

CCLXXIII. THE EXCRETION OF VITAMIN C IN HUMAN URINE AND ITS DEPENDENCE ON THE DIETARY INTAKE.

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PREVIOUS papers from this laboratory have dealt with the application of the titration method with 2:6-dichlorophenolindophenol to the estimation of vitamin C in various animal materials [Harris and Ray, 1933, 1, 2; Birch *et al.*, 1933; Birch and Dann, 1933; Harris, 1933]. Results on human urines are discussed in the present communication. A principal purpose we had in mind in undertaking this enquiry was to discover whether any relation could be found between urinary loss and the state of nutrition of the individual in respect to vitamin C sufficiency or subnormality. Any such index would obviously be of great value for practical human dietetics.

Previous biological work has failed to demonstrate the presence of vitamin C in urine [van der Walle, 1922], but a note just published by Eekelen *et al.* [1933] mentions, without giving actual data, that the reducing substance in urine is higher in persons using much fruit.

METHOD.

The micro-method of Birch *et al.* [1933] was used throughout. The urine to be examined was made acid with trichloroacetic acid (final concentration of latter, 5 %) and titrated from a micro-burette reading to 0.01 cc. against 0.05 cc. of the indicator, which had been previously standardised against ascorbic acid. Titrations were carried out immediately after, or within a few minutes of, urination, as we found that the titre tended to fall in urine which had been allowed to stand for long. It is essential to carry out the titration rapidly and reach the end-point within about 1 min., otherwise erroneously high values will be caused by phenolic or similar substances in the urine reducing the indicator slowly. Urines are sometimes encountered which are too dilute to give a satisfactory end-point. If a reading is required for such an individual it is necessary to restrict his fluid intake.

The results to be described make it clear that the titration figure bears a genuine relation to vitamin C metabolism, but in expressing our results for convenience in terms of so many mg. of ascorbic acid we wish to make it clear that it is without prejudice to the question of the invariable specificity or otherwise of the reaction.

EXPERIMENTAL.

Excretion after large dose. Our most detailed observations were undertaken with the object of obtaining a quantitative picture of the course of excretion of the vitamin after the administration of a single large dose. These results will be

described first. In the experiment represented in Fig. 1, A. W. drank 600 cc. of orange juice at 9 a.m., restricted himself for several days thereafter to a vitamin

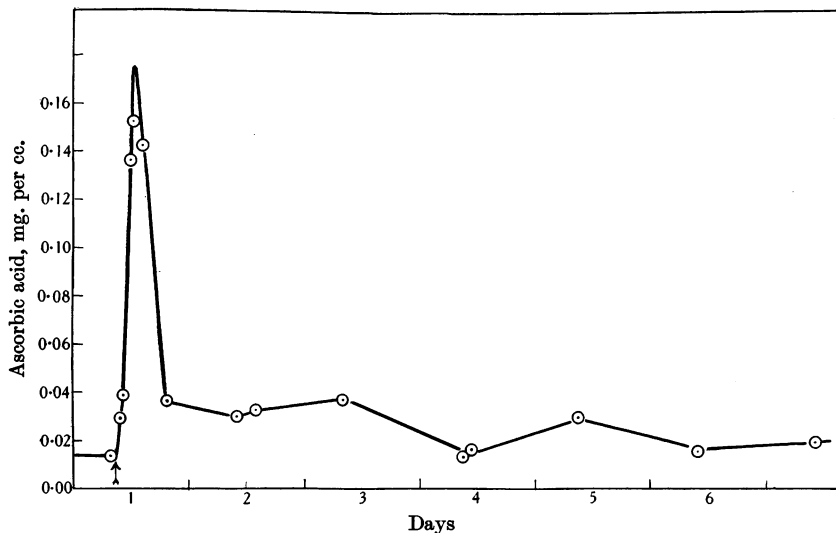


Fig. 1. Sudden rise in concentration of vitamin C in urine following large dose of orange juice (given at arrow).

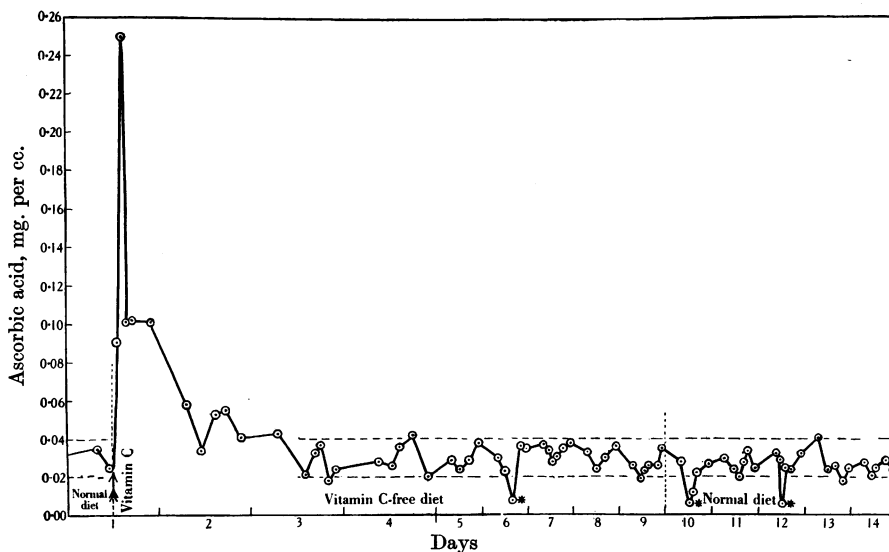


Fig. 2. Complete 14-day (half scale) record of vitamin C concentration in urine. Sudden rise in concentration after large dose of orange juice (given at arrow), followed by fall to within normal limits within 48 hours. * = diuresis after beer drinking.

C-free diet, and estimated the vitamin C content of his urine at frequent intervals. The results show that the vitamin C excretion had already begun to rise markedly after 1 hour, had reached a maximum in about 3 hours, and fallen to the original level again by the end of the third day. At the third hour, at the peak of the

curve, the concentration of vitamin C in the urine was no less than seven times greater than the normal figure. In nine hours, 76 mg. out of the original 300 mg. taken (the latter value being based on the titration figure) had been voided, and in the course of 3 days about 250 mg.

A second experiment was conducted along similar lines (Figs. 2 and 3), and the same typical sudden rise followed by rapid fall to a level value was again seen. In this experiment 556 mg. of ascorbic acid were taken in one dose

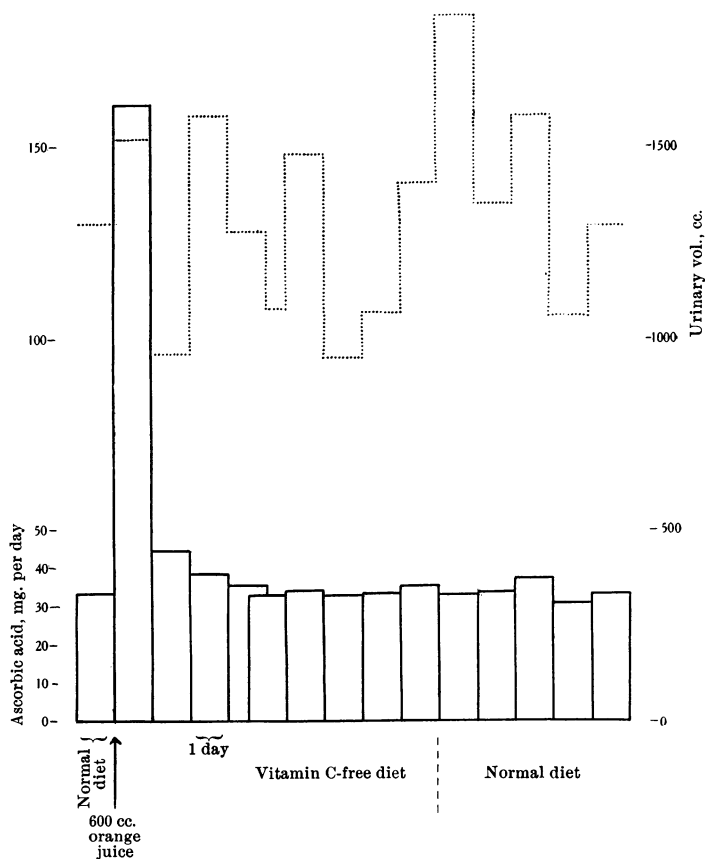


Fig. 3. Total daily outputs of vitamin C in urine, showing increase after large dose, and, later, steady output of about 33 mg. per day.

(600 cc. orange juice) and observations were continued for over a fortnight afterwards. It will be noted that the maximum rate of excretion was reached in 3 hours, that after 24 hours the excretion was only little above normal, and that it had quite dropped back to normal after 50 hours. (At the peak the urine contained as much as eight or ten times the normal concentration of vitamin C and the total weight of ascorbic acid excreted in the first 10 hours was 132.4 mg. compared with a normal of about 20 mg. for a corresponding day-time period.) For a further $6\frac{1}{2}$ days the vitamin C-free regimen (eggs, cheese, butter, bread, various cereals) was continued, and it was found that the amount of vitamin C excreted continued to remain extremely constant, between limits of

32.5 and 35.3 mg. per day (average 33.6), and with an average concentration of about 0.03 mg. per cc., a level we find to be characteristic of most of the normal specimens we have examined.

No departure from this steady state occurred when the subject recommenced a normal diet, 10th to 15th day of experiment.

Normal urines. Tests on urines from normal individuals on ordinary mixed diets are recorded in Table I. All are males. The first four are laboratory

Table I. *Urinary excretion of vitamin C by men on normal diets.*

Subject	Time	Total volume of urine passed cc.	Weight of vitamin C in total specimen mg.	Concentration of vitamin C in urine mg. per cc.
G. G. G.	10.30 a.m.	165	8.35	0.050
	2.45 p.m.	130	6.96	0.053
	9 a.m.	610	12.85	0.021
				Av. 0.032
S. G. I.	12 noon	140	2.71	0.019
	1 p.m.	75	1.45	0.019
	4.30 p.m.	255	7.26	0.028
	9 a.m.	360	10.11	0.028
	1 p.m.	235	4.77	0.020
				Av. 0.024
A. W. D.	10.30 a.m.	230	10.44	0.045
	1 p.m.	115	5.03	0.044
	4.30 p.m.	160	2.55	0.016
	5.30 p.m.	110	4.64	0.042
	9 a.m.	725	15.47	0.021
				Av. 0.028
S. A. C.*	10.30 a.m.	115	—	} <0.014
	1 p.m.	210		
	2.45 p.m.	305		
	4 p.m.	260		
	5 p.m.	260		
	9 a.m.	740		
1 p.m.	500			

* Diuretic specimen.

assistants. It will be seen that the figure is generally very close to 0.03 mg. per cc. of urine, and the daily output does not vary far from about 30 mg. (see Table II). An example is included of one subject (S. A. C.) who had diuretic tendencies, and the concentration was insufficient to permit a clear end-point being reached in the titration.

Table II. *Average concentration and daily output of vitamin C in urine in normal males.*

Subject	mg. of vitamin C per cc. of urine	Approximate total output (calculated) (mg.)
A. W. D.	0.028	38.1 in 24 hours
G. G. G.	0.032	28.2 "
S. A. C.*	<0.01	—
S. G. I.	0.024	ca. 10.0 in 10-12 hours
S. N. R.	0.027†	—
T. W. B.	0.022†	—
W. J. D.	0.017†	—
L. J. H.	0.016†	—

* Diuretic specimen.

† Based on single titrations only.

Results quoted in the last section show also that the urine which has collected in the bladder during the night and is excreted first thing in the morning often tends to become rather more concentrated as regards the vitamin.

Since the daily output of vitamin C in the urine seems to be at so constant a level in many individuals it is of interest to compare it with the reputed minimum daily need. This is stated to be "1 oz. of orange or lemon juice per day" (*i.e.* about 20 mg. of ascorbic acid). It would appear therefore that the average individual excretes daily in his urine an amount of vitamin C of a similar order to, but definitely somewhat greater than, the bare amount needed to prevent scurvy. It may be expected from this that whenever the vitamin C intake falls below a safe margin an effect should be readily apparent on the urine. We hope to present data of urinary excretion in Barlow's disease and the response after vitamin C as compared with normals in illustration of this. The most accurate general procedure would appear to be to determine the total daily output after 48 hours on a vitamin C-free regimen. This rules out irregularities due to abrupt changes in urinary volume and to the more immediate effects which follow directly on the administration of a single dose of the vitamin; if necessary also the fluid intake may be restricted in order to give a urine concentrated enough to ensure a sharp end-point in the titration.

SUMMARY.

1. The vitamin C content of human urines has been determined by the titration method.

2. In a number of normal individuals the amount of vitamin C excreted in the urine was surprisingly constant, generally in the neighbourhood of 30 or 33 mg. per day, or roughly 0.02–0.03 mg. per cc. of urine (this concentration being naturally lowered in diuresis, and being sometimes rather higher in the more concentrated early morning urine).

3. If a normal individual is given a single very large dose of vitamin C (*e.g.* 600 cc. of orange juice, 30 times the reputed daily requirement), the concentration of vitamin C in the urine sharply rises, reaching in about 3 hours a maximum as high as, *e.g.*, 8 or 10 times the "normal"; it then rapidly drops again and within a day or so reaches the "normal" resting level of about 33 mg. excreted per day where it remains remarkably steady although the subject may be restricted, *e.g.* for a week or more, to a vitamin C-free diet.

4. This "normal" daily loss of about 33 mg. is somewhat greater than the reputed minimum daily requirement for man (*viz.* "1 oz. of orange or lemon juice").

5. The technique described has possible applications for dietetics, for the diagnosis of hypovitaminosis-C in human beings.

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