts of the body. The inability of the tissues to produce and maintain the intercellular material causes softening of bone and disintegration of muscle fibres and connective tissue. Gums become swollen and bleed easily and teeth loosen. The walls of the blood vessels are weakened and hemorrhages occur everywhere. Such a marked deficiency is not often seen at the present time, but there are many cases where a deficiency exists to a lesser degree, cases which, though pathological changes have actually begun to take place within the body, show little or no clinical signs of a deficiency.

It is the detection and treatment of these milder or subclinical cases of scurvy which most concerns physicians at the present time.

The earliest method used to determine vitamin C nutrition was a measurement of the strength of the blood capillaries. This method was originated in 1951 before the chemical identity of the vitamin was known and is based on the fact that the capillaries are among the first tissues affected by a continued lack of ascorbic acid in the diet. The fragility of the capillaries is expressed in terms of the number of petechiae, small cutaneous hemorrhages, which appear in a given area of the skin as a result of increased intravascular pressure. The increase in pressure is brought
about either by applying a tourniquet to the upper arm, which is known as the positive pressure technique and was originated by Göthlin (22)(23), or by the negative pressure technique perfected by Dalldorf (10). In the latter procedure negative pressure is applied to a small area of the skin by means of a suction cup. It was first thought that the measurement of capillary fragility would be practical for testing vitamin C nutrition in large numbers of people but such has not proved to be the case. Farmer and Abt (1) who checked it against the ascorbic acid content of the blood plasma and Sloan (48) who measured capillary fragility in a number of patients suffering from known degrees of deficiency showed that the capillary fragility technique will, in most cases, indicate the presence or absence of vitamin C depletion but that it does not indicate the degree of the depletion. Sloan (48) found that the test gave falsely negative results in the presence of severe anemia.

With the knowledge of the chemical identity of vitamin C more satisfactory tests for its presence became possible. The method of determination which has received widest application was originated by Tillmans, Hirsch, and Hirsch (54) and is based on the fact that the dye-indicator, 2,6-dichlorobenzenoneindophenol, is quantitatively reduced by ascorbic acid.
Ascorbic acid has also been determined by the use of such compounds as methylene blue (35), phosphotungstic acid (38) and ferrocyanide (51).

As pointed out above there is a constant excretion of ascorbic acid in the urine, the amount of the excretion depending first, upon the immediate intake of the vitamin and second, upon the degree of saturation of the tissues. If the maximum storage capacity of the tissues, or saturation, has been reached, an increase in ascorbic acid intake causes a rise in urinary excretion within one to three hours. If the tissues are not in a state of saturation, the urinary excretion does not increase until the saturation state has been reached.

It was thought by early workers in the field that a measurement of the day-by-day excretion of ascorbic acid would be a fairly accurate index of the adequacy of the intake. However, this "resting level" has been found to vary so much among different individuals that it is now considered of little value. It is now thought (49) that a better picture can be obtained by the measurement of excretion following the ingestion of a large dose of ascorbic acid (200-600 mg.), which is known as the test dose method, or by the saturation method in which large doses of ascorbic acid are given over a period of several days until saturation, as determined by excretion, has been reached. In the
former method the state of nutrition is indicated by the proportion of the test dose which is excreted during the following 24 hours. In the latter, the daily requirement is calculated by dividing the total amount of ascorbic acid required to bring about saturation by the number of days. A combination of these two methods may also be used. In this case the test dose is employed as a measure of saturation, a large percentage of it being excreted if the maximum storage capacity of the tissues has been reached.

The determination of urinary excretion would not, however, be very satisfactory for testing the vitamin C nutrition of large numbers of subjects. Analysis of a single specimen is of no value because excretion may vary considerably during a 24-hour period, and the collection and analysis of a 24-hour specimen presents many difficulties. Then too, if urinary excretion figures are to have meaning the ascorbic acid intake must be carefully controlled over a period of at least several days.

The method which seems best adapted to clinical use in the measurement of the ascorbic acid concentration in the blood. For normal blood this concentration appears to be directly proportional to the ascorbic acid content of the previous diet (1)(28). A single specimen serves the same purpose as a 24-hour specimen of urine and micromethods have now been devised which require but a few drops of blood. Regarding the efficiency of blood analysis as a
test for vitamin C nutrition Sloan (49) who compared the results obtained on normal subjects and cases of known degrees of deficiency says, "It would seem that of the single determinations the blood assay is the most informative".

There appears to be a definite relationship between the amount of ascorbic acid excreted in the urine and the fasting concentration in the blood. As ascorbic acid intake is increased the blood concentration rises and the urinary excretion increases until tissue saturation is reached. At this point the urinary excretion, as mentioned before, rises rapidly but the blood level remains constant. This threshold level of urinary excretion was thought by Faulmer and Taylor (18) to correspond to a definite concentration in the blood. Other workers have since shown that the concentration varies among different individuals.

An intradermal test for vitamin C deficiency was designed by Rotter (47). In this test a small amount of standard indophenol dye solution is injected beneath the skin and the fading time is recorded. The technique was perfected in the hope that it would prove a simple test for clinical use, but other investigators (44)(17)(59) have found it unsatisfactory.

ASCORBIC ACID CONTENT OF BLOOD. The first determinations of the ascorbic acid content of human blood were reported by Gabbe (20) who measured the ascorbic acid content of the serum of a number of adult patients suffering
from various illnesses and found it to range from 0.14 to 1.21 mg. per 100 ml.

Farmer and Abt (15), seeking a method which would be clinically practical, simplified the procedure used by some of the other workers and developed a micro-method which could be used on small samples of capillary blood (16)(17). They found (1) that among a group of 59 infants and children on adequate diets, ranging in age from birth to puberty, the reduced ascorbic acid content of the blood plasma ranged from 0.752 to 2.416 mg. per 100 ml. In another group of children, six weeks to eleven years of age, apparently normal but on low ascorbic acid intake, the plasma values ranged from 0.515 to 0.769 mg. per 100 ml. The same paper (1) also reports findings among a group of young adult medical students, 21 to 32 years of age. On their regular diets the plasma values ranged from 0.687 to 2.29 mg. per 100 ml. These results led the authors to the conclusion that there seemed to be no correlation between age and ascorbic acid content of blood, but that diet seemed to have a very definite effect. Regarding the influence of diet they made the following statement, "The ascorbic acid (reduced) content of the blood plasma varies directly with the vitamin C content of the previous diet." They also concluded that blood plasma values less than 0.75-0.80 mg. per 100 ml. indicated suboptimal vitamin C intake.
Mirsy, Swadesh and Soskin (40) made observations on total blood ascorbic acid in about 100 different individuals. Values which they considered normal ranged from 1.19 to 2.66 and those in patients suffering from a variety of chronic diseases ranged from 1.11 to 2.38 mg. per 100 ml. They were unable to observe any correlation between total blood ascorbic acid and dietary regime.

Van Ecklon (56) kept a subject on a vitamin C-free diet plus measured supplements of ascorbic acid for a period of three months and observed changes in urinary excretion and blood concentration. He believed that a blood level of about 1.5 mg. per 100 ml. or above signified saturation, that levels below 0.4 mg. per 100 ml. could be considered insufficient and that values between these two extremes would be more or less sufficient.

From his observations on a number of patients, Deggeler (12) reported a variation in blood concentration from 0.13 to 1.7 mg. per 100 ml. and suggested the following ranges as representing the various nutritional states: a level higher than 1.3 mg. per 100 ml., excellent; 1.0-1.3, good; 0.5-1.0, sufficient; and less than 0.5, insufficient. The saturation point of the blood he believed to lie between 0.9 and 1.5 mg. per 100 ml.

According to Greenberg, Rinehart and Phatak (25) who made many determinations on plasma, the blood plasma ascorbic acid curve after the ingestion of a test dose of 500 mg.
per 100 pounds of body weight is similar in type to that for blood sugar, with the peak coming two to four hours after ingestion. They concluded that reduced ascorbic acid levels below 0.7 mg. per 100 ml. of plasma were probably suboptimal, that levels ranging between 0.7 and 0.9 would appear adequate but that the optimal level was probably above this range, and that values less than 0.5 must be considered low.

Taylor, Chase and Paulkner (52) whose normal subjects included adults of both sexes and all ages, all free from infections or clinical evidence of dietary deficiency, found the average serum level to be 1.61 mg. per 100 ml. with extremes of 0.83 and 2.45. In a group of 10 adults with clinical manifestations of scurvy the levels were considerably lower, varying from 0.11 to 0.55 mg. per 100 ml.

Figures for whole blood, red cells and white cells as well as plasma were given by Stephens and Hawley (50) whose purpose was to determine the partition of reduced ascorbic acid within the blood. They found the concentration in the white cells to be much higher than that in either red cells or plasma. The red cell content was found to be slightly lower than that of plasma, values for which were similar to those reported by other investigators. In 30 specimens of blood obtained from a group of normal individuals and patients with a variety of pathologic conditions the values were 0.66-5.45 mg. per 100 ml. for whole blood,
0.70-5.75 mg. per 100 ml. for plasma, 0.72-4.25 mg. per 100 ml. for red cells and 6.95 to 75.0 mg. per 100 gm. for white cells. The higher values for white cells (26.4-75.0 mg. per 100 gm.) were found in patients suffering from leucemia. This high white cell content was thought to account for the higher values for whole blood in these patients, 1.66-5.45 mg. per 100 ml.

The findings of Pijoan and Eddy (45) also indicated the ascorbic acid content of plasma to be slightly higher than that of red cells. In a series of carefully controlled analyses of blood from normal individuals they found values of 1.31-2.80 mg. per 100 ml. for plasma and 0.86-

1.70 mg. per 100 ml. for red cells.

Wright, Lilienfeld and MacKenathan (60) observed plasma levels from 0.27 to 1.54 mg. per 100 ml. in a group of 49 patients in various nutritional states. Among those whose dietary history was considered excellent the average value was 1.54, for those whose diets were rated good, the values ranged from 0.84-1.4, and for those with a fair to poor dietary history the values were 0.70 and below.

Ingalls (31) studied the concentration of ascorbic in the plasma of three groups of infants comprising well babies, babies picked at random from the ward and patients with scurvy. The plasma levels ranged from amounts too low to determine to 1.32 mg. per 100 ml. From these results he grouped the levels representing the various nutritional
states as follows: optimum level, signifying saturation, 2.00-1.00, normal, 1.00-0.70, low normal, 0.70-0.50, suboptimum, 0.50-0.30, and deficiency level, 0.30-0.00 mg. per 100 ml.

In a review covering the use of ascorbic acid in clinical medicine Wright (59) stated that "the normal blood plasma level for ascorbic acid in adults lies between 0.70 and 1.5 mg. per 100 ml."

A comparison of the various methods used in grading subclinical scurvy was made by Sloan (48) who tested patients suffering from known degrees of deficiency by various methods in an effort to evaluate each method. From the results of this investigation he concluded that "blood assay is the simplest dependable procedure and roughly indicates the degree of depletion". He believed that a plasma level of 0.50 mg. per 100 ml. represented the lower limit of normal and 0.80 to 1.0 the average fasting normal value. For the mildest cases of scurvy he found the level to be 0.36 and for the more severe cases, 0.20 mg. per 100 ml.

Using a modified method in which plasma values were determined by means of the photoelectric colorimeter, Mindlin and Butler (59) found the plasma concentration to vary from 0.1 to 1.6 mg. per 100 ml.

Heinemann (29) observed blood levels of two subjects over considerable periods of time. One subject was
saturated and thereafter supplied with 0.53 mg. of ascorbic acid per kilogram daily for 32 days, and on another occasion with 0.5 mg. per kilogram for 53 days. During these periods the blood concentration fell from 1.4-1.5 to 0.72 and from 1.4-1.5 to 0.34 mg. per 100 ml., respectively. Another subject was partially depleted as a result of a diet devoid of vitamin C and was then given 40 mg. of ascorbic acid (0.5 mg. per kilogram) daily for 16 days during which period the blood level remained unchanged at 0.54-0.55 mg. per 100 ml.

Portnoy and Wilkinson (43), investigating vitamin C balance in patients with peptic ulceration and haematemesis, found a lower plasma level in these patients, 0.14-0.69 mg. per 100 ml., than in a control group, 0.60-1.84 mg. per 100 ml. All of the ulcer cases tested showed indications of deficiency.

Wortis, Liebmann and Wortis (58) who determined the ascorbic acid content of blood, spinal fluid and urine in a number of individuals in various states of nutrition divided the values obtained into three groups, the normal with plasma levels of 0.70 mg. per 100 ml. and above, the intermediate subnormal with values 0.4-0.69, and the subnormal with values less than 0.4 mg. per 100 ml. Of the 153 patients tested 30 were in the upper group, 53 in the intermediate and 68 in the lower. The plasma levels of 14 healthy medical students were found to range from 0.74 to 1.38 mg. per 100 ml.
Nouweiler (42) studied the ascorbic acid content of the blood plasma in subjects in various degrees of saturation and came to the conclusion that about 0.8 mg. per 100 ml. was a good normal plasma concentration but did not denote saturation. He regarded values below 0.55 mg. per 100 ml. as pathological and those below 0.4 mg. per 100 ml. as definitely scorbutic.

Drigalski (13) studied six subjects and found that although the ascorbic acid concentration in the plasma was always low in those receiving diets poor in vitamin C and higher in those having adequate diets, there was no regular variation according to the vitamin C content of the diet. He concluded that plasma values more than 1.0 mg. per 100 ml. indicated good vitamin C nutrition and those less than 0.5, a mild state of deficiency.

Faulkner and Taylor (18) cited evidence to support their statement that ascorbic acid has a definite renal threshold corresponding to a serum level of about 1.4 mg. per 100 ml. They believed that serum values above this figure indicated saturation and those below it, various degrees of undersaturation.

Goldsmith and Ellinger (21) also considered that there is a definite relation between the level of reduced ascorbic acid in the blood and the amount excreted by the kidneys. They observed that when the plasma level reached about 1.4 mg. urinary excretion increased. In a series of tests on
25 individuals they found the level of reduced ascorbic acid in the plasma to vary all the way from 0.04 to 1.38 mg. per 100 ml. For the group considered normal according to findings obtained on examination of the urine, the plasma values ranged from 0.43 to 1.98, with an average of 1.11 mg. per 100 ml. Only one member of this group showed a level below 0.65. For the group considered deficient, the plasma level varied from 0.04 to 0.52 with an average of 0.17 mg. per 100 ml. Six of the subjects in the latter group showed values less than 0.1 mg. per 100 ml. and four of these exhibited clinical evidence of vitamin C deficiency.

In a study concerning the effect of low vitamin C intake on wound healing, Hartzell, Winfield and Irvin (26) determined plasma concentrations in a group of apparently healthy adults and a group of patients with various benign conditions. In the hospitalized group the plasma levels ranged from 0.49 to 1.6 and in the normal group, 0.64 to 1.86 mg. per 100 ml.

Ralli, Friedman and Shoryy (46) report a long-time investigation in which three normal male adults were hospitalized and fed a diet containing not more than five milligrams of vitamin C daily. During certain periods the basic diet alone was given and during others the basic diet was supplemented with known amounts of ascorbic acid. Blood plasma ascorbic acid concentration was determined three times a week. The plasma levels varied from 0.20
when no supplement was given to values as high as 1.50 mg. per 100 ml. when the supplement was 150 mg. From these results the authors concluded that a plasma concentration of 1.0 mg. per 100 ml. indicates saturation. When the daily intake was 50 mg. the plasma concentration averaged 0.4 mg. per 100 ml.; the patients appeared normal and there were no signs of a deficiency. It was therefore suggested that this plasma level be considered the lower limit of normal.

Working with three women on controlled diets, Toddhunter and Robbins (55) found the plasma ascorbic acid concentration at saturation to be from 1.29 to 1.84 mg. per 100 ml. When the total daily intake was 60 mg. the plasma level did not drop below 1.0 mg. per 100 ml.

Pincus (19) has also studied the plasma levels of subjects on controlled intake. At saturation the values in three subjects were 0.99 to 1.35 mg. per 100 ml. At the end of a six-day period when the intake was 60 mg. levels in two of the subjects were 0.63 and 0.81 mg. per 100 ml. An intake of 80 mg. for the same length of time resulted in a plasma level of 0.93 in one subject, and when the supplement was 90 mg. plasma values of 0.91 and 1.03 mg. per 100 ml. were found in two of the subjects. Daily plasma determinations were made on two of the subjects. In one, on an intake of 110 mg. the plasma concentration averaged 0.37 mg. per 100 ml. over a period of six days and when the supplement was raised to 150 mg. for the same
length of time the average plasma value was 1.05. In the other subject a daily intake of 90 mg. maintained an average plasma value of 1.0 mg. per 100 ml.

Daily plasma determinations were made by Hauck (27) on two subjects whose intake was kept at the saturation level for four weeks. The average plasma values were found to be 1.6 and 1.0 mg. per 100 ml.

It is suggested by Butler and Cushman (8) that in deficiency and undersaturation the ascorbic acid content of whole blood would be a more reliable index of nutritional status than that of plasma. This suggestion is based on the fact that in subjects suffering from acute vitamin C deficiency the white layer (white cells plus platelets) of centrifuged blood was found to contain measurable amounts of what was apparently ascorbic acid after the plasma level had become zero. Similar findings were reported by Grandy and his coworkers (9)(34) who produced experimental scurvy in a normal active man by keeping him on a vitamin C-free diet for a period of six months. Grandy found that the plasma level fell rapidly from a normal value of 1.0 mg. per 100 ml., reaching zero at the end of 41 days but that the ascorbic acid content of the white cell-platelet layer which was normally 30-40 mg. per 100 ml. fell much more gradually, reaching zero only after 122 days which was just 10 days before scurvy set in.
The findings and recommendations of the investigators who determined concentration of ascorbic acid in the blood plasma of normal individuals are summarized in Table I.

**REQUIREMENT.** There seems to be a considerable difference of opinion regarding the vitamin C requirement of humans. One reason, no doubt, is the fact that there still seems to be a disagreement among investigators as to the basis on which requirement should be expressed. Many workers believe that it should be expressed as the amount required to maintain tissue saturation, which is the truly optimal state, while others think that it should be the minimum amount needed for protection against scurvy; and there are those who believe that in order to provide for adequate nutrition the intake must be somewhat above the indispensable minimum but need not reach the optimum value.

Gothlin and his associates (24) investigated the requirements of 4 adults, two male and two female, on controlled intake and found that at the point where restoration to normal of lowered capillary resistance took place the intake of ascorbic acid was 0.39–0.48 mg. per kilogram of body weight. This, they believed, represented the indispensable minimum requirement.

The Technical Commission on Nutrition of the League of Nations' Health Organization (55) reported that "the adult's requirement of ascorbic acid is no doubt covered by about 30 mg. daily, which has been shown to be a
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<td>Pijean and Eddy</td>
<td>1.31-2.80 Normal</td>
</tr>
<tr>
<td>Portnoy and Wilkinson</td>
<td>0.60-1.84 Normal</td>
</tr>
<tr>
<td>Ralli, Friedman and Sherry</td>
<td>1.0 Saturation 0.4 Lower limit of normal</td>
</tr>
<tr>
<td>Sloan</td>
<td>0.80-1.00 Average normal 0.50 Lower limit of normal</td>
</tr>
<tr>
<td>Stephens and Hawley</td>
<td>0.70-1.35 Normal</td>
</tr>
<tr>
<td>Taylor, Chase and Faulkner</td>
<td>0.83-2.43 Normal</td>
</tr>
<tr>
<td>Tocchunter and Robbins</td>
<td>1.29-1.84 Saturation</td>
</tr>
<tr>
<td>Wortis, Liebmann and Wortis</td>
<td>0.74-1.38 Normal</td>
</tr>
<tr>
<td>Wright</td>
<td>0.7-1.3 Normal</td>
</tr>
<tr>
<td>Wright, Lilienfeld and MacLenathen</td>
<td>1.54 Excellent 0.84-1.40 Good</td>
</tr>
</tbody>
</table>
curative dose for adults suffering from scurvy.

According to Kellie and Zilva (32) who studied urinary excretion and blood plasma levels in two subjects, a daily intake of 30 to 40 mg. will "insure a margin of safety". It was found that this amount maintained a more or less constant excretion in the urine.

Nowciler (42) calculated the ascorbic acid content of the diets of a number of persons with a blood plasma ascorbic acid level of about 0.8 mg. per 100 ml., which he regarded as a good normal value without denoting saturation, and found that their daily intake was 30 to 35 mg. in winter and 50 to 55 mg. in summer. He therefore favored the recognition of 30 to 35 mg. as the minimum daily requirement for normal healthy persons with efficient metabolism.

Recently, Grandon, Lund and Dill (9) produced experimental scurvy by keeping a normal, healthy adult on a vitamin C-free diet for about six months. They estimated the maximum daily utilization of the vitamin by determining the amount required to resaturate the subject after the first signs of scurvy appeared, subtracting from this figure the total amount excreted in the urine during the depletion period, and dividing the result by the length of the depletion period. The daily requirement, on this basis, was found to be between 50 and 45 mg.

Van Eekelen (56) used a test dose method in which the subject was first saturated on a daily intake of 250 mg.
of ascorbic acid and then kept on a vitamin C-free diet for a period of about three months following which the intake was again increased until tissue saturation had been reached. From observations of both urinary excretion and blood concentration made during this period he concluded that about 60 mg. represented the daily requirement for adults weighing 70 kg.

Heinemann (28), employing a similar test dose procedure with two male subjects, also found the requirement for re-saturation after depletion to be 60 mg. per day for a 70 kg. man. In another study (29) in which he measured the concentration of ascorbic acid in the blood while following the same test dose procedure, he concluded that at least 0.8 mg. per kilogram of body weight is used daily by healthy subjects saturated with the vitamin, but that 0.4 mg. per kilogram will protect against scurvy.

Belser, Hauck and Storvick (4) determined the intake required to keep the tissues in a state of saturation as measured by urinary excretion. The method used was a modification of the test dose procedure in which the tissues of the subjects were first saturated by daily intakes of 200 mg. of ascorbic acid for a period of three to six days, following which a 400 mg. test dose was given. A series of similar tests on graded doses of ascorbic acid were then made until the least quantity was found which would produce a response to the 400 mg. test dose similar to that obtained
in the preliminary period. The maximum requirement of seven normal adults, five men and two women, were found, by this method, to range from 70 to 100 mg. or 1.0 to 1.6 mg. per kilogram of body weight.

Todhunter and Robbins (55) who used the same method reported that the minimum intake required to maintain tissue saturation in three women was 90, 80, and more than 110 mg., or 1.7, 1.6, and more than 1.6 mg. per kilogram of body weight, respectively.

Fincke (19) studied the requirement of three women, employing the same procedure as that used by Hauck, supplemented with measurements of blood plasma ascorbic acid, and found it to be 110, 110, and 130 mg. per day, respectively.

From the results of a prolonged study in which three normal adult male subjects were kept on a controlled diet and urinary excretion and blood plasma concentration were determined, Ralli, Friedman and Sherry (46) concluded that the optimal daily intake was about 100 mg.

After an extensive review of the literature on the subject Smith (49) listed three levels of requirement: 1. the physiologically indispensable—to prevent the slightest decrease in capillary resistance or to maintain uniform excretion of the vitamin barely above that on a vitamin C-free diet; 2. the adequate—to maintain uniform excretion after a depletion period following a preliminary saturation period; and 3. the saturation or luxus consumption
level—to maintain the tissues in a state of saturation as determined by various modifications of the test dose method. For an average adult, Smith considered that intakes of 28, 50-60, and 100 mg. daily would roughly represent these three levels of requirement.

It is outside the scope of this discussion to consider the influence on requirement of factors such as illness. Suffice it to say, that increased metabolism, whether it results from infection, fever or increased muscular activity, does appear to have an effect on ascorbic acid requirement.

PURPOSE. Inasmuch as it is quite generally agreed that a blood plasma concentration of 0.7 to 0.9 mg. per 100 ml. seems adequate to take care of the body's needs, it would appear that an intake sufficient to maintain this blood plasma level would represent adequate nutrition.

It was the purpose of this investigation, therefore, to determine the ascorbic acid intake which would maintain the blood plasma concentration at an average of about 0.8 mg. per 100 ml.
PROCEDURE

GENERAL PLAN OF INVESTIGATION. Four college students, MLS, LS, WL, and RS, were placed on a constant diet of known low ascorbic acid content but adequate in all other respects, supplemented with known amounts of ascorbic acid, for a period of twenty days and the concentration of ascorbic acid in the blood plasma was determined daily.

For the first three days, large amounts of ascorbic acid were given in order to raise the blood plasma level well above 0.8 mg. per 100 ml. This eliminated the possibility of the ascorbic acid intake being utilized to resaturate tissue rather than maintain blood concentration. On the first and second days the supplement consisted of orange juice, one cup after breakfast and one cup at lunch, and on the third day, 100 mg. of ascorbic acid was administered after breakfast.

This preliminary period was followed by a seven-day experimental period when a daily supplement of ascorbic acid which it was estimated would be sufficient to maintain the concentration in the blood plasma at an average level of 0.8 mg. per 100 ml. was given in the morning after breakfast. This supplement was 50 mg. for the women, MLS and LS, and 60 mg. for the men, WL and RS.

* The crystalline ascorbic acid used in this investigation was furnished by Merck and Company.
On the eleventh and twelfth days the ascorbic acid intake was again increased to bring the plasma levels above 0.8 mg. per 100 ml. Orange juice, as above, was given on the eleventh day and 100 mg. of ascorbic acid, on the twelfth. It was necessary to keep subject RS on a high intake for one more day, because his plasma ascorbic acid concentration had not reached the desired level on the morning of the thirteenth day. He was given 70 mg. of ascorbic acid after breakfast and 100 mg. at lunch.

After the first period, it was decided to decrease the supplement for HLS and LS to 40 mg., and that for VL to 50 mg., and increase the supplement for RS to 80 mg. per day during the second experimental period, the thirteenth through the twentieth days.

Blood samples were collected each morning, after breakfast but before any supplement had been given, and analyzed for ascorbic acid as soon as possible.

Each subject was weighed daily, and a record was kept of any unusual activity or other factors which it was thought might affect the ascorbic acid requirement.

About three weeks before the period on controlled diet began a preliminary test was made to determine the ascorbic acid concentration in the blood plasma of each subject while on his regular diet. The subject was asked to keep a record of all food eaten for a week before this test was made.
BASIC DIET. The basic diet, a modification of that used by Belser, Hauck, and Storvick (4), consisted of the following foods:

<table>
<thead>
<tr>
<th>Food</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canned whole beets</td>
<td>100 gm.</td>
</tr>
<tr>
<td>Juice</td>
<td>10</td>
</tr>
<tr>
<td>Canned shoestring carrots</td>
<td>100</td>
</tr>
<tr>
<td>Juice</td>
<td>10</td>
</tr>
<tr>
<td>Canned pears</td>
<td>100</td>
</tr>
<tr>
<td>Juice</td>
<td>50</td>
</tr>
<tr>
<td>Cooked dried prunes</td>
<td>100</td>
</tr>
<tr>
<td>Juice</td>
<td>10</td>
</tr>
<tr>
<td>Evaporated milk</td>
<td>60</td>
</tr>
<tr>
<td>American cheese</td>
<td>60</td>
</tr>
<tr>
<td>Ground beef</td>
<td>100</td>
</tr>
<tr>
<td>Egg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>at least one plus a limited number in cooking</td>
</tr>
</tbody>
</table>

Shredded Ralston  
Kraft  
Rice, brown and white  
Whole wheat and white flour  
Butter  
Cooking fat  
Nuts  
Brown and white sugar  
Coffee and tea  
Chocolate  
Seasonings  

[Ad libitum]

The canned beets, carrots, pears, and milk were purchased in case lots and two representative cans from each case were analyzed for ascorbic acid. A 25-pound box of dried prunes was purchased, and the prunes were cooked in a standard manner about once a week and stored in a covered
kettle in the refrigerator until used. Each lot cooked was analyzed for ascorbic acid. Freshly-ground beef was pur-
chased every other day and a check made on its ascorbic acid content once each week.

The food analysis for ascorbic acid was carried out according to the method described by Bessey (5). For the solid materials, an extract was prepared by grinding a weighed portion, 10 to 25 gm., with a small amount of 3 per cent metaphosphoric acid in a glass mortar. The solids were thrown down by centrifuging and the clear extract was de-
canted into a 100 ml. volumetric flask. The solids were re-
turned to the mortar, reground with a second portion of meta-
phosphoric acid, centrifuged, and the clear extract added to the first. This procedure was repeated until the solids had been reduced to a smooth paste (4 or 5 extractions). The combined extracts were then made up to volume and an aliquot titrated with a standard solution of the dye, 2,6-dichloro-
benzenoneindophenol, until a faint pink end point lasting at least 20 seconds was reached.

The beet, carrot, pear and prune juices were strained through a clean cheesecloth to remove any solid matter; a weighed amount, 10 to 25 gm., was diluted to 100 ml. with 3 per cent metaphosphoric acid and an aliquot was titrated with the dye as above.

In the case of the evaporated milk, 20 gm. were weighed out in a 50 ml. centrifuge tube and an equal volume of 3
per cent metaphosphoric acid was added. The mixture was stirred thoroughly and allowed to stand for several minutes, centrifuged to separate the precipitate, and the clear liquid decanted into a 100 ml. volumetric flask. Another portion of metaphosphoric acid was mixed with the solids, the mixture centrifuged, and the clear liquid combined with the first extract. This procedure was repeated twice, the combined extracts were made up to volume, and an aliquot was titrated with the dye.

The beet and prune extracts, because of the presence of red-colored pigments, were titrated according to the method of Eilhenry and Graham (37). This procedure is based on the fact that most red-colored plant pigments are insoluble in chloroform, whereas the dye is more soluble in chloroform than in water, which makes it possible to detect the pink end point of the titration by mixing the extract with a layer of chloroform.

A small quantity of chloroform, 5 or 10 ml., was placed in a 50 ml. centrifuge tube, 5 or 10 ml. of extract were added, and the dye solution run in slowly. After the addition of the estimated amount of dye (determined from a preliminary titration) the aqueous layer was stirred for 20 to 30 seconds to allow for the complete reaction of the dye with any ascorbic acid present. The two liquids were then thoroughly mixed with a stirring rod for about 30 seconds and separated again by centrifuging for a minute. If the
chloroform layer showed no color a few drops of dye solution were added and the layers mixed and separated as before. The end point was reached when the chloroform layer showed a definite pink tinge.

In all of the food analysis a 10 ml. burette of small bore was used for measuring the dye.

The average ascorbic acid content of the foods analyzed was found to be as follows:

<table>
<thead>
<tr>
<th>Food</th>
<th>Ascorbic Acid Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boots Juice</td>
<td>0.036 mg. per gm.</td>
</tr>
<tr>
<td>Carrots Juice</td>
<td>0.008</td>
</tr>
<tr>
<td>Pears Juice</td>
<td>0.010</td>
</tr>
<tr>
<td>Prunes Juice</td>
<td>0.021</td>
</tr>
<tr>
<td>Milk</td>
<td>0.011</td>
</tr>
<tr>
<td>Beef</td>
<td>-</td>
</tr>
</tbody>
</table>

The basic diet, therefore, contained an average of 9.2 mg. ascorbic acid daily.

**BLOOD ANALYSIS.** The blood samples were taken each morning after breakfast but before any ascorbic acid was given. The analysis of the plasma was carried out according to the micromethod of Farmer and Abt (16). A few drops of capillary blood (0.3 to 0.8 ml.) were collected from a lancet wound in a finger tip into a hard-glass vial containing a small amount of lithium oxalate to prevent clotting. The blood was stirred once with the broad end of a
toothpick, taking care not to touch the glass which would cause hemolysis, and the vial was stoppered and centrifuged for five minutes. By means of a capillary pipette, 0.1 ml. of plasma was transferred to a 15 ml. conical centrifuge tube. With the same pipette, 0.1 ml. of redistilled water (freshly boiled and cooled) was added, thus rinsing the pipette. The proteins were precipitated by adding 0.2 ml. of 5 per cent metaphosphoric acid. The solutions were thoroughly mixed by tapping the centrifuge tube against the hand, and centrifuged for five minutes. The procedure up to this point was carried through as rapidly as possible. If it was not possible to titrate the deproteinized plasma immediately, the tubes were stoppered and placed in the refrigerator until used.

The titration was carried out by pipetting 0.2 ml. of deproteinized plasma into one of the depressions of a white porcelain titration tile. Standard dye solution was run in from a 0.1 ml. microburette until a faint pink color was obtained. A blank was prepared by titrating 0.2 ml. of 2.5 per cent metaphosphoric acid (0.1 ml. 5 per cent metaphosphoric acid plus 0.1 ml. redistilled water) in an adjacent depression in the tile until the color matched that of the unknown. From these data the ascorbic acid content of the plasma was computed thus:

\[
\frac{\text{mg. ascorbic acid per}}{100 \text{ ml. plasma}} = (\text{ml. dye} - \text{ml. blank}) \times 3 \times 2000
\]
where S represents the milligrams of ascorbic acid equivalent to 1 ml. of dye. Duplicate samples of plasma were deproteinized and titrated as checks.

STANDARDIZATION OF DYE. Due to the fact that aqueous solutions of the dye change slowly even though kept in a dark bottle in the refrigerator, it was found advisable to standardize the dye solution every day it was used. This was done by titration with a solution of ascorbic acid of known strength. The dye reacts with ascorbic acid according to the equation:

\[ C_6H_8O_6 + 0.1C_2H_2Cl_2·H·C_6H_4·OMe \rightarrow C_6H_8O_6 + H_2O·C_2H_2Cl_2·MMe·C_6H_4·OMe \]

The strength of the ascorbic acid solution was checked daily against a solution of 0.001 normal iodine prepared as recommended by Bessey and King (6). Ascorbic acid reacts with iodine according to the equation

\[ C_6H_8O_6 + I_2 \rightarrow C_6H_8O_6 + 2 HI \]

which makes 1 ml. of 0.001 normal iodine equivalent to 0.038 mg. of ascorbic acid.

The strength of the ascorbic acid solution was so adjusted that the same size aliquot could be used for titration with both dye and iodine, thus simplifying both procedure and calculation. Twenty-five milliliters of ascorbic acid solution were pipetted into a 100 ml. Erlenmeyer flask and the dye was run in from a 50 ml. burette until a faint pink end point lasting at least 30 seconds had been reached.
Another 25 ml. portion of ascorbic acid solution was pipetted into a 100 ml. Erlenmeyer flask, 1 ml. of fresh 2 per cent starch solution was added, and iodine solution dropped in from a 10 ml. burette until a faint bluish tinge became visible. In both of these cases the usual procedure of running a blank following the titration was reversed. A 25 ml. blank containing the same amount of acetic acid as the ascorbic acid solution was prepared by adding a known quantity of dye or iodine, as the case might be. The unknown was then titrated until an end point which matched the blank had been reached. Because the relative size of the blank was so much larger in these titrations than in most, it was believed that the latter method would be more accurate. It was also easier to obtain exact checks when this procedure was used.

From the results of the above titrations the ascorbic acid equivalent of the dye (S) was calculated from the equation

\[ S = \frac{\text{ml. iodine} \times R}{\text{ml. dye}} \]

where R represents the milligrams of ascorbic acid equivalent to 1 ml. of iodine solution.

The iodine solution was standardized every four weeks according to the procedure given in Fales and Kenny’s Quantitativo Analysis (14). In this method the iodine solution is titrated with standard 0.001 molar sodium
thiosulfate solution which in turn is standardized against 0.0001666 molar potassium dichromate solution prepared by weighing out an exact amount of the pure moisture-free salt.

For standardization of the iodine solution, 25 ml. were pipetted into a 100 ml. Erlenmeyer flask and the standard sodium thiosulfate solution was run in from a 50 ml. burette until the brownish color of the iodine had faded to pale yellow. One milliliter of 2 per cent starch solution was added and the titration continued until the blue color had disappeared. This reaction is represented by the equation

$$I_2 + 2S_2O_3^2- \rightarrow 2I^- + S_4O_6^2-$$

Here, 1 ml. of iodine solution is equivalent to 2 ml. of thiosulfate (of an equal molarity) and the normality of the iodine would, therefore, be calculated:

$$\text{molarity } I_2 = \frac{(\text{ml. } Na_2S_2O_3)(\text{molarity } Na_2S_2O_3)}{2 \times \text{ml. } I_2}$$

Normality $I_2 = 2 \times$ molarity $I_2$

and the ascorbic acid equivalent of the iodine solution (R) would be obtained by multiplying the normality of the iodine by 0.088.

For standardization of the thiosulfate, 5 ml. of 1.8 molar potassium iodide solution and 1.7 ml. of 12 molar hydrochloric acid were added to 50 ml. of distilled water in a 250 ml. beaker; 25 ml. of standard dichromate solution
were added with constant stirring, and the mixed solutions allowed to stand, out of direct sunlight, for three minutes. The mixture was diluted immediately with 50 ml. distilled water and titrated with the thiosulfate solution until the brownish color of the iodine had diminished to a pale yellow. One milliliter of 2 per cent starch solution was added and the titration was continued until the blue color disappeared. This reaction takes place in two steps

\[ \text{Cr}_2\text{O}_7^{2-} + 14 \text{H}^+ + 6 \text{I}^- \rightarrow 2 \text{Cr}^{3+} + 3 \text{I}_2 + 7 \text{H}_2\text{O} \]

\[ 3 \text{I}_2 + 6 \text{S}_2\text{O}_3^2- \rightarrow 6 \text{I}^- + 3\text{S}_4\text{O}_6^2- \]

Thus 1 ml. of potassium dichromate solution would be equivalent to 6 ml. of sodium thiosulfate solution of an equal molarity. The strength of the dichromate solution being known, the molarity of the thiosulfate solution would be determined as follows:

\[
\text{molarity Na}_2\text{S}_2\text{O}_3 = \frac{6(\text{molarity K}_2\text{Cr}_2\text{O}_7)(\text{ml. K}_2\text{Cr}_2\text{O}_7)}{\text{ml. Na}_2\text{S}_2\text{O}_3}
\]

SOLUTIONS. The water used in making up all of the solutions was redistilled from glass to make sure that any traces of heavy metals, particularly copper, would be removed. The water for the ascorbic acid and metaphosphoric acid solutions was boiled and cooled just prior to making up the solutions in order to remove any dissolved oxygen which might be present.
Dye. Approximately 25 mg. of sodium 2,6-dichlorobenzenoneindophenol, technical, obtained from Eastman Kodak Company and purified by repeated extractions with ether, were dissolved in 980 ml. of hot redistilled water. The solution was cooled to room temperature or below and 20 ml. of phosphate buffer, pH 6.8, was added. The solution was then filtered into a dark bottle and stored in the refrigerator. Two liters of the solution were prepared at a time. This amount lasted 7 to 8 days.

Ascorbic Acid. Approximately 20 mg. of crystalline ascorbic acid were dissolved in a liter of freshly boiled and cooled redistilled water containing 10 ml. of 6 per cent acetic acid. The solution was stored in a dark bottle in the refrigerator. A liter of this solution lasted 7 to 8 days.

Metaphosphoric Acid. The required amount of pure stick metaphosphoric acid from which the coating of orthophosphoric acid had been removed by scraping and dissolving in redistilled water was dissolved in a small quantity of freshly boiled and cooled redistilled water. The solution was made up to volume and stored in the refrigerator. Both 5 per cent and 5 per cent solutions were prepared fresh every 7 days. The 5 per cent solution was made up in 100 ml. lots and the 5 per cent, 1 or 2 liters at a time.

Iodine. Approximately 0.1269 gm. of iodine crystals were dissolved in a small amount of redistilled water
containing 15 gm. of potassium iodide and the resulting solution was made up to a liter.

**Sodium Thiocyanate.** Approximately 0.2432 gm. of the pure crystalline salt were dissolved in a small quantity of redistilled water and the solution made up to a liter.

**Potassium Dichromate.** 0.0490 gm. of the pure, moisture-free salt were dissolved in a small quantity of redistilled water and the solution made up to a liter. The moisture-free salt was prepared by heating a small quantity of the salt (analytical grade) in a clean, dry evaporating dish to a point just above that at which it fused, taking care to exclude all dust and organic matter. The fused salt, after it had cooled, was crushed in a clean, dry glass mortar and stored in a glass-stoppered bottle in a desiccator until used.

**DESCRIPTION OF SUBJECTS.** Subject HLS was a young woman, 22 years of age. She was the smallest of the group, height 61.5 in., weight 100 lb. Though she moved about briskly she did not appear to be nervous.

The other young woman, LS, was 21 years of age, 65.5 in. tall, 135 lb. in weight. She did not move about as briskly as HLS but appeared to be very efficient and not at all nervous.

The two men were, in many respects, quite distinct contrasts to one another. UL was a small, rather wiry individual, 22 years of age, 67.25 in. tall and 151 lb. in
weight. Nothing appeared to worry him and he never seemed
to be in a hurry, yet, at the same time, he appeared to be
efficient in his movements.

RS, on the other hand, was a highly strong individual.
He was 22 years of age and the largest of the group, being
70.5 in. tall and weighing 165 lb. He drank large amounts
of coffee and, although he had no noticeable cold during the
course of the experiment, he was ordinarily subject to fre-
quently colds and some headaches. His skin seemed to be very
sensitive to cuts and bruises, and when it was necessary
for him to increase the blood flow through his hand by warm-
ing and exercise at the time the blood samples were taken,
he complained of a dull pain in his fingers. The lancet
wounds in his finger tips took longer to heal than those
in the fingers of the other subjects.
RESULTS AND DISCUSSION

The period on controlled diet began January 6, immediately following the vacation. The preliminary test to determine blood plasma ascorbic acid concentration while the subjects were on their regular diets was made on December 18 just before the vacation began. This allowed twenty days for building up the plasma levels of the men, UL and RS, which were found at that time to be 0.37 and 0.44 mg. per 100 ml., respectively. MLS was found to be in good nutrition, her plasma level being 0.95 mg. per 100 ml. A preliminary test was not made on subject LS because she was not added to the group until January 6. She was substituted for another subject who was eliminated at the last minute due to the presence of an infection. Her first blood test showed her to be in good nutrition.

The low plasma levels in the men were explained by the records of food consumed during the previous week. The diet had been practically the same for both since they lived together and did their own cooking. Fruit had been limited to an apple about every other day and the only fresh vegetable was a prepared salad once during the week. Some sort of canned vegetable was eaten every day, but only once was this vegetable tomatoes. During the vacation they consumed considerable amounts of oranges and grapefruit and when a rough determination was made on January 4
without standardizing the dye solution the plasma levels had risen to about 1.0 mg. per 100 ml.

The day-to-day variation in the blood plasma ascorbic acid concentrations of the subjects when the intake was controlled is shown in Tables II-V.

When HLS was given a supplement of 50 mg. ascorbic acid the plasma concentration decreased gradually from 1.06 to 0.82 mg. per 100 ml., averaging 0.83 for five days (see Table II). When the supplement was changed to 40 mg., the plasma concentration again dropped to 0.82 and the average level was 0.84 during the last four days of the experimental period. Just why the plasma level dropped no lower on an intake of 40 mg. than on 50 mg. is hard to explain.

There is a possibility that the after-effects of a slight attack of influenza which the subject suffered during the vacation period may have influenced the requirement during the first experimental period.

In LS (Table III) the plasma concentration decreased about 0.2 mg. per 100 ml. from the original value of 1.14 when the supplement was 50 mg. Though the level reached 0.86 on the last day, the average for the last five days was 0.93 mg. per 100 ml. The 40 mg. supplement maintained an average value of 0.87 for the last four days.

The requirement of the men seemed to be somewhat higher. As shown in Table IV, an intake of 60 mg. was required to maintain an average plasma level of 0.82 mg. per 100 ml. in
TABLE II
DAILY BLOOD PLASMA ASCORBIC ACID LEVELS IN SUBJECT M L S

Height  61.5 in.  Age  22 yrs.

<table>
<thead>
<tr>
<th>Day</th>
<th>Ascorbic Acid Supplement</th>
<th>Plasma Ascorbic Acid</th>
<th>Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mg.</td>
<td>Per 100 Ml.</td>
</tr>
<tr>
<td>1</td>
<td>Orange Juice*</td>
<td>0.92</td>
<td>99</td>
</tr>
<tr>
<td>2</td>
<td>Orange Juice*</td>
<td>1.07</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>1.06</td>
<td>101</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>1.02</td>
<td>101</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>1.00</td>
<td>101</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>0.99</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>0.82</td>
<td>101</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>0.85</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>50</td>
<td>0.85</td>
<td>101</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>0.82</td>
<td>102</td>
</tr>
<tr>
<td>11</td>
<td>Orange Juice*</td>
<td>0.82</td>
<td>101</td>
</tr>
<tr>
<td>12</td>
<td>100</td>
<td>1.00</td>
<td>101</td>
</tr>
<tr>
<td>13</td>
<td>40</td>
<td>1.02</td>
<td>101</td>
</tr>
<tr>
<td>14</td>
<td>40</td>
<td>0.99</td>
<td>100</td>
</tr>
<tr>
<td>15</td>
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<td>101\frac{1}{2}</td>
</tr>
<tr>
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</tr>
<tr>
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<td>0.82</td>
<td>100\frac{1}{2}</td>
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<tr>
<td>18</td>
<td>40</td>
<td>0.85</td>
<td>100\frac{1}{2}</td>
</tr>
<tr>
<td>19</td>
<td>40</td>
<td>0.85</td>
<td>100</td>
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<tr>
<td>20</td>
<td>40</td>
<td>0.86</td>
<td>99</td>
</tr>
</tbody>
</table>

* 1 Pint
<table>
<thead>
<tr>
<th>Day</th>
<th>Ascorbic Acid Supplement</th>
<th>Plasma Ascorbic Acid</th>
<th>Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mg.</td>
<td>Mg. Per 100 Ml.</td>
<td>Lb.</td>
</tr>
<tr>
<td>1</td>
<td>Orange Juice*</td>
<td>–</td>
<td>134</td>
</tr>
<tr>
<td>2</td>
<td>Orange Juice*</td>
<td>1.19</td>
<td>135</td>
</tr>
<tr>
<td>3</td>
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<td>1.14</td>
<td>134</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>1.12</td>
<td>134</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>1.10</td>
<td>133 ½</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>0.98</td>
<td>132</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>0.89</td>
<td>133 ½</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>0.93</td>
<td>132 ½</td>
</tr>
<tr>
<td>9</td>
<td>50</td>
<td>0.99</td>
<td>132</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>0.92</td>
<td>132 ½</td>
</tr>
<tr>
<td>11</td>
<td>Orange Juice*</td>
<td>0.86</td>
<td>132</td>
</tr>
<tr>
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<td>100</td>
<td>1.00</td>
<td>132 ½</td>
</tr>
<tr>
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<td>1.06</td>
<td>133</td>
</tr>
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<td>14</td>
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<td>1.05</td>
<td>133</td>
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<td>40</td>
<td>0.91</td>
<td>132</td>
</tr>
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<td>40</td>
<td>0.89</td>
<td>133</td>
</tr>
<tr>
<td>17</td>
<td>40</td>
<td>0.86</td>
<td>132 ½</td>
</tr>
<tr>
<td>18</td>
<td>40</td>
<td>0.85</td>
<td>132</td>
</tr>
<tr>
<td>19</td>
<td>40</td>
<td>0.86</td>
<td>131 ½</td>
</tr>
<tr>
<td>20</td>
<td>40</td>
<td>0.90</td>
<td>150</td>
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</tbody>
</table>

* 1 Pint
**TABLE IV**

DAILY BLOOD PLASMA ASCORBIC ACID LEVELS IN SUBJECT W L

<table>
<thead>
<tr>
<th>Day</th>
<th>Ascorbic Acid Supplement</th>
<th>Ascorbic Acid Plasma</th>
<th>Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg.</td>
<td>mg. Per 100 ml.</td>
<td>Lb.</td>
</tr>
<tr>
<td>1</td>
<td>Orange Juice*</td>
<td>1.09</td>
<td>133(\frac{1}{2})</td>
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<tr>
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<td>Orange Juice*</td>
<td>1.16</td>
<td>132</td>
</tr>
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<td>3</td>
<td>100</td>
<td>1.08</td>
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<tr>
<td>4</td>
<td>60</td>
<td>1.19</td>
<td>131(\frac{1}{2})</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>1.04</td>
<td>131(\frac{1}{2})</td>
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<td>6</td>
<td>60</td>
<td>1.03</td>
<td>131(\frac{1}{2})</td>
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<td>60</td>
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</tr>
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<td>8</td>
<td>60</td>
<td>0.80</td>
<td>130</td>
</tr>
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<td>9</td>
<td>60</td>
<td>0.81</td>
<td>131</td>
</tr>
<tr>
<td>10</td>
<td>60</td>
<td>0.82</td>
<td>132</td>
</tr>
<tr>
<td>11</td>
<td>Orange Juice*</td>
<td>0.84</td>
<td>130(\frac{1}{2})</td>
</tr>
<tr>
<td>12</td>
<td>100</td>
<td>1.02</td>
<td>129(\frac{1}{2})</td>
</tr>
<tr>
<td>13</td>
<td>50</td>
<td>1.00</td>
<td>129</td>
</tr>
<tr>
<td>14</td>
<td>50</td>
<td>0.95</td>
<td>130(\frac{1}{2})</td>
</tr>
<tr>
<td>15</td>
<td>50</td>
<td>0.83</td>
<td>129</td>
</tr>
<tr>
<td>16</td>
<td>50</td>
<td>0.83</td>
<td>129</td>
</tr>
<tr>
<td>17</td>
<td>50</td>
<td>0.82</td>
<td>129(\frac{1}{2})</td>
</tr>
<tr>
<td>18</td>
<td>50</td>
<td>0.76</td>
<td>130</td>
</tr>
<tr>
<td>19</td>
<td>50</td>
<td>0.77</td>
<td>129(\frac{1}{2})</td>
</tr>
<tr>
<td>20</td>
<td>50</td>
<td>0.76</td>
<td>128</td>
</tr>
</tbody>
</table>

*1 Pint
### TABLE V

**DAILY BLOOD PLASMA ASCORBIC ACID LEVELS IN SUBJECT R S**

<table>
<thead>
<tr>
<th>Day</th>
<th>Ascorbic Acid Supplement</th>
<th>Plasma Ascorbic Acid</th>
<th>Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg.</td>
<td>mg. Per 100 ml.</td>
</tr>
<tr>
<td>1</td>
<td>Orange Juice1</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Orange Juice1</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>1.06</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>1.02</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>60</td>
<td>0.87</td>
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</tr>
<tr>
<td>9</td>
<td>60</td>
<td>0.79</td>
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<tr>
<td>10</td>
<td>60</td>
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</tr>
<tr>
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<td>Orange Juice1</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
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<td>100</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>170</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
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<td>80</td>
<td>0.87</td>
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</tr>
<tr>
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<td>80</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
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<td>80</td>
<td>0.85</td>
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<tr>
<td>17</td>
<td>80</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>80</td>
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<tr>
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<td>0.81</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>80</td>
<td>0.76</td>
<td></td>
</tr>
</tbody>
</table>

* 1 Pint
subject WL for the last four days of the experimental period. When the dosage was lowered to 50 mg, the plasma level reached 0.76 mg. per 100 ml. on the last day and averaged 0.78 for the last four days.

There seemed to be more variation in the plasma concentration of RS from day to day than in that of the other subjects (see Table V). During the first experimental period when the supplement was 60 mg, his plasma level dropped steadily from 1.06 to 0.66 mg. per 100 ml. and three days on increased ascorbic acid intake raised the level to 0.87. When the allowance was increased to 80 mg, the plasma concentration varied somewhat but did not drop below 0.72 mg. per 100 ml. and averaged 0.79 mg. during the last six days of the period.

With the exception of WL there was little variation in the body weight of the subjects during the course of the experiment (see Tables II-V). WL lost about 5 1/2 pounds, his weight decreasing gradually from 138 to 123.

It would therefore appear that, for the men, the minimum daily supplement which would maintain an average blood plasma concentration of about 0.8 mg. per 100 ml. would be 50 mg. in the case of WL and 80 mg. for RS. For the women, a supplement of 40 mg. maintained the desired plasma concentration. Whether a lower dosage would also have been sufficient is a question which could be answered only by further experimentation which circumstances did not permit
at the time. Adding to those amounts the ascorbic acid content of the basic diet, which was about 9.2 mg., the total amounts required would be 49.2 mg. for MLS and LS, 59.2 mg. for WL, and 89.2 mg. for RS, or, in terms of body weight, 1.1, 0.8, 1.0, and 1.2 mg. per kilogram, respectively.

From the above figures it would appear that ascorbic acid requirement is definitely related to body weight. There would also seem to be a relationship with nervous temperament and muscular activity; e.g., MLS who, though apparently not of a nervous temperament, moved about more rapidly than the others, required 0.3 mg. per kilogram more than LS who moved more slowly but apparently more efficiently; and RS whose requirement was the highest of the group, was of a definitely nervous temperament.

In her review of the literature on vitamin C requirement Smith (49) states that "a comparison of the absolute and relative values for different ages suggests that vitamin C requirements are related both to body weight and to metabolic rate, the effect of the latter being the more pronounced". With these subjects there obviously were other factors besides body weight which affected the requirement. Whether or not these other differences could have been explained by differences in metabolic rate is, of course, a question. Had it been possible to include measurements of basal metabolism in this study, more light
might have been thrown on the subject.

Judging from the results obtained with these four subjects, and assuming that a blood plasma concentration of 0.8 mg. per 100 ml. indicates adequate nutrition, the daily ascorbic acid requirement for adequate nutrition of the average normal adult would appear to be about 1.0 mg. per kilogram of body weight. Due allowance must of course be made for individual variations, but this would seem to be a fair statement of the average.

The results of this investigation would also indicate the advisability of expressing requirement in terms of body weight rather than total daily consumption.
SUMMARY AND CONCLUSIONS

The ascorbic acid requirement for adequate nutrition of four normal, healthy subjects was determined by placing them on a constant diet of known low ascorbic acid content supplemented with weighed amounts of the crystalline vitamin for a period of twenty days and measuring the blood plasma ascorbic acid concentration daily.

To maintain an average fasting plasma level of 0.8 mg. per 100 ml., which was considered to constitute an adequate state of nutrition, the women each required a total of 49.2 mg. and the men, 59.2 and 69.2 mg., respectively, of ascorbic acid daily. Expressed in terms of body weight the requirements of the women were 1.1 and 0.8 and of the men 1.0 and 1.2 mg. per kilogram.

On the basis of these findings, it would appear that the ascorbic acid requirement for adequate nutrition of the average normal adult would be about 1.0 mg. per kilogram of body weight.
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    question of vitamin C deficiency.) Klin. Wochschr.
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    bic acid as a test for latent vitaminosis C.
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    the renal threshold for ascorbic acid in man.
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    blood and urine after oral administration of a test
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    standard and requirement of physically healthy indi-
    viduals by testing the strength of their cutaneous
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    nation of the strength of the skin capillaries and
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