THE VITAMIN C REQUIREMENT OF MAN.[1] ESTIMATED AFTER PROLONGED STUDIES OF THE PLASMA CONCENTRATION AND DAILY EXCRETION OF VITAMIN C IN 3 ADULTS ON CONTROLLED DIETS

By ELAINE P. RALLI, GERALD J. FRIEDMAN, AND SOL SHERRY

(From the Department of Medicine, New York University College of Medicine and the Third (New York University) Medical Division, Bellevue Hospital, New York City)

(Received for publication August 1, 1939)

There has been a wide variation in the daily estimated vitamin C requirement of man (1). This appears to be due to several factors. The first of these is the lack of uniformity in the criteria used for estimating the amount of vitamin C required, some observers using changes in capillary fragility, others using the urinary excretion following test doses of vitamin C, and still others using the amount of vitamin C excreted in 24-hour periods. Secondly, in a great many of the reports the diets are not carefully controlled. Thirdly, no prolonged studies have been done in which the plasma concentration and the urinary excretion of vitamin C have been correlated when the patients were kept on vitamin C-free diets and fed quantitative amounts of vitamin C.

The question of the requirement of vitamin C can be considered from two aspects: (*1*) the amount necessary to maintain the normal plasma concentration, and (*2*) the amount necessary to raise the plasma concentration from a subnormal to a normal level. The concensus of opinion at the present time is that the normal plasma concentration of vitamin C should not be below 0.8 mgm. per cent (*2*). In most groups of normals reported, including a group of students observed in this laboratory, the average plasma vitamin C was about 1.2 mgm. per cent.

In a previous study (3) we found that the amount of vitamin C excreted in the urine depended on the plasma concentration of the vitamin, on the rate of glomerular filtration, and on the maximal rate of tubular reabsorption. These two latter factors were found to be fairly constant in normal adults and a sharp increased excretion of the vitamin did not occur until the plasma level had exceeded about 1.4 mgm. per cent. On the basis of these observations it seemed to us that the amount of vitamin C required daily by an adult would be the smallest amount necessary to maintain the normal plasma level. It follows that at this level the amount excreted should be small and should remain fairly constant so that the maximal reabsorptive capacity of the kidney tubules would not be exceeded. Furthermore, if more than the daily requirement of vitamin C were given, the excretion of vitamin C should rise promptly.

Obviously, in order to determine the amount of vitamin C required daily by adults, the dietary intake should be controlled, the vitamin should be fed quantitatively, the 24-hour urinary excretion of the vitamin should be determined daily, and this should be correlated with the plasma concentration at frequent intervals.

METHODS

Three normal adults were hospitalized and fed diets containing not more than 5 mgm. of vitamin C (Table I). The vitamin C content of these diets was determined by direct analysis except for the meat, the figure for which was obtained from the publication of the Department of Agriculture No. 275 (4). The 24-hour urinary excretion of vitamin C was determined daily in each case during the entire period of observation. The urines were collected in dark bottles to which was added enough glacial acetic acid so that, by the end of the 24 hours, the concentration of acid was approximately 10 per cent by volume. As the urinary volume was fairly constant in each individual from day to day, the amount of acid necessary was readily calculated. The bottles were kept in the icebox and the urines were added when voided. The plasma concentration was determined three times weekly throughout the study.

In the early part of the study vitamin C was determined in both blood and urine by titration with dichlorphenolindophenol. Later the determinations of vitamin C were done in blood and urine in the photoelectric colorimeter, using the methods described by Mindlin and Butler (5) for blood plasma and by Evelyn (6) for urine. In 2 patients on the vitamin C-free diet the urinary vitamin C was determined both by titration and

[1] This research was aided by a grant from the Josiah Macy, Jr. Foundation.

TABLE I

Diet

	Carbohydrate	Protein	Fat	Vitamin C
	grams	*grams*	*grams*	*milligrams*
BREAKFAST				
30 grams Cereal				0
2 Eggs				0
60 grams Bread				0
240 grams Milk				0.24
10 grams Butter				0
20 grams Sugar				0
Coffee				0
	88	20	30	
LUNCH				
150 grams Meat (medium fat)				4
150 grams Rice *or* spaghetti (plain)				0
480 grams Milk				0.48
120 grams Bread				0
10 grams Butter				0
1 Custard				0
Tea, with 10 grams sugar				0
	143	53	68	
SUPPER				
60 grams Cheese *or* 3 eggs				0
150 grams Rice *or* spaghetti (plain)				0
240 grams Milk				0.24
120 grams Bread				0
10 grams Butter				0
1 Jello				0
Tea, with 10 grams sugar				0
	121	27	40	
Total	352	100	138	5

by the photoelectric colorimeter. By the latter method the excretion of the reducing substance averaged 10 mgm. less than by the titration method. The difference is accounted for by the fact that there is a small amount of non-vitamin C reducing substance in the urine that reduces the dye within the titration period of 1–2 minutes. With the photoelectric colorimeter this error is reduced appreciably as shown by Evelyn. For this reason, in the study in which the urinary vitamin C was determined by titration alone (Case 1), 10 mgm. was subtracted from the daily amount excreted to correct for the amount of non-vitamin C reducing substance. This same error does not occur in blood, since there does not appear to be an appreciable amount of non-vitamin C reducing substance in the plasma filtrate. The determinations were done on fasting specimens of blood.

Vitamin C was fed in the form of cevitamic acid (Merck). Never more than 50 mgm. was given in one dose, the total amount being divided throughout the day.

RESULTS

The first case, L. R., age 57, height 72¼ inches, weight 160 pounds, surface area 1.93 square meters (Figure 1), was observed for a total of 110 days. The diet, as has already been shown, contained not more than 5 mgm. of available vitamin C daily, and in discussing the intake we are discounting this small amount. When the observations were begun on this patient his blood plasma vitamin C was at the low normal level (0.85 mgm. per cent). During the first period of 19 days, when on a daily intake of 50 mgm. of vitamin C, the plasma concentration varied from 0.76 mgm. per cent to 0.97 mgm. per cent, the average being 0.85 mgm. per cent. The daily average excretion was 11 mgm. with a Standard Deviation of $\pm$ 4.6; the daily retention averaged 39 mgm. When the vitamin C intake was raised to 100 mgm. daily (Period 2, 53 days), the average daily excretion rose to 20 mgm. with a Standard Deviation of $\pm$ 5.3; the average daily retention of vitamin C was 80 mgm. The plasma vitamin C rose so that the average plasma level was 1.12 mgm. per cent, the variations being from 0.93 mgm. per cent to 1.22 mgm. per cent. In the third period of 22 days the daily intake was increased to 200 mgm. As a result of this, the daily excretion rose to 109 mgm. with a Standard Deviation of $\pm$ 20. The retention, therefore, was not increased to any extent, averaging 91 mgm. The average blood plasma concentration during this period amounted to 1.14 mgm. per cent. The fact that doubling the intake of vitamin C did not increase the retention of the vitamin or effect a significant increase in the plasma level indicates that the ingestion of the vitamin in amounts exceeding 100 mgm. daily is not necessary in normal people. Further substantiation that larger doses of vitamin C were not effective is provided in the next period of 16 days when the intake was increased to 350 mgm. daily. The daily excretion rose sharply, averaging 259 mgm., so that the average retention remained at approximately 91 mgm. The blood level toward the end of this period reached a peak of 1.41 mgm. per cent, the average during the period being 1.15

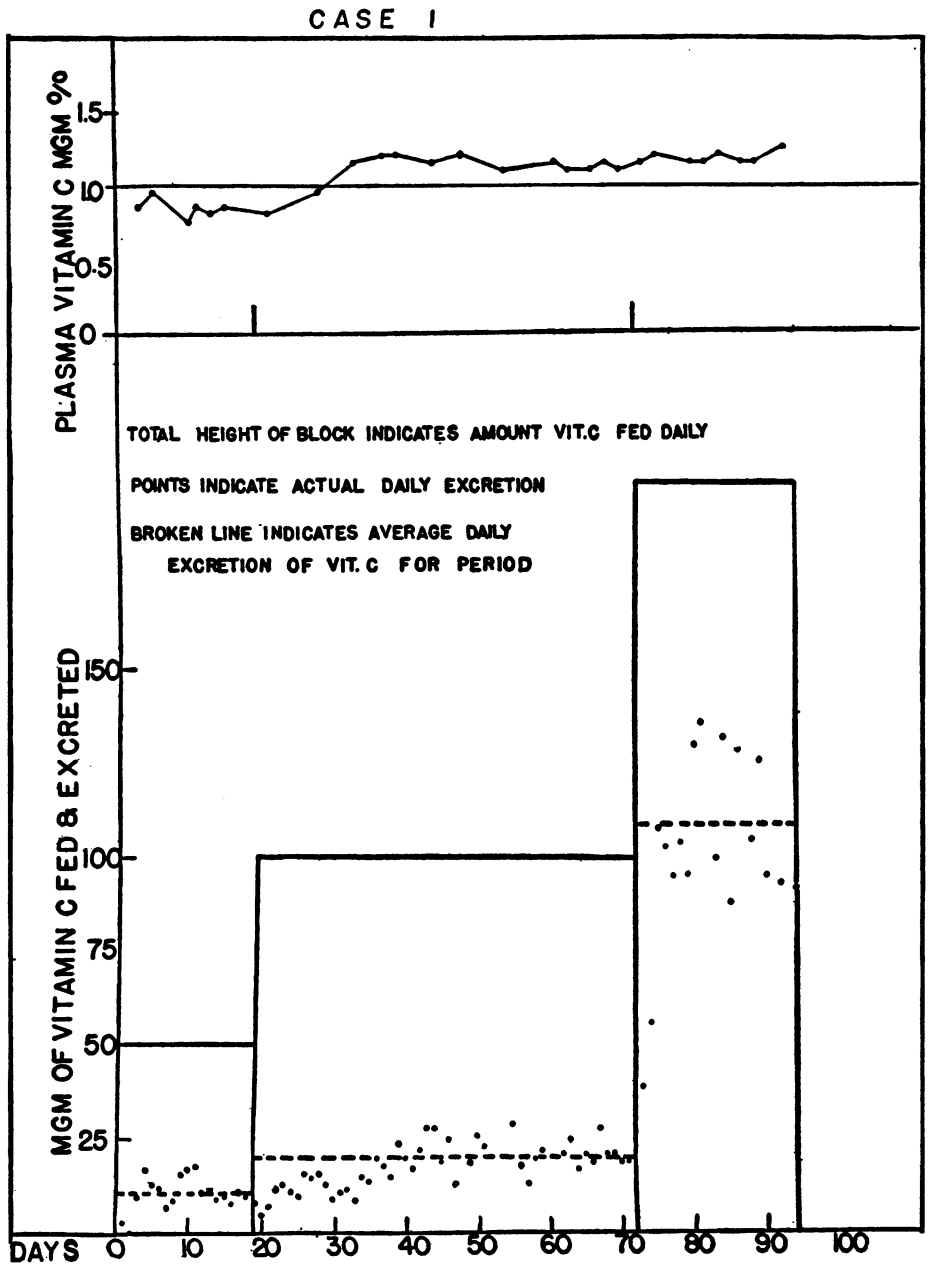

FIG. 1. CASE 1. MGM. OF VITAMIN C FED AND EXCRETED DAILY AND PLASMA LEVELS OF VITAMIN C DURING EACH PERIOD

mgm. per cent. Apparently the daily peak of retention had been reached when 100 mgm. was administered and no further increase in the amount retained occurred in spite of the increased intake. In using the word *retention* we are referring to the difference between the amount fed and the amount excreted in the urine. The recent work of Chinn and Farmer (12) has shown that very little is excreted in the feces. Therefore, the amount that is not excreted in the urine probably represents the amount retained and utilized by the body.

The second patient was a male 42 years of age, height 66½ inches, weight 160 pounds, surface area 1.8 square meters (Figure 2). The vitamin C studies were begun when the blood plasma con-

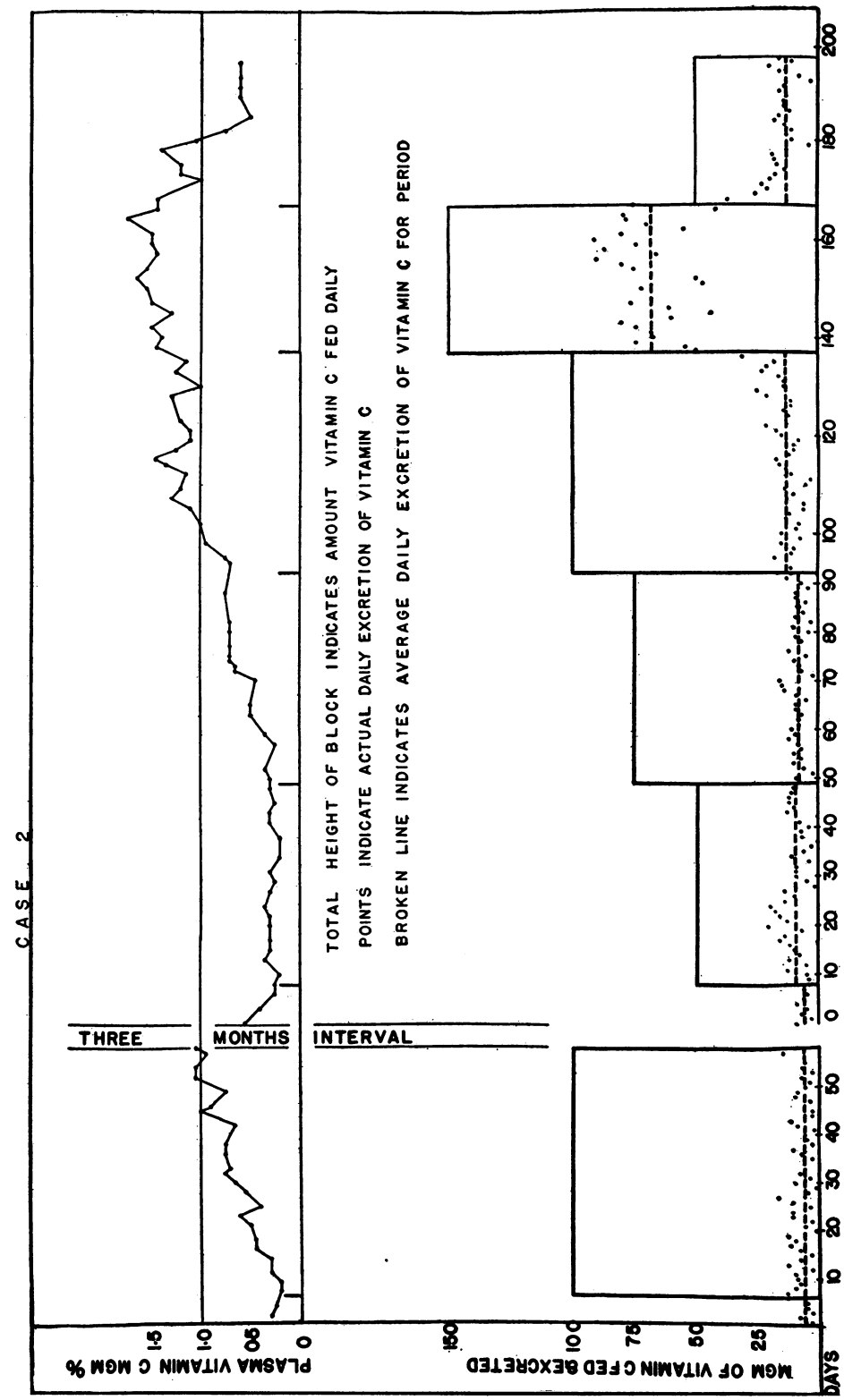

FIG. 2. CASE 2. MGM. OF VITAMIN C FED AND EXCRETED DAILY AND PLASMA LEVELS OF VITAMIN C DURING EACH PERIOD

centration was 0.30 mgm. per cent. For the first 6 days the patient was maintained on diet alone. The daily excretion was 6.4 with a Standard Deviation of ± 3.9. The patient was then fed 100 mgm. of cevitamic acid daily for a period of 52 days. The average daily urinary excretion for this period was 6.0 ± 4.4 mgm. The retention averaged 94 mgm. daily. After 25 days the plasma concentration was 0.77 mgm. per cent. Nineteen days later the plasma concentration was 1.07 mgm. per cent, and it remained at approximately this level. The studies were then discontinued for a period of 2 months. In the second part of the study, during the first period of 8 days on diet alone, the patient excreted an average of 6 mgm. with a Standard Deviation of ± 2.0 daily and the plasma concentration of vitamin C fell from 0.56 to 0.27 mgm. per cent. For the next 41 days he received 50 mgm. daily. The average excretion during this period was 9.6 ± 5.2 mgm. and the average retention was 40 mgm. The average plasma concentration of vitamin C was 0.28 mgm. per cent, and the blood plasma varied from 0.24 mgm. per cent to 0.34 mgm. per cent. In the third period, consisting of 43 days, the daily intake of vitamin C was increased to 75 mgm. of cevitamic acid. The daily excretion averaged 7.9 ± 3.6 mgm. so that the daily retention was 67 mgm. The blood plasma concentration of vitamin C rose from 0.30 mgm. per cent to 0.70 mgm. per cent and was maintained at this latter figure for a period of 20 days. The vitamin C intake was then increased to 100 mgm. daily for a period of 45 days. The average daily excretion was 13 ± 3.7 mgm. and the daily retention averaged 87 mgm. The plasma concentration of the vitamin rose from 0.70 mgm. per cent to 1.20 mgm. per cent within 16 days and was maintained at this level for 24 days.

During the next period of 31 days the patient received 150 mgm. of cevitamic acid daily. The average daily excretion was 68 mgm. ± 14 mgm. and the daily retention averaged 82 mgm. The blood plasma concentration of vitamin C promptly rose from 1.20 mgm. per cent to 1.50 mgm. per cent and was maintained at the higher level for the rest of the period.

For the sixth and last period of 30 days the vitamin C intake was reduced to 50 mgm. daily. The purpose of decreasing the intake for a period of time was to find out whether once the plasma concentration had been elevated a smaller daily dose would maintain the higher plasma level. As can be seen in the chart this did not occur. On the 15th day the plasma concentration fell to 0.76 mgm. per cent and leveled off at 0.6 mgm. per cent. The urinary excretion fell more promptly than the blood and by the 7th day was 13 mgm., which was the average daily excretion for the period, with a Standard Deviation of ± 4.3 mgm.

A third patient, A. T., 35 years of age, height 65 inches, weight 135 pounds, surface area 1.65 square meters (Figure 3), was kept on the diet alone for a period of 12 days. His blood plasma concentration of vitamin C at the start of the study was 1.7 mgm. per cent due to the fact that he had received massive doses of cevitamic acid for several days prior to the study. On diet alone the blood plasma concentration of the vitamin fell rapidly to 0.30 mgm. per cent. The urinary excretion fell during this time and by the 11th day he was excreting none.

In the second period of 18 days he was given 50 mgm. of cevitamic acid daily. The plasma concentration of vitamin C was maintained throughout this period at a level of about 0.30 mgm. per cent. The average daily excretion of vitamin C was 5.9 ± 2.3 mgm. and the average daily retention was 44 mgm.

The daily intake of vitamin C was then raised to 75 mgm. for a period of 25 days. The blood plasma concentration of vitamin C rose slightly to 0.50 mgm. per cent. The average daily excretion was 6 ± 2.2 mgm. and the average daily retention was 69 mgm.

In the fourth period of 42 days the patient was given 100 mgm. of cevitamic acid daily. The daily urinary excretion averaged 12 ± 3.3 mgm. and the average daily retention was 88 mgm. The blood plasma concentration began to rise rapidly from 0.50 mgm. per cent and after 32 days had reached a concentration of 1.0 mgm. per cent. For the last 10 days of the period the average plasma level was 1.2 mgm. per cent. During this fourth period the plasma concentration fell on the 25th and 27th days and we found that through an error only 50 mgm. of cevitamic acid was given for 2 days prior to this fall.

In the fifth period of 18 days the vitamin C in-

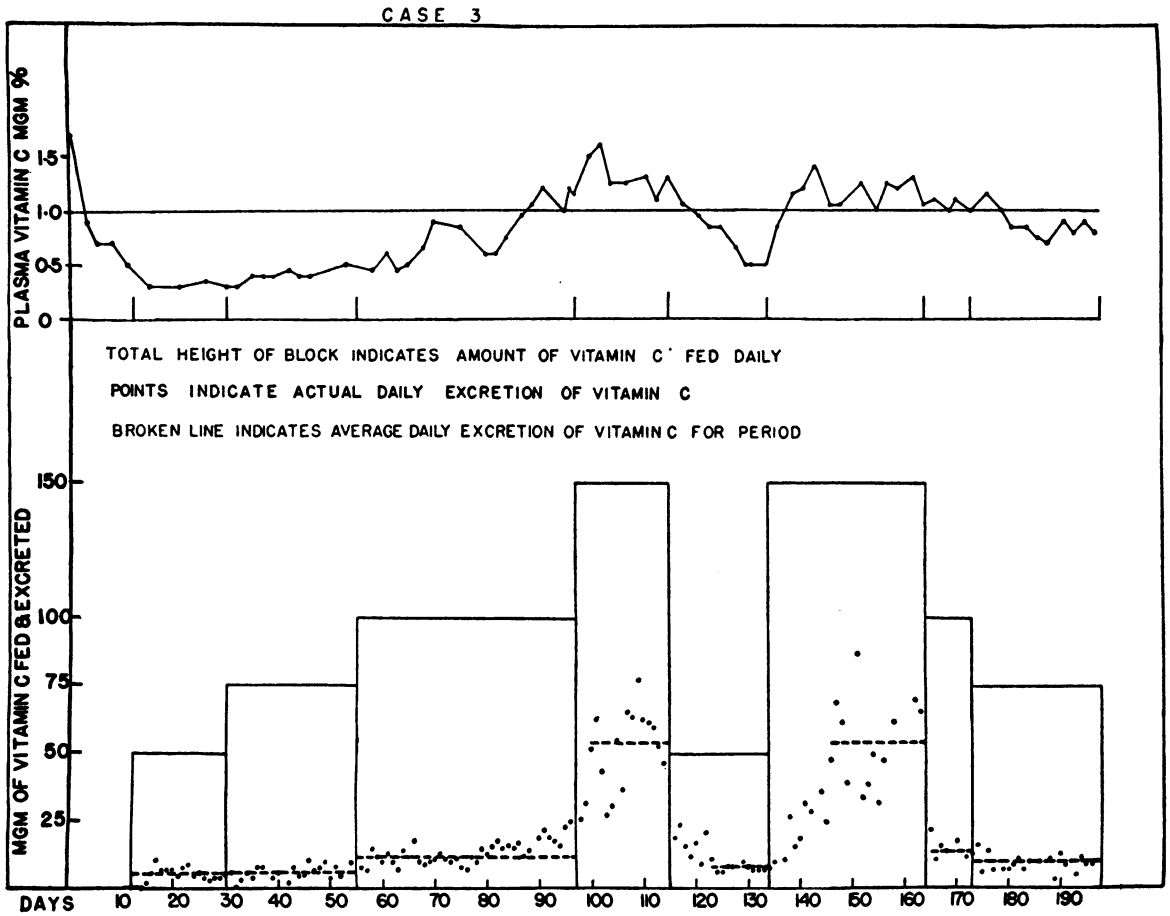

FIG. 3. CASE 3. MGM. OF VITAMIN C FED AND EXCRETED DAILY AND PLASMA LEVELS OF VITAMIN C DURING EACH PERIOD

take was increased to 150 mgm. daily. The plasma concentration rose to a peak of 1.58 mgm. per cent on the 6th day, but the average of the period was 1.25 mgm. per cent. The daily urinary excretion rose after the first day. The average daily excretion of vitamin C for the period was 54 mgm. ± 14. The retention averaged 96 mgm. daily.

We then reduced the intake of the vitamin to 50 mgm. daily and the same fall in the plasma concentration occurred as had occurred in Case 2. By the 6th day the plasma level was 0.96 mgm. per cent, and on the 15th day it had reached a level of 0.5 mgm. per cent, which was maintained for the rest of the period. The urinary excretion fell gradually during the first 9 days and then leveled off, so that for the last 10 days of the period the average excretion was 7 mgm. ± 1.3. The daily retention was 43 mgm.

The intake of vitamin C was then raised again to 150 mgm. daily for 30 days. The plasma concentration rose rapidly, reaching 1.0 mgm. per cent by the 5th day and leveling off for the rest of the period at about 1.25 mgm. per cent. The urinary excretion also rose promptly and for the last 19 days reached a level of 54 mgm. ± 15. The daily retention was 96 mgm.

For the next 9 days the patient's intake was reduced to 100 mgm. daily. The plasma concentration was maintained above 1.0 mgm. per cent for the whole period, *but the urinary excretion immediately fell to a level of 14 mgm. ± 2.9.* The retention averaged 86 mgm. daily.

In the last period of 24 days the patient was placed on a daily intake of 75 mgm. It was previously shown in this case and Case 2 that 50 mgm. of vitamin C daily would not maintain a plasma level of 1.0 mgm. per cent and we were

anxious to determine if possibly 75 mgm. daily would be sufficient to maintain this plasma level. This dose proved not to be sufficient and on the 8th day the plasma concentration had fallen to 0.86 mgm. per cent and for the remaining 16 days the plasma level remained at about 0.8 mgm. per cent, which is the lowest limit of normal. The average daily urinary excretion was 10 mgm. ± 3.2 and the retention averaged 69 mgm.

The results on the 3 cases by periods are summarized in Table II.

TABLE II

Summary of the excretion, retention and plasma levels of vitamin C in the 3 cases by periods

Case	Period	Number of days	Daily intake vitamin C	Average daily excretion vitamin C	Average daily retention vitamin C	Plasma range vitamin C
			milligrams	*milligrams*	*milligrams*	*milligrams per cent*
L.R.	I	19	50	11±4.6	39	0.97→0.80
	II	53	100	20±5.3	80	0.80→1.12
	III	22	200	109±20	91	←1.14→
	IV	16	350	259±48	91	1.25→1.41
W.J.	I	6	0	6.4±3.9	0	0.30→0.20
	II	52	100	6.0±4.4	94	0.20→1.07
			INTERVAL OF 2 MONTHS			
	I	8	0	6.0±2.0	0	0.56→0.27
	II	41	50	9.6±5.2	40	←0.28→
	III	43	75	7.9±3.6	67	0.30→0.70
	IV	45	100	13±3.7	87	0.70→1.20
	V	31	150	68±14	82	1.20→1.50
	VI	30	50	13±4.3	37	1.45→0.60
A.T.	I	12	0			1.70→0.35
	II	18	50	5.9±2.3	44	←0.30→
	III	25	75	6.2±2.2	69	0.30→0.50
	IV	42	100	12±3.3	88	0.50→1.20
	V	18	150	54±14	96	1.15→1.25
	VI	19	50	7.0±1.3	43	1.25→0.50
	VII	30	150	54±15	96	0.50→1.25
	VIII	9	100	14±2.9	86	←1.05→
	IX	24	75	10±3.2	65	1.10→0.80

DISCUSSION

The results in these 3 cases suggest that the optimum daily intake of vitamin C in adults should be 100 mgm. Ingestion of this amount was accompanied by the maximum retention of the vitamin, approximately 90 mgm. daily, and by a constant low excretion averaging 8 to 13 mgm. daily. Feeding more than this daily was apparently of no advantage in these normal adults since, when the amount of vitamin C ingested daily exceeded 100 mgm., there was a prompt rise in the amount excreted, and the excretion continued to parallel any increase in the amount ingested. When the feeding of 100 mgm. daily was begun at a plasma level below 1.0 mgm. per cent there was a slow gradual rise until this level had been reached. The number of daily doses of 100 mgm. required to raise the plasma level depended on the plasma concentration when the feeding was begun. The daily and not the total amount of the vitamin fed was the deciding factor in raising and maintaining a higher plasma level. For example, in Case 2, after 100 mgm. had been fed daily for a period of 17 days, giving a total intake of 1,700 mgm., the plasma level rose from 0.20 to 0.61 mgm per cent. However, when 50 mgm. was fed daily for 41 days, giving a total intake of 2,050 mgm., the plasma concentration remained unchanged at 0.30 mgm. per cent. Apparently the tissues required fully 50 mgm. daily for their own needs, and even on an intake of 75 mgm. the tissue stores were still not saturated as the plasma level could neither be raised to nor maintained at 1.0 mgm. per cent. This suggests that, on an intake of less than 100 mgm. daily, the tissue stores are not completely saturated, so that enough vitamin C is not available for the blood concentration to be maintained at 1.0 mgm. per cent.

In normal individuals these results suggest that a fasting plasma level of 1.0 mgm. per cent or over indicates complete saturation of the tissues by vitamin C.

These observations have led us to question whether the present lower limit of the normal plasma vitamin C level has not been set too high. The average of all the plasma determinations on the 3 cases when receiving 50 mgm. of vitamin C daily was 0.4 mgm. per cent. This covered a total period of 127 days. During this period of time there was no clinical evidence of vitamin C deficiency. Many observers have previously considered 50 mgm. of vitamin C daily an adequate intake, and yet at this intake in these 3 cases it was impossible to maintain the accepted normal plasma concentration of the vitamin. It would be equally impossible to produce scurvy on the amount and, furthermore, Göthlin (8) has shown that less than this amount (22 mgm. daily) will maintain a normal capillary fragility. It seemed

reasonable, as a result of these observations, to reconsider our ideas of the normal plasma limit and to consider a plasma concentration of 0.4 mgm. per cent as the low limit of normal. We do not wish to create the impression that this is a desirable plasma level of vitamin C for an individual. In fact these studies have shown that at this plasma level the tissues are not saturated and, when increasing amounts of vitamin C were fed, the plasma concentration continued to rise without any parallel increase in the excretion of the vitamin until the daily intake exceeded 100 mgm. Even when the plasma vitamin C level was 0.8 mgm. per cent the tissues were still apparently not saturated, as the excretion of vitamin C did not increase until a fasting plasma level of 1.2 mgm. per cent had been reached. On the basis of these facts we would not recommend that a normal individual should only ingest enough vitamin C to maintain such a low plasma concentration. On the contrary we feel that *100 mgm. of vitamin C daily* is the desirable intake and, if one expects to maintain a plasma concentration of 1.0 mgm. per cent, this is the amount required.

In previous studies from many laboratories (9)

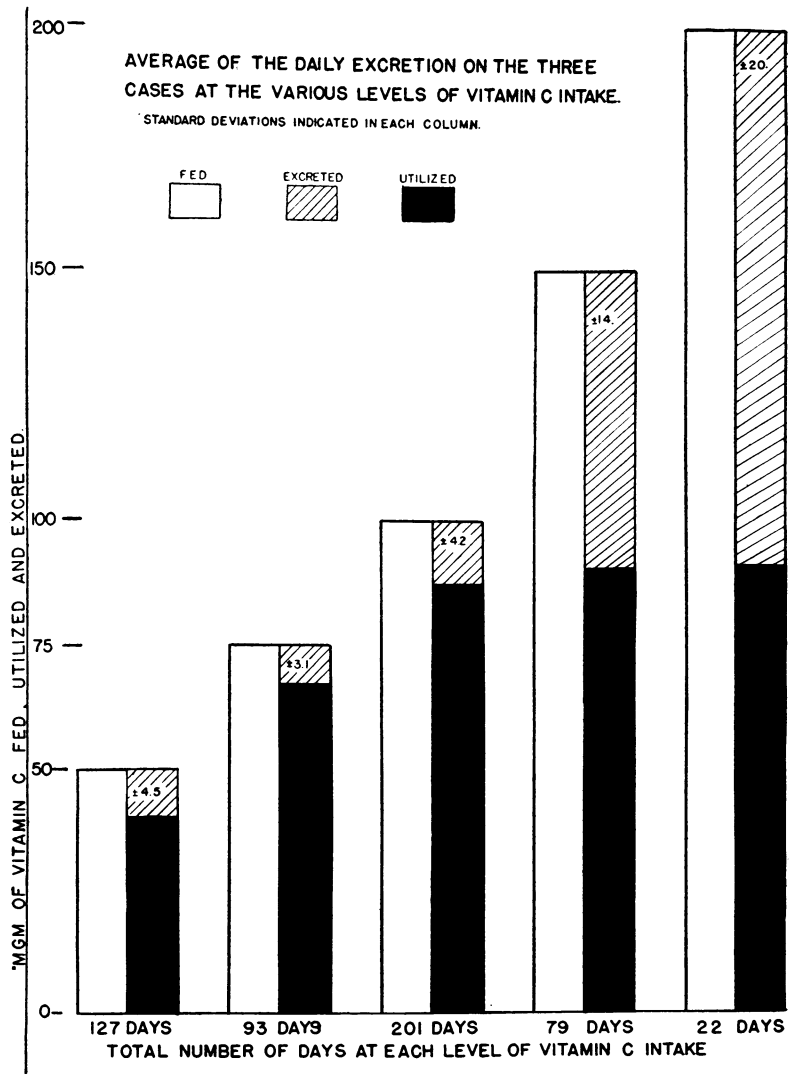

FIG. 4. AVERAGE OF THE DAILY EXCRETION ON THE THREE CASES AT THE VARIOUS LEVELS OF VITAMIN C INTAKE

the vitamin C status of the individual has been estimated on the basis of the urinary excretion of the vitamin. Obviously, the excretion of the vitamin depends on the amount ingested. However, until the ingestion exceeded 100 mgm. of vitamin C daily, the excretion remained fairly constant and rarely exceeded 15 mgm. of vitamin C in 24 hours. Therefore the 24-hour urinary excretion of vitamin C without any knowledge of the plasma level does not give complete information of the vitamin C status of the individual. An excretion of 15 mgm. daily can occur at intakes varying from 50 to 100 mgm. of vitamin C and at fasting plasma levels ranging from 0.3 to 1.0 mgm. per cent. This is shown in the summary of all the periods in all 3 cases (Figure 4), where the average daily excretion of vitamin C at intakes of 50, 75, and 100 mgm. daily varied from 8 to 13 mgm. It is only when the intake exceeded 100 mgm. daily that there was any significant increase in the 24-hour urinary excretion of the vitamin. Below an intake of 100 mgm. of vitamin C daily, the plasma level was a more accurate index of the vitamin C intake. When the intake exceeded 100 mgm. daily, the urinary excretion reflected more accurately the increases in the ingestion of the vitamin.

Apparently, in the full-grown adult, the daily vitamin C requirement is not dependent on age, height and weight. In these 3 cases the ages varied from 35 to 57 years; the heights from 65 inches to 72¼ inches, and the weights from 135 to 160 pounds. Case 3 gained 20 pounds during the 200 days that he was under observation and this had no effect on the vitamin C requirement. For this reason we have not estimated the daily requirement on a per kilogram basis as was suggested by Heinemann (10).

SUMMARY

The daily excretion of vitamin C and the plasma concentration of the vitamin were determined on 3 male adults kept on control diets and fed varying amounts of vitamin C in the form of ascorbic acid.

When the amounts of vitamin C fed daily did not exceed 100 mgm. the 24-hour excretion of vitamin C did not exceed an average of 13 mgm. When more than 100 mgm. was fed daily, there was a sharp rise in the urinary excretion of the vitamin and the rise continued to parallel any increase in the ingestion of the vitamin.

When less than 100 mgm. was fed daily, it was impossible either to raise or maintain a plasma vitamin C level of 1.0 mgm. per cent.

When fed 50 mgm. of vitamin C daily, the plasma concentration of vitamin C averaged 0.4 mgm. per cent. As the patients were normal, and as there were no symptoms of vitamin C deficiency, it is suggested that this plasma level be considered the lower limit of normal. At this plasma level, however, the body tissues are not saturated with vitamin C and saturation apparently does not occur until the plasma concentration is at a level of 1.0 mgm. per cent.

As a plasma concentration of 1.0 mgm. per cent can only be obtained and maintained on a daily intake of at least 100 mgm. of vitamin C, it is suggested that this be considered the optimum daily intake.

BIBLIOGRAPHY

1. Smith, S. L., Human requirement of vitamin C. J. A. M. A., 1938, **111**, 1753.
2. Abt, A. F., Farmer, C. J., and Epstein, I. M., Normal cevitamic (ascorbic) acid determinations in blood plasma and their relationship to capillary resistance. J. Pediat., 1936, **8**, 1.
3. Ralli, E. P., Friedman, G. J., and Rubin, S. H., Mechanism of excretion of vitamin C by human kidney. J. Clin. Invest., 1938, **17**, 765.
4. U. S. Department of Agriculture, Vitamin content of foods, Miscellaneous Publication, No. 275, June 1937.
5. Mindlin, R. L., and Butler, A. M., Determination of ascorbic acid in plasma; macromethod and micromethod. J. Biol. Chem., 1938, **122**, 673.
6. Evelyn K. A., Malloy, H. T., and Rosen, C., Determination of ascorbic acid in urine with photoelectric colorimeter. J. Biol. Chem., 1938, **126**, 645.
7. (a) Van Wersch, H. J., Determinations of the daily requirements for ascorbic acid of man. Acta brev. Neerland, 1936, **6**, 86.
   (b) Heinemann, M., Human Requirements for vitamin C. Biochem. J., 1936, **30**, 2299.
8. (a) Göthlin, G. F., Method of establishing vitamin C standard and requirement of physically healthy individuals by testing strength of their capillaries. Skandinav. arch. f. Physiol., 1931, **61**, 225.
   (b) Göthlin, G. F., Human daily requirements of dietary ascorbic acid. Nature, 1934, **134**, 569.

(c) Göthlin, G. F., Frisell, E., and Rundquist, N., Experimental determinations of indispensable requirements of vitamin C (ascorbic acid) of physically healthy adult. Acta med. Scandinav., 1937, **92**, 1.

9. Abbasy, M. A., Harris, L. J., Ray, S. N., and Marrack, T. R., Diagnosis of vitamin C subnutrition by urine analysis: Quantitative data. Lancet, 1935, **2**, 1399.
10. Heinemann, M., Requirements for vitamin C in man. J. Clin. Invest., 1938, **17**, 671.
11. Everson, G. J., and Daniels, A. L., Vitamin C studies with children of preschool age. J. Nutrition, 1936, **12**, 15.
12. Chinn, H., and Farmer, C. J., Determination of ascorbic acid in feces. Its excretion in health and disease. Proc. Soc. Exper. Biol. and Med., 1939, **41**, 561.