The Assessment of Vitamin C Nutrition in Man

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As our knowledge of the part played by ascorbic acid in the maintenance of the intercellular architecture grows [Aschoff & Koch, 1919; Dalldorf, 1938] and in turn the importance of the latter in wound healing [Lund & Crandon, 1941; Bartlett, Jones & Ryan, 1942], the need for a reliable and simple assessment of the nutritional status with respect to this vitamin is obvious. A diminished supply of vitamin C-rich foodstuffs in the war-time diet of some adolescents and adults increases the urgency.

Two methods have been widely used: (1) the urinary saturation test, and (2) an examination of the concentration of ascorbic acid in whole blood, white cell-platelet layer or plasma.

Many of the conflicting reports in the literature concerning the urinary saturation test are undoubtedly attributable to the administration of widely different dosages of ascorbic acid ranging from 50 to 1000 mg./day, orally or intravenously in whole or divided doses and to differences in times of collection of urine. Data for normal daily urinary excretion of ascorbic acid vary from zero [Roe & Hall, 1939] to 40 mg. [Portnoy & Wilkinson, 1938].

Whole-blood determinations present some technical difficulties which are not easily offset. Haemolysis causes oxidation of ascorbic acid and inhibition of this by saturation with CO leads to indefiniteness in the end-point of the titration. Determination of the ascorbic acid content of the white cell-platelet layer involves a more complex technique and requires special apparatus which most laboratories would not possess.

It would be of great advantage to replace the somewhat laborious and unsatisfactory technique of the urinary saturation test, even in its most abbreviated form [Harris & Abbasy, 1937], by one determination. We have therefore endeavoured to establish whether a single determination of the plasma ascorbic acid concentration could be used to assay reliably the status of vitamin C nutrition of a subject. Opinion as to the value of isolated determinations of the plasma ascorbic acid content is equally divided; Portnoy & Wilkinson [1938], Ralli & Sherry [1941], and others hold the view that it is of value. The first named say that it may prove to be better than 'saturation' tests, whilst Kellie & Zilva [1939], Storvick & Hauck [1942], among

others, hold the opposite view. Our results indicate that the plasma ascorbic acid determination can be used to assay the standard of vitamin C nutrition, in contrast to the unreliability of observations of the so-called 'resting' urinary daily output.

The work here reported on is compiled from data obtained from 50 subjects and falls into two categories: (a) the correspondence between the results of urinary 'saturation' tests and those of the determination of plasma ascorbic acid content, and (b) the assessment of the nutritional status of the subject with respect to ascorbic acid.

METHODS

Plasma ascorbic acid. 10 ml. of blood were run into a tube containing 0.1 ml. 20% potassium oxalate and 0.05 ml. 5% KCN, and the whole was mixed and immediately centrifuged. To 4 ml. of the plasma, which should show no haemolysis, were added 16 ml. of freshly prepared 3% HPO_3 in glass-distilled water. After centrifugation, 5 ml. portions were titrated with 0.01% 2,6-dichlorophenolindophenol. No difficulty was experienced in determining a stable end-point if the titration was carried out rapidly, and the results were highly reproducible by both of us. Visual titration was found to be as accurate as photo-electric methods [cf. Mindlin & Butler, 1937-8].

PiJoan & Klemperer [1937] added KCN (5 mg.) to inhibit oxidation of ascorbic acid in freshly drawn venous blood. Barron, Barron & Klemperer [1936] considered that addition of KCN may enhance the ascorbic acid value. Golden & Garfinkel [1942], who added ascorbic acid to the plasma, confirmed Borsook, Davenport, Jeffreys & Warner [1936-7] and Kassan & Roe [1940] in finding that ascorbic acid was preserved best in whole blood, especially at low temperatures of the order of 5-8°, whereas once the plasma had been separated little loss occurred in the first 3 hr. at these temperatures, though there was a rapid loss at room temperature. Our experiments on blood containing a high, but physiological, concentration of ascorbic acid, indicate that KCN does not enhance the reduction titre under the conditions employed, but stabilizes the plasma value.

Urine ascorbic acid and dehydroascorbic acid. Urine was collected in dark-coloured Winchester containing 70 g. HPO_3 . 0.05 ml. 0.10% 2,6-dichlorophenolindophenol was titrated with the urine, the latter diluted with a suitable volume of 3% HPO_3 if necessary [cf. Harris & Ray, 1935].

For dehydroascorbic acid a convenient volume of urine was treated with H_2S for 30 min. and then with N_2 for 15 min. after all traces of H_2S had been removed as shown by lead acetate paper. The resulting solution was used to titrate the dye.

The dye solutions used for blood and urine determinations were found to be stable for several weeks in the refrigerator if made in freshly boiled and cooled glass-distilled water. They were standardized in the usual way against ascorbic acid which in turn was standardized with $N/10 I_2$ solution.

Dosage and blood sampling. Unless stated to the contrary, a standard dose of 700 mg. of ascorbic acid/140 lb. body weight was given orally each day. The dose, in 50 mg. tablets, was administered as nearly as possible in two equal portions, one at the commencement of each 24 hr. collection of urine, the other approximately 12 hr. later. Blood samples were taken, either at the end of the 24 hr. period before the first dose of the subsequent 24 hr., or, if no dose was given, 3 hr. after taking food.

Todhunter, Robbins & McIntosh [1942] showed that after ingestion of synthetic ascorbic acid a maximum rise occurs in the plasma within 1–1½ hr., which may be delayed until 2½ hr. if a natural source of the vitamin, such as cauliflower, is employed. Initially high plasma levels (0.9 mg./100 ml.) may be raised above the level for the renal threshold by a 400 mg. test dose. The effective rise in the plasma level is correspondingly less if a smaller dose is ingested, or the initial level is appreciably lower. These findings justify our division of the standard dose of 700 mg./140 lb. body weight into two approximately equal parts at 12-hourly intervals in order to reduce the sudden overflow through the kidney to a minimum, an event which is more likely to occur the more nearly 'saturated' the subject.

Belser, Hauck & Storvick [1939] and Fincke & Landquist [1942] employed a test dose of 400 mg. and Goldsmith & Ellinger [1939] 300 mg. In general, a 50% excretion of the test dose has been taken as evidence of 'saturation'. Harris took as his criterion the first sharp rise in the urinary excretion, but later modified this to the excretion of 50 mg. or more in the period of 3 hr. commencing 4 hr. after the administration of the test dose. Wright, Lillienfeld & MacLenathen [1937], to avoid disturbance due to faulty absorption, employed a dose of 1000 mg. intravenously, 'saturated' subjects excreting more than 50% in the subsequent 5 hr.

RESULTS

(a) *Urinary and plasma ascorbic acid contents during extended saturation tests.* Investigations were carried out on nine subjects to study the behaviour of the plasma ascorbic acid during extended saturation of the subject with ascorbic acid. All subjects took, in addition to the test dose, the normal hospital diet. Evidence is presented elsewhere [Prunty & Vass, 1943] that such a diet is poor in vitamin C. It was thought necessary to establish the fact that the plasma ascorbic acid does not reflect the violent fluctuations which are well known to occur in the urinary output under these conditions, and therefore accurately reflects the intake of ascorbic acid at these levels.

Data typical of our results are given in Table 1 and Fig. 1. The latter brings out the point that the plasma ascorbic acid does in fact remain constant during prolonged saturation, very marked fluctuations in the urinary excretion being shown. Table 1 also indicates the importance of another fact in the

interpretation of saturation. During the first period of saturation, 8–16 June 1942 (saturation being demonstrable on the latter date), the rise in the daily urinary excretion was relatively slow and no point of sharp demarcation was obvious. In this case the plasma ascorbic acid at the beginning of the test was 0.00 mg./100 ml., and such a low initial level tended to be associated with this phenomenon in other cases. A second saturation test carried out on the same subject with the plasma level initially at 0.26 mg./100 ml. shows on 15 August 1942 a spectacular rise in the excretion of ascorbic acid, an event easy of interpretation. Furthermore, on attaining saturation some of our subjects did not excrete as much as 50% of the test dose at any time. Therefore we have arbitrarily defined 'saturation' as the excretion of 33½% of the test dose based on 700 mg./140 lb. body weight, administered as outlined above.

Fig. 1. Saturation test in subject J. Female. Abdominal sinuses. ••••• Plasma ascorbic acid, mg./100 ml. — Urine ascorbic acid, mg./24 hr. ----- Urine ascorbic acid + dehydroascorbic acid, mg./24 hr.

The data given in Fig. 1 serve to confirm the fact that the fluctuations in the urinary excretion of ascorbic acid during extended saturation are not due to compensating excretions of dehydroascorbic acid.

(b) *The relation of the plasma ascorbic acid to the 'state of saturation' of the subject and resting urinary excretion of 'ascorbic acid'.* The data on this point are compiled in part from that given in the last section and in part from additional data of which Fig. 2 is illustrative. It shows the usual saturation test carried out in six normal subjects with initial plasma levels ranging from 0.31 to 1.46 mg./100 ml. The striking correlation between the time of demonstration of saturation and the initial plasma level is

Table 1. *Extended ascorbic acid saturation test*

Subject B. Male. Gastric ulcer, partial gastrectomy. Body wt. 124 lb.

Date	Ascorbic acid				Average urinary conc. over 24 hr. (mg./100 ml.)	Remarks
	Dose (mg./day)	Urinary excretion (mg./day)	Percentage of dose excreted	Plasma conc. (mg./100 ml.)		
5. vi. 42	—	—	—	0.00	—	Confined to bed Medical treatment for gastric ulcer
6. vi. 42	—	16	—	—	0.64	
8. vi. 42	600	12	2	—	0.45	
9. vi. 42	600	11	2	0.55	0.50	
10. vi. 42	600	18	3	—	0.73	
11. vi. 42	600	17	3	—	0.65	
12. vi. 42	600	85	14	—	3.68	
13. vi. 42	600	118	20	1.54	3.51	
14. vi. 42	600	—	—	—	—	
15. vi. 42	600	186	31	—	3.36	
16. vi. 42	600	248	41	—	11.42	Got up
17. vi. 42	600	268	45	—	10.05	
18. vi. 42	600	356	60	—	11.18	
19. vi. 42	600	269	45	1.99	11.00	
20. vi. 42	600	329	55	—	19.40	
21. vi. 42	600	—	—	—	—	
22. vi. 42	600	244	41	—	9.20	
23. vi. 42	600	348	58	—	17.60	
24. vi. 42	600	188	31	1.94	15.20	
25. vi. 42	600	267	45	—	11.70	
26. vi. 42	600	244	41	—	11.15	Operation
27. vi. 42	600	257	43	—	11.36	
28. vi. 42	300	—	—	—	—	
29. vi. 42	300	103	34	—	5.64	
30. vi. 42	300	186	62	1.36	10.00	
1. vii. 42	300	88	29	—	5.16	
2. vii. 42	300	129	43	—	6.90	
3. vii. 42	300	134	45	—	8.55	
4. vii. 42	300	48	16	—	2.35	
5. vii. 42	300	—	—	—	—	
6. vii. 42	300	106	35	1.29	5.76	Home
7. vii. 42	300	95	32	—	11.06	
8. vii. 42	50	—	—	—	—	
to	50	—	—	—	—	
17. vii. 42	50	—	—	1.26	—	
to	50	—	—	—	—	
23. vii. 42	50	18	36	—	0.93	
24. vii. 42	50	15	30	—	0.60	
25. vii. 42	50	12	24	0.90	0.62	
27. vii. 42	—	—	—	—	—	
30. vii. 42	50	20	40	—	0.86	Home
to	50	—	—	—	—	
10. viii. 42	50	10	20	—	0.66	
11. viii. 42	50	10	20	0.26	0.90	
12. viii. 42	50	18	3	—	0.88	
13. viii. 42	600	33	6	—	2.10	
14. viii. 42	600	70	12	—	5.73	
15. viii. 42	600	421	70	—	23.10	
16. viii. 42	600	460	77	—	32.60	
17. viii. 42	600	—	—	—	—	
to	100	—	—	—	—	
6. x. 42	100	—	—	0.62	—	

self-evident. Subject L., with a plasma ascorbic acid of 0.31 mg./100 ml., required 2 days to become saturated, whilst subject V. showed this in one day, the plasma level being initially 0.80 mg./100 ml.

Initial plasma ascorbic acid levels and the corresponding urinary ascorbic acid excretions before saturation with large doses of ascorbic acid, the dosage required to bring to a 'state of saturation',

and the corresponding days required to demonstrate saturation are given in Table 2.

Fig. 3 shows the dose required to bring the subject to a 'state of saturation', and the initial plasma level. This dose was computed by determining the doses necessary to produce an excretion of one-third of the test dose (700 mg./140 lb. body weight) in 24 hr. If on a given day the excretion

was less than one-third and on the succeeding day the excretion was greater than one-third, the figure was obtained by interpolation. From the total dose

ordinate therefore represents the plasma concentration at the 'saturated state' corresponding to 0.8 mg./100 ml. Thus if the plasma ascorbic acid concentration of the subject, whose saturation is being investigated, is 0.8 mg./100 ml., administration of the test dose results in the excretion of one-third of the test dose. The plasma level reaches or even exceeds the renal threshold, placed by Ralli, Friedman & Sherry [1939] at 1.2-1.4 mg./100 ml.

Fig. 2. Plasma level of six normal subjects before and after urinary saturation test. •-----• Plasma ascorbic acid, mg./100 ml. — Urinary ascorbic acid, mg./24 hr.

are deducted 700 mg., which represent the single test dose per 140 lb. body weight necessary to demonstrate 'saturation' in a subject who has reached a 'state of saturation'. The point at which the curve crosses the

Table 2. Initial plasma concentration and daily urinary excretion of ascorbic acid compared with total dosage mg./140 lb. body weight, and number of days required to bring subjects to a state of saturation

Subject	Ascorbic acid			Days to saturate
	Initial plasma conc. (mg./100ml.)	Initial urinary excretion (mg./day)	Dosage (mg.) required to bring to saturation level/140 lb. body wt.	
S.	0.0	50	4680	8
B.	0.0	24	4200	8
*D.	0.0	18	3620	6
Da.	0.05	24	2600	5
W.	0.08	31	1580	4
Ch.	0.18	13	1700	6
Sa.	0.18	18	1500	4
B.	0.28	13	1580	4
J.	0.26	17	1230	3
L.	0.31	26	580	2
C.	0.33	29	440	2
M.	0.36	24	520	2
Co.	0.56	7	870	3
F.	0.57	27	300	2
A.	0.59	—	230	2
P.	0.76	—	40	1
V.	0.80	27	40	1
Pr.	1.46	85	-430	1

* A case of clinical scurvy.

Fig. 3. Initial plasma ascorbic acid concentration against dose to bring to saturation level, with large doses. (700 mg./140 lb. body wt.)

The curve indicates that the relationship between the two variables is not linear, a point that may partially account for the divergence of opinion in the literature as to the validity of 'resting' plasma ascorbic acid concentration as an index of vitamin C nutrition. It must be noted that the only marked deviation from the curve is that of case Co., a child aged 7 years, whereas the other points given are all for adults. The point B. was obtained in the second saturation test (Table 1). The point D. relates to a case of scurvy [Prunty & Vass, 1943].

In spite of the presence of unknown interfering substances, several authorities regard the titre of the 'resting urine' as significant an index as the 'saturation' test itself [Harris, Abbasy, Yudkin & Kelly, 1936; Portnoy & Wilkinson, 1938]. The former stated that 'as under ordinary circumstances the response to a test dose is proportional to the resting level, a measurement of the latter alone is sufficient for most routine tests of the kind here described'. If in an adult 13.8 mg. ascorbic acid are excreted each day, a good response to the test dose of 700 mg./140 lb. body weight is obtained on the first or second day. Table 2 shows that the 'resting' urinary excretion affords little evidence of the status of the vitamin C nutrition of any subject. D., who was clinically frankly scorbutic, excreted as much as the equivalent of 18 mg./day of ascorbic acid, whilst S. and B., who showed no such signs,

both requiring at least 6 days to become saturated, excreted much more than this in the 'resting urine' (see Table 2).

(c) *Comparison of the ascorbic acid intake with the maintenance of a known plasma ascorbic acid concentration.* During the course of this work it was possible to maintain only relatively few subjects on controlled intakes of ascorbic acid for prolonged periods during which observations of the plasma ascorbic acid concentration were made. A summary of the results obtained is presented in Table 3. In many cases the greater part of the ascorbic acid ingested was the synthetic product; the remainder of the intake was estimated from a dietetic history [cf. Harris & Olliver, 1943]. The figures fall into two groups, those obtained when the plasma level prior to the period of known intake was lower than the final level and vice versa.

shows that these fluctuations are not due to any interchange between these substances, which is in agreement with the findings of Johnson & Zilva [1934]. Examination of the two saturation tests in subject B. (Table 1) shows the differing urinary excretion responses which are met with in markedly unsaturated subjects (saturation not reached until the sixth day or later) compared with less unsaturated subjects (saturation reached by the fourth day) and illustrates the difficulty, in the earliest urinary saturation test, of noting the first sharp urinary response as an index of the achievement of saturation.

The data given in Table 2 and Fig. 3 establish the fact that the initial plasma ascorbic acid concentration is related to the dose of ascorbic acid required to bring the body to a 'state of saturation', which is attained when the plasma ascorbic acid

Table 3. *The relation between ascorbic acid ingested and the plasma ascorbic acid concentration*

Ascorbic acid							
Subject	Initial plasma conc. (mg./100ml.)	Daily dose (mg.)	Estimated dietary intake (mg./day)	Total daily intake (mg.)	Final plasma conc. (mg./100ml.)	Duration of exp. (days)	Remarks
A. Plasma concentration previously lower							
P.	0.15	300	50	350	1.52	88	
Ja.	—	150	70	220	1.26	1000	
De.	0.67	200	20	220	1.02	6	Received 75 mg./day for 14 days, 3 weeks prior to this
De.	—	100	20	120	0.67	7	
C.	—	50	50	100	0.33	7	
J.	—	—	70	70	0.26	28	Two oranges daily
B. Plasma concentration previously higher							
B.	—	100	50	150	0.62	52	Saturated 52 days before
B.	0.90	50	20	70	0.26	38	Dose omitted for 3 days after partial gastrectomy, 3 days after plasma value was 0.90 mg./100 ml.
B.	1.29	50	20	70	1.26	9	
B.	1.26	50	20	70	0.90	21	
Ch.	2.17	50	20	70	1.31	8	Saturated 8 days before
Da.	1.24	50	20	70	1.06	6	Saturated 6 days before
Pr.	1.52	50	100	150	1.46	17	
Pr.	—	—	75	75	0.86	26	Saturated 26 days before
Pr.	0.86	—	50	50	0.58	23	
Pr.	0.58	—	50	50	0.41	23	

DISCUSSION

Our results show that the marked urinary fluctuations met with in the 'saturation' test are not mirrored in the plasma level. This supports the idea that the 'saturated state' is maintained and emphasizes the reliability of the plasma ascorbic acid content as an index of this state. Indeed, Harris [1942], in answer to his critics, considers that these fluctuations do not in any way invalidate the saturation test. An examination of the urinary excretion of dehydroascorbic acid and ascorbic acid

reaches 0.8 mg./100 ml. Harris [1942] stated that the necessity of maintaining a state of saturation for optimum nutrition is not established. Ralli & Sherry [1941, p. 290] maintained that in a 'normal' adult the optimal requirement of vitamin C was 100 mg. daily, 'this being the smallest daily dose on which saturation of the body tissues was attained and maintained'. These authors have endeavoured to assess the standard of nutrition in regard to vitamin C by examining the urinary excretion after administration of 100 mg. of the vitamin intravenously. Although they state that in normal (i.e. presumably saturated) subjects 50 % of the injected

vitamin is excreted within 3 hr., 8 out of 12 of their normal subjects failed to satisfy this criterion.

It is widely accepted that the dosage of ascorbic acid required to saturate the subject is a measure of the depletion of the available vitamin C reserves of the body, even though these may be small. It therefore follows from Fig. 3 that the lower the initial plasma ascorbic acid concentration the greater the degree of depletion of the available reserves of the body.

A summary of some of the 'normal' values for the ascorbic acid content of plasma which are to be found in the literature is tabulated below; this range is so great that it is of little clinical value.

Normal plasma values of ascorbic acid, mg./100 ml.

Secher [1942]	Down to 0
Wright, Lilienfeld & MacLenathen [1937]	0.27-1.54
Croft & Snorf [1939]	0.4
Ingalls [1937]	0.5-2.0
Butler & Cushman [1940]	0.6
Portnoy & Wilkinson [1938]	0.6-1.85
Abt & Farmer [1938]	0.7-2.0
Fincke & Landquist [1942]	0.8
Abt, Farmer & Epstein [1936]	0.8 upwards

It is obvious from Fig. 3 that a plasma ascorbic acid value greater than 0.8 mg./100 ml. denotes an intake of ascorbic acid more than sufficient to satisfy the body's requirements and that at values at or near zero the ascorbic acid intake is grossly inadequate. This is borne out by the fact that scurvy does occur when the dosage requirement to bring to saturation is less than that of non-scorbutic subjects (see D., Fig. 3).

Examination of this curve reveals that the maximum change in the dosage of ascorbic acid required to produce a 'state of saturation' at a given plasma level occurs at a value slightly less than 0.4 mg./100 ml. plasma. In view of these facts it is clear that a plasma value of not less than 0.4 mg./100 ml. is compatible with a desirable standard of nutrition with respect to the vitamin. When the initial plasma ascorbic acid concentration is 0.4 mg./100 ml., 450 mg. of ascorbic acid per 140 lb. of body weight will bring the subject to a 'state of saturation' which can be demonstrated by the ingestion of a further 700 mg. of ascorbic acid. This is equivalent to a good saturation response in 2 days with the urinary saturation test of Harris [1943], who states that 'the number of days beyond the second may be taken as an index of the relative deficit in the past intake'.

The data presented in Table 3, although deficient in evidence relating to plasma ascorbic acid levels below 0.26 mg./100 ml., explain certain discrepancies met in the literature. Whereas Bryan, Turner, Huenemann & Lotwin [1941] and Ralli & Sherry [1941] found that a daily intake of 100 mg. of ascorbic acid stabilized the plasma level at approximately 1 mg./100 ml., our results suggest that a

daily intake of at least 100 mg. is required to attain a 'state of saturation' with a plasma ascorbic acid content of 0.8 mg./100 ml. Our results also show that if the subject has previously been on a higher intake of ascorbic acid and reverts to a lower one, the plasma ascorbic acid tends to remain at a higher level than that reached when the procedure is reversed, unless a considerable period on the lower intake is maintained (probably at least 30 days). For example, after 21 days on an intake of 70 mg. ascorbic acid daily (subject B), where the initial plasma level was 1.26 mg./100 ml., the plasma level only fell to 0.90 mg./100 ml. This intake, continued for a total of 38 days, gave a final plasma level of 0.26 mg./100 ml. Subject J., on the other hand, who had been receiving the equivalent of 70 mg. daily, had a plasma content of 0.26 mg./100 ml. after 28 days, the dietetic history indicating that the initial plasma level was below that at the end of the test period.

Göthlin [1934], on the basis of his capillary fragility test, suggested at least 19-27 mg. of ascorbic acid daily were necessary to prevent scurvy in the adult. Fox, Dangerfield, Gottlich, Jokl & Suzman [1940] showed that a daily intake of 15-25 mg. of ascorbic acid did not prevent scurvy in a group of Witwatersrand miners. By contrast, Kellie & Zilva [1939] estimated that daily doses of 15 mg. ascorbic acid would suffice to prevent scurvy and maintain good health. They endeavoured to ascertain the minimum daily dose required to maintain 'saturation' and considered this to be achieved when, on a given dose, the ascorbic acid voided in the urine became constant from day to day. Having regard to the overlapping of an increased or decreased daily excretion as the dosage was stepped up or down, they studied the level of excretion reached on ascending from a state of 'unsaturation' and vice versa, with daily doses of 30, 50 and 100 mg. As a result they stated that 'it may be assumed that the minimum dose necessary to bring about "saturation" lies between 30 and 50 mg./day, probably nearer the former figure'. Their interpretation of the results obtained on A.E.K. do not agree with our results on subject B., Table 1, who on 23-25 July 1942 inclusive was excreting diminishing amounts of ascorbic acid in the urine in spite of the fact that the plasma level at the time was 0.9 mg./100 ml. and that B. had been receiving at least 50 mg. ascorbic acid per day for 16 days following a period of 'saturation'. Moreover, the high plasma levels observed in A.E.K. on a continually falling dose agree with our findings in Table 3. Ralli & Sherry [1941] have determined the plasma ascorbic acid values when the subject was on a known intake of vitamin C. With a daily intake of 50 mg., the plasma concentration varied from 0.28 to 0.30 mg./100 ml. when the initial and final plasma concentrations were not appreciably different. When the initial values were 0.97 and 1.45 mg./100 ml. the final values were 0.80 and 0.60 mg./100 ml. (cf. Pr., Table 3, initial value 0.86 mg./100 ml., daily dose 50 mg., value in 23 days 0.58 mg./100 ml., after 46 days 0.41 mg./100 ml.).

On 75 mg. daily intake, when the initial values were 0.3, the final values were 0.5 and 0.7 mg./100 ml.; when the initial value was 1.10 mg./100 ml., the final value was 0.8 mg./100 ml. On 100 mg. daily intake the initial values given are 0.2, 0.5, 0.7, 0.8, 1.25 and the respective final values, 1.07, 1.2, 1.2, 1.12, 1.05 mg./100 ml. These authors computed the daily requirement for an optimal nutrition at 100 mg. of ascorbic acid per day in the adult, by determining the amount retained on a known daily intake, a hazardous procedure in our opinion, in view of the marked

urinary fluctuations they also observed and by reason of the fact that it is not yet possible to determine quantitatively all the metabolic products of ascorbic acid, other than dehydroascorbic acid, which may be eliminated in the urine. Their results also show, as we have observed, that during prolonged saturation the urinary ascorbic acid content tends to fall to a lower value than that initially obtained, which is a further possible source of error in the computation of the ascorbic acid retention.

The most widely accepted figures of the daily requirements for vitamin C in this country and in America are respectively those of The Technical Commission of The League of Nations, Geneva [Bigwood, 1939] (30 mg./day) and The Committee on Food and Nutrition, National Research Council (U.S.A.) [1941] (75 mg./day). Whilst our evidence presented in Table 3 is insufficient a basis on which to dogmatize, it does suggest that at least 70 mg. of ascorbic acid daily are required for a plasma level of the order of 0.4 mg./100 ml. This conclusion, with that of Ralli & Sherry [1941] quoted above, together with the confirmatory observations of Bryan *et al.* [1941] that an intake of approximately 70 mg./day maintained a plasma level of 0.6 mg./100 ml., suggests that at least 60 mg./day are required to maintain a plasma level of as much

as 0.4 mg./100 ml. This is in agreement with the view of Bacharach & Drummond [1940] and the Committee on Food and Nutrition (U.S.A.) quoted above, that the requirement of ascorbic acid is 75 mg./day, and that a well-balanced diet should contain this amount.

SUMMARY

1. The plasma ascorbic acid concentration is in man an index of the nutritional state with respect to vitamin C, unless, within the previous 30 days, the subject has been on an appreciably higher intake of ascorbic acid than he is receiving at the time of the test. For clinical purposes this determination is more easily carried out than a urinary saturation test.

2. A plasma ascorbic acid level of 0.8 mg./100 ml. represents a 'state of saturation'.

3. Evidence is presented for the desirability of a plasma ascorbic acid concentration of not less than 0.4 mg./100 ml.

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