the vitamin is high. A very large oral dose can be similar in effect to an intravenous injection of vitamin C, both elevating the blood concentration so suddenly that an overflow into the urine occurs even when saturation has not actually been attained (van Eekelen and Heinemann, 1938). Intramuscular injections of vitamin C have been reported by Lilienfeld et al. (1936) to be as satisfactory as oral dosage and probably superior to the intravenous route in that absorption is slower to take place and the height of increase of blood plasma vitamin C is sustained longer.

van Eekelen and Heinemann (1938) state that many investigators prefer to use a 300-mg. dose of vitamin C or less at one time. Even unsaturated tissues have a limited absorptive capacity for the vitamin. Baumann is reported by Smith (1938) as considering the best method of determining vitamin C nutrition to consist of orally administering small doses of ascorbic acid over a period of several days. An excretion of 60 to 80 per cent of a test dose on the third or fourth day is indicative of saturation.

Differences in individual response to a test dose have been reported by Storvick and Hauck (1942) who noticed that the smallest amount of ascorbic acid necessary to maintain tissue saturation ranged in five subjects from 75 to 160 mg. when an excretion of 50 per cent of a test dose in 24 hours was used as the criterion of saturation. Todhunter and Robbins (1940) and Todhunter and Fatzer (1940) found a

variation in response to a test dose of ascorbic acid in the same individual even when intake remained constant.

The excretion of ascorbic acid in urine after a test dose depends on the degree of saturation of tissues and the immediate intake of the vitamin (Bessey, 1938a). It appears that the amount of vitamin required to induce saturation in a given subject is a measure of the vitamin C storage of that individual. van Eekelen and Heinemann (1938) state that after depletion of ascorbic acid many and repeated doses can be given before an increase in urinary excretion occurs and that the test dose required for tissue saturation is inversely proportional to tissue storage of vitamin C. Kellie and Zilva (1939) report that urinary excretion in their subjects was lower after a test dose of ascorbic acid when the subject was ascending from an unsaturated condition or from a state of saturation induced by small doses of ascorbic acid than when the test dose was ingested after the subject had reached saturation with a higher dose.

Saturation tests by Zilva, Abbasy, Harris and Ray (Kajdi et al., 1939) measure urinary clearance after a test dose of ascorbic acid. The time required for more than 50 per cent of the dose to be excreted is considered an index of body stores of ascorbic acid. Urinary tolerance tests are similar to saturation tests in that they consist in administering a massive dose of vitamin, in this case parenterally, and observing the excretion which occurs in the three to five hours that follow.

Goldsmith and Ellenger (1939) state that in normal subjects ascorbic acid excretion begins to increase within one hour after the administration of a test dose, reaches a peak between the first and sixth hours and gradually levels off to the original concentration within 24 hours. This phenomenon is not observed in the individual with deficient vitamin C stores. In the latter case only a slight rise or none at all occurs in urine after a test dose. These same authors found that excretion during a 24-hour period paralleled the excretion in a six-hour period for the same individual. They report a 61-per cent average excretion within six hours after a test dose for normal persons and a 44-per cent excretion for vitamin C deficient persons, a 100-per cent excretion of the test dose being considered by them as indicative of partial or actual saturation.

That ascorbic acid might have a diuretic effect was first suggested in English literature by Abbasy who observed that urinary volumes significantly increased after large doses of the vitamin (Shaffer, 1944). The diuretic action observed corresponded to the extent of tissue reserve of vitamin C in subjects studied. No diuretic action of the vitamin was produced by giving doses parenterally because excretion of it was too rapid. Shaffer reports a diuretic action of vitamin C in patients in cardiac decompensation. He gave vitamin C orally to ten patients who were receiving a 50 mg. daily intake of the vitamin and observed a small diuresis.

Intravenous injections of the vitamin did not cause a similar effect. When Shaffer administered vitamin C to his patients in combination with a recognized diuretic as mercupurin, a comparatively large diuresis occurred which was significantly greater than the diuresis possible with mercupurin alone. Shaffer cites studies with experimental animals which have indicated that the diuresis caused by ascorbic acid is due to an alteration in osmotic pressure. Evans (1938) compared the diuretic effect of vitamin C to that produced by established diuretics as digitalis, ammonium chloride and theobromine. He noted an increased urinary output of vitamin C in 9 patients. Greater diuresis was induced by the vitamin than by digitalis but less than by the other diuretics studied.

c. Significance of saturation. Vitamin C saturation probably represents the maximum amount of vitamin that can be absorbed and retained by the body, any excess being voided in urine. Since vitamin C is non-toxic even in large doses (Abt and Farmer, 1938) numerous investigators have recommended maintaining tissues in constant saturation with it. The desirability of such a high allowance of vitamin C has at present no adequate justification (Belser et al., 1939) and is economically not within the reach of many people.

Hathaway and Meyer, Ralli, Friedman and Sherry are reported by Lewis (1943) as finding higher utilization values in individuals whose tissues were in a state of saturation. Lewis found utilization varied for the four individuals that

he studied, one utilizing the same amount on a 74-mg. daily intake as on a 200-mg. intake. According to Lewis' criterion, three out of six subjects given 74 mg. ascorbic acid daily were able to maintain tissue saturation at that intake while there was evidence of some depletion of vitamin C reserves in the other three subjects. Levcowich and Batchelder (1942) observed that excretion tended to parallel a rise in intake, but that the increments were not constant enough to assume better utilization at higher levels. Their three subjects showed a sharp drop in urinary excretion values on the first day during a vitamin C deficient diet, but a comparatively constant rate of excretion was reached on the second day. It may be possible that there is a lower concentration of vitamin C in body tissues than is entirely compatible with good health (Howe, 1943).

3. Plasma values

Proposed values for normal ascorbic acid content of blood plasma vary so widely that it is difficult to determine an optimal range of concentration. Table 2 tabulates some of the reported estimates for plasma content of vitamin C in relation to intake.

It would appear from Table 2 that it should be possible to maintain plasma concentrations of ascorbic acid around 0.8 mg. per cent or higher at a vitamin intake of above 50 mg. This plasma figure has been more or less accepted as a value

Table 2

Plasma Concentrations of Ascorbic Acid as Reported in the Current Literature

Citation	Intake of Ascorbic Acid	Plasma Ascorbic Acid	Notes
	mg./24 hrs.	mg./24 hrs.	
Crandon and Luna (1940)	"C deficient"	0.19	
Goldsmith and Ellenger (1939)	C deficient	0.17	
Hinehart and Greenberg (1942)	self-chosen	0.30	
Goldsmith and Ellinger (1939)	self-chosen	0.65-2.0	
Holmes et al. (1941)	self-chosen	0.25-0.75	
Farmer et al. (1936)	"low" intake	0.75-0.80	
Abt and Farmer (1938)	"adequate"	0.70	
Kajdi et al. (1939)	"adequate"	0.70-0.90	
Horwitt (Kyhos et al., 1944)	25	0.40	
Fincke and Landquist (1942)	38-61	0.80	Women
Ralli et al. (1939)	50-75	0.50	
Kyhos et al. (1944)	50	0.80	
Goldsmith et al. (1941)	50		Maintained saturation as determined by urinary excretion
Todhunter and Robbins (1940)	60	1.0	
Roberts and Roberts (1942)	65-75	0.70	Saturation as determined by 50% excretion of a test dose
Ralli et al. (1939)	75	0.85	
Fincke and Landquist (1942)	69-89	0.80	Men
Kyhos et al. (1944)	75	0.80	
Belser et al. (1939)	70-100		Saturation as determined by 50% excretion of a test dose

(Continued)

Table 2 (Cont'd)

Plasma Concentrations of Ascorbic Acid as Reported in the Current Literature

Citation	Intake of Ascorbic Acid mg./24 hrs.	Plasma Ascorbic Acid mg./24 hrs.	Notes
Todhunter and Robbins (1940)	10-110		Saturation
Ralli et al. (1939)	100	1.0	Saturation
Fincke and Landquist (1942)	111-131	1.0	
Farmer et al. (1936)	"high"	1.3	
Ralli et al. (1939)	200	1.14	
Storvick and Hauck (1942)	200	1.07-1.58	
Faulkner and Taylor (1938)	300	1.40	Renal threshold
Mirsky et al. (Farmer et al. (1936)		1.19-2.66	Found no relation of plasma concentration to intake.

compatible with good functional health. Plasma values and intake of vitamin C seem to be correlated up to the point where the renal threshold of ascorbic acid is reached since fasting plasma concentrations rise with intake up to a level of about 1.4 mg. per cent but higher plasma concentrations are not maintained. A larger proportion of the studies would suggest that saturation can be maintained on 50- to 100-mg. intakes of vitamin C; that, conversely, intakes below 50 mg. yield plasma vitamin C concentrations which are lower than saturation values. However, at these lower intakes no signs of a C avitaminosis were observed so possibly a revision of the low limit of normal for plasma vitamin C is indicated.

In connection with low plasma concentrations of ascorbic acid and their significance in human nutrition, the controlled study conducted by Crandon and Lund (1940) is particularly interesting. During a period of four months on a vitamin C deficient diet their normal adult demonstrated a decrease to zero in the ascorbic acid content of blood plasma, white cells and platelets. No clinical symptoms of scurvy developed, increased fatigue due to muscular exertion was absent, and the oral condition remained good. A wound experimentally made when zero plasma readings had been observed for more than forty days showed good healing. Crandon and Lund suggest from their study that a plasma concentration below 0.5 mg. per cent is not necessarily a danger signal since they believe that the plasma ascorbic acid determination indicates the

extent of tissue saturation only.

4. Simultaneous plasma and urinary determinations for vitamin C

Simultaneous plasma and urinary determinations have been recommended for the most reliable information as to the state of vitamin C nutrition in an individual (Smith, 1938). However, tables 1 and 2 would seem to indicate that a straight line relationship exists between plasma vitamin C and urinary excretion of vitamin C and that an analysis of either might be a measure of vitamin C nutrition. In either case, the mean of a series of determinations is probably the most valid index of vitamin C stores since urinary and plasma concentrations of the vitamin vary markedly in the same individual from day to day (Storvick and Hauck, 1942).

F. Adult Requirement for Vitamin C

The quantity of ascorbic acid that protects against scurvy is not sufficient to prevent a partial C avitaminosis or subclinical scurvy. According to Bessey and King (1933) the time required for a depletion of tissue reserves of ascorbic acid is much shorter than the time required for the symptoms of scurvy to appear. They indicate that further investigation is needed in this zone between a low vitamin C intake and the appearance of scorbutic symptoms. Dalldorf

(1938) states that deviation from health may be slight in subclinical scurvy but it is definitely a pathologic state. He concludes from his investigation that even in the mildest degree of a vitamin C deficiency the anatomic effects peculiar to C avitaminosis are prompt to appear. Smith (1938) reports that it was early found that children receiving sufficient ascorbic acid to protect against scurvy might still develop a form of deficiency characterized by severe tooth injury. She states that much larger allowances of vitamin C are necessary for good nutrition than for the prevention of scurvy.

On the other hand, there is no direct evidence that maintenance of tissues in a state of saturation with vitamin C is advisable. According to Belser and associates (1939) it is self-evident that requirement cannot be greater than the amount necessary to maintain tissue saturation. They determined this amount to be 70 to 85 mg. for two subjects, 85 to 100 mg. for three subjects, and more than 100 mg. for two subjects. On a basis of body weight, therefore, Belser and associates would place the normal adult vitamin C requirement at 1 to 1.6 mg. per Kg. per day.

Other estimates for normal adult vitamin C requirement define a wide range of intake. Ralli and associates (1939) feel that an adult requirement should be the minimum amount that would maintain plasma at normal concentrations or, i.e., 0.8 mg. per cent or above, an amount which would result in a small and comparatively constant excretion of ascorbic acid.

These investigators report that they found that 100 mg. daily is required by normal adults for good nutrition. This intake was accompanied by maximum retention and a low, constant excretion of from 8 to 13 mg. daily. The daily amount of ascorbic acid necessary to maintain tissues of five subjects in adequate vitamin C nutrition was found by Fincke and Landquist (1942) to range from 38 to 89 mg. per day or 0.8 to 1.2 mg. per Kg. of body weight. The criterion used by these authors for a state of adequate vitamin C nutrition was a plasma concentration of 0.8 mg. per cent. Heinemann (1938) states that healthy individuals require at least 0.8 mg. per Kg. of body weight to maintain saturation and 0.4 mg. per Kg. of body weight to protect against scurvy.

The minimal daily intake of ascorbic acid required by healthy male adults for satisfactory plasma response, 0.8 mg. per cent, and health of gum tissue is said to be about 75 mg. (Kyhos et al., 1944). This requirement was estimated from plasma ascorbic acid determinations on 71 subjects over a period of 17 months. A few cases were found to require 100 mg. daily. Levcowich and Batchelder (1942) studied the urinary ascorbic acid of eight women on self-chosen diets, on a basal diet containing large quantities of vitamin C-high fruits and vegetables, on low-vitamin C diets, after the administration of massive doses of crystalline ascorbic acid, and at other various controlled intakes of vitamin C. On the basis of their observations they estimate that 50 mg. of vitamin C

daily plus 50 per cent for a safety factor or, i.e., a requirement of 75 mg. is satisfactory for a fairly active college woman. Rinehart and Greenberg (1942) state that it has not been determined whether plasma concentrations less than 1 mg. per cent or, i.e., saturation, are sufficient for optimum metabolism. They propose a 50- to 75-mg. daily intake as a range wherein tissue depletion is negligible.

These variations in calculated requirement for ascorbic acid are due to a lack of agreement on criteria, use of experimental diets that have not been carefully controlled, and the lack of long-time studies on plasma and urinary content of ascorbic acid where subjects are kept on a vitamin C-free diet and fed quantitative amounts of vitamin C (Ralli et al., 1939). Certainly more evidence is needed concerning the prolonged effects of ingesting quantities of the vitamin which will not maintain tissue saturation.

G. Effect of Exercise on Requirement

There is no experimental proof in the literature that would support an effect of exercise on vitamin C requirement. Several investigators have, however, suggested from chance observations that high energy expenditure increases body need for the vitamin and some have proposed a loss of vitamin C in sweat as a possible explanation.

Belser, Hauch and Storvick (1939) were unable to

duplicate previous results in saturation experiments during certain summer months when environmental temperatures were high. For example, the same subject excreted 182 mg. in response to a test dose when the mean temperature was 77° F and 231 mg. when the temperature was 55° F. Belser and associates believed therefore that urinary excretion of ascorbic acid is affected by high temperatures. Bernstein (1937) investigated the loss of vitamin C in laborers on Witwatersrand gold mines. Incidence of scurvy and subclinical deficiencies of vitamin C were high among these men even at an average intake of 20 to 30 mg. daily. Since a miner may lose two and a half to five pounds of weight, mostly sweat, during an eight-hour shift, Bernstein pointed out that loss of vitamin C in sweat may be an important factor in the vitamin nutrition of these miners particularly since their intake is not liberal.

Later work by Wright and MacLenathen (Belser et al., 1939) demonstrated that loss of ascorbic acid in sweat is minimal. This conclusion was confirmed by Tennent and Silber (1943) who found no ascorbic acid in sweat samples during a well-controlled experiment on healthy young males. Some loss in dehydroascorbic was found in all subjects but the average amount was not great, 0.23 mg. per hour. These authors deemed it unlikely that even excessive sweating could precipitate a vitamin C deficiency. They believed that the high values reported in previous experiments for vitamin C in

sweat were based on faulty analytical procedures.

Since sweat has been eliminated as a route of significant vitamin C loss, there remains to be found another explanation for reduced vitamin stores during exercise as noted in the investigations reviewed in the introduction to this paper. However, the data reported were not based on conclusive experimental evidence and so do not have much practical value. It is very possible that exercise by heightening metabolic activity and increasing exchange of nutrients in the body does create a greater need for vitamin C, but the hypothesis needs proof. It is hoped that this study will contribute information concerning vitamin C requirement during exercise.