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Excretion of Vitamin C in Urine Following Repeated Administration of Big Test Doses

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The vitamin C saturation test as introduced by Harris & Ray (1935) is based on counting the number of days necessary for administration of standard test doses of vitamin C before a large overflow of the vitamin appears in the urine, this number increasing in proportion to the lowness of the past intake. For convenience in examining large groups of subjects, a simplified procedure, involving the collection of a single specimen of urine each day during the peak of excretion rather than of the whole day's output, was recommended by Harris & Abbasy (1937) as sufficiently accurate for routine surveys. Thus modified, the saturation test is easily applicable to group surveys and has been found capable of distinguishing between groups of children or adults at slightly different levels of intake (e.g. Harris, 1940, 1943; Atkins, 1943). The procedure adopted by various workers has, however, differed somewhat, not only as to the size of the test dose but also in choice of excretion period.

In carrying out a small-scale survey using the simplified saturation test as outlined by Harris & Abbasy (1937), it was thought that some supplementary tests, involving the estimation at graded intervals of the total 24 hr. output, might be of interest. This would give some idea of the relation in time between the peak of excretion on approaching saturation and the test period selected (e.g. a 3 hr. period, from the 4th to the 7th hour after the test dose); at the same time information might be obtained about the reliability of this short-period excretion as a criterion of the total overflow.

EXPERIMENTAL

Subjects and their treatment. Five male students at a residential college, 20–21 years of age were given big test doses of ascorbic acid by mouth on each of 4 consecutive days (6–9 June 1950).

The test doses amounted to 10 mg./kg. body-weight and were taken in the morning except on the 1st day when they were taken at noon. Immediately before receiving the test dose the subject emptied his bladder; subsequently urine was collected at frequent intervals and analysed for vitamin C. All the subjects had undergone saturation tests about 4 months before. The dietary intakes of vitamin C were estimated to be about 20 mg./day, and serum values below 0·2 mg. vitamin C/100 ml. were found in all cases when tested just before administering the first dose.

Ascorbic-acid estimation. The ascorbic acid in the urine was estimated by the indophenol method, the reduction of the dye being determined by using a photo-electric colorimeter (Lumetron, Photovolt Corporation, New York City) instead of by titration. It was not thought necessary to correct for the blank (that is any non-specific indophenol-reducing substances).

The following procedure was used. To a measured quantity of urine (varying from 1 to 8 ml., according to a preliminary rough estimation of the concentration of the ascorbic acid) were added 2 ml. of a 20 % (w/v) solution of trichloroacetic acid, and the volume was then made up to 10 ml. with distilled water. Of the urine so diluted a suitable portion, not exceeding 2 ml., was transferred to each of a matched pair of test-tubes containing 4 ml. of an acetic acid-sodium acetate buffer of pH about 4·1. When less than 2 ml. were used, sufficient 4 % (w/v) solution of trichloroacetic acid was added to make the volume up to 6 ml. One of the tubes, after the addition of 2 ml. water, was used for zero adjustment of the colorimeter (density scale, green filter 530), and 2 ml. of a solution of dichlorophenolindophenol were then added to the other: the density reading was next taken as rapidly as possible. The difference between this reading and that of the dye control (read against water as the blank) is a measure of the extent of reduction of the dye, the dye control having been put up in the same way except that the diluted urine was replaced by an equal amount of the 4 % trichloroacetic-acid solution. The concentration of the dye solution was such as to give a reading of 5 % on the transmission scale. The dye control then gave a reading of about 2·7 on the density scale.

By this method readings can be taken rapidly, within 5–10 sec., so as to minimize the interfering action in normal urine of reducing substances other than ascorbic acid. When normal urine with low ascorbic-acid concentration (less than 1 mg./100 ml.) was tested by this method, it was found that the amount of reduction might increase considerably within the first 2 min. or so—the time often required for titration. At higher concentrations, when only small amounts of urine, diluted to one-fifth or one-tenth, were used in the test, the increase in reduction after the first reading was, however, negligible.

RESULTS

The values found for vitamin C in urine collected at intervals after the test doses are given in Table 1. Fig. 1 shows the cumulative excretion of each successive day.

On the 1st day the excretion was low in all subjects, varying from 8·7 to 19·2 mg. in the 20½ hr. Already on the 2nd day three of the subjects gave a marked response. Their response was considerably greater on the following day, but after the fourth test dose

Period (hr. after dose)	Length of period (hr.)	Subject: H.G. (65 kg.) Urine (ml.)	H.G. Ascorbic acid mg./100 ml. urine	H.G. Ascorbic acid mg.	J.A. (80 kg.) Urine (ml.)	J.A. Ascorbic acid mg./100 ml. urine	J.A. Ascorbic acid mg.	K.S. (69 kg.) Urine (ml.)	K.S. Ascorbic acid mg./100 ml. urine	K.S. Ascorbic acid mg.	V.P. (77 kg.) Urine (ml.)	V.P. Ascorbic acid mg./100 ml. urine	V.P. Ascorbic acid mg.	G.I. (74 kg.) Urine (ml.)	G.I. Ascorbic acid mg./100 ml. urine	G.I. Ascorbic acid mg.
Before first test dose																
—	—	—	0·43	—	—	0·43	—	—	0·18	—	—	0·54	—	—	—	—
After first test dose																
0– 4	4	584	0·57	3·33	80	3·39	2·71	194	4·64	9·00	334	1·61	5·38	250	0·71	1·78
4– 7	3	106	3·21	3·40	88	3·03	2·67	60	4·11	2·47	544	0·76	4·13	115	2·14	2·46
7–10	3	270	1·43	3·86	130	2·14	2·78	168	1·43	2·40	208	1·25	2·60	157	1·79	2·81
10–20½	10½	555	0·54	3·00	770	0·53	4·08	500	1·07	5·35	440	0·46	2·02	307	0·54	1·66
Total	20½	1515	—	13·59	1068	—	12·24	922	—	19·22	1526	—	14·13	829	—	8·71
After second test dose																
0– 2	2	180	14·3	25·7	205	1·4	2·9	85	11·4	9·7	144	1·7	2·4	167	0·9	1·5
2– 4	2	157	22·6	35·5	120	2·1	2·5	85	37·5	31·9	160	30·4	48·6	75	1·1	0·8
4– 7	3	149	6·0	8·9	169	1·9	3·2	200	13·1	26·2	220	2·9	6·4	193	0·9	1·7
7– 9	2	145	6·8	9·9	94	3·4	3·2	95	5·3	5·0	245	4·3	10·5	120	1·3	1·6
9–11	2	85	3·2	2·7	48	3·6	1·7	67	3·2	2·1	175	1·1	1·9	89	2·3	2·0
11–13	2	133	1·8	2·4	90	1·8	1·6	130	1·8	2·3	500	0·14	0·7	100	0·9	0·9
13–24	11	710	0·4	2·6	560	0·9	5·0	365	0·93	3·4	540	0·54	2·9	360	0·50	1·8
Total	24	1559	—	87·7	1286	—	20·1	1027	—	80·6	1984	—	73·4	1104	—	10·3
After third test dose																
0– 2	2	204	7·1	14·5	64	1·4	0·9	133	19·3	25·7	125	12·1	15·1	120	1·5	1·8
2– 4	2	190	42·9	81·5	120	53·6	64·3	156	89·3	139·3	172	96·4	165·8	250	12·9	32·3
4– 7	3	617	25·0	154·3	212	53·6	113·6	520	24·3	126·4	542	25·7	139·3	384	17·9	68·7
7– 9	2	170	13·6	23·1	90	8·6	7·7	146	16·9	24·7	327	4·8	15·7	115	15·7	18·1
9–11	2	205	8·6	17·6	130	8·6	11·1	158	12·9	20·4	135	3·6	4·9	223	1·0	2·2
11–13	2	222	5·7	12·7	82	4·3	3·5	202	7·9	16·0	193	7·5	14·5	120	2·1	2·5
13–23	10	685	1·1	7·5	602	2·1	12·6	460	3·5	16·1	275	3·6	9·9	655	0·43	2·8
Total	23	2293	—	311·2	1300	—	213·7	1775	—	368·6	1769	—	365·2	1867	—	128·4
After fourth test dose																
0– 2	2	} 710	28·6	203·1	{ 90	19·6	17·6	585	7·9	46·2	} 80	150·0	120·0	{ 120	9·3	11·2
2– 4	2				90	28·6	25·7	122	114·3	139·4				110	150·0	165·0
4– 7	3	213	78·6	167·4	190	50·0	95·0	255	35·7	91·0	370	26·1	96·6	105	152·9	160·5
7– 9	2	100	14·3	14·3	103	45·7	47·0	77	32·1	24·7	116	35·7	41·4	70	46·4	32·5
9–11	2	72	14·7	10·6	20	28·6	5·7	85	19·1	16·2	80	21·4	17·1			
Total		1005		395·4	493		191·0	1124		317·5	646		275·1	405		369·2

only one of them still showed some further increase in excretion, but the other two showed a slight decline. Two of the subjects showed almost no response until after the third test dose. It was not found worth while to calculate all the values to a standard weight (70 kg.) as the main conclusions arrived at would obviously not have been thereby affected.

Table 2 shows the amounts of vitamin C excreted at certain periods on the 2nd and 3rd days, as percentages of the total output in 24 or 23 hr., respectively.

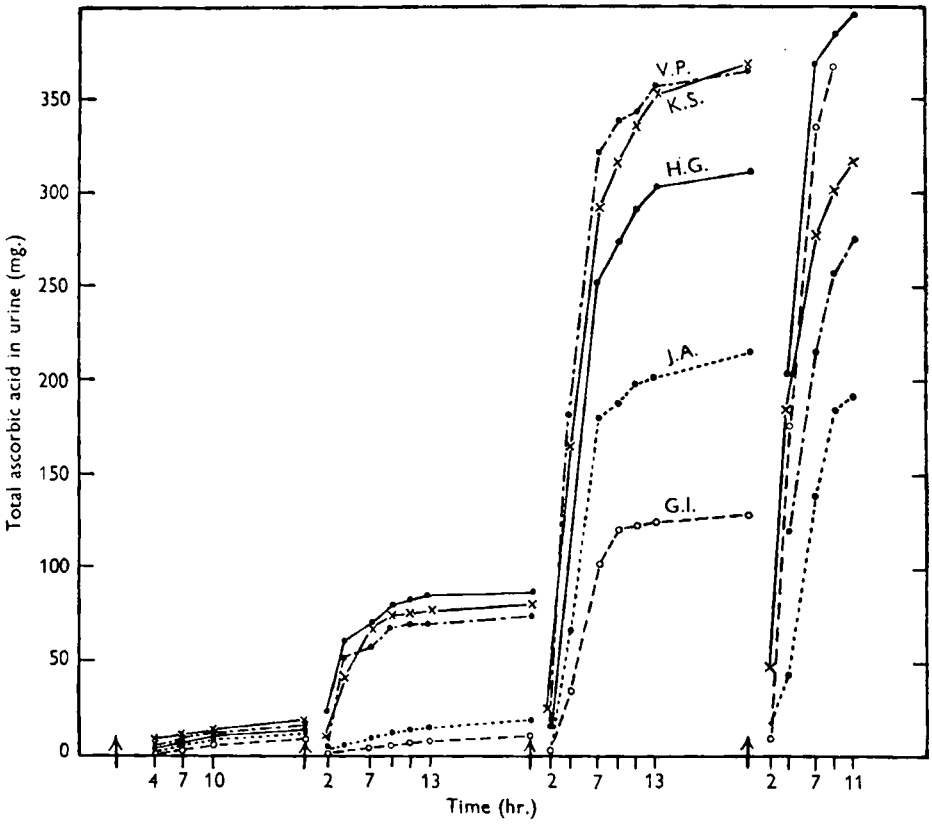

Fig. 1. Cumulative daily excretion of ascorbic acid in the urine of five male students 20–21 years old, following the administration of big test doses (10 mg./kg. body-weight) on each of 4 consecutive days, as indicated by the arrows (cf. also Table 1).

DISCUSSION

From Table 2 it is seen that when a marked response is reached (in three subjects on the 2nd day and in all on the 3rd day) a remarkably constant proportion, 94·1–97·8 %, of the 23–24 hr. output is excreted within the first 13 hr. following administration of the test dose. Also, the percentage excreted in the first 7 hr. is relatively constant, ranging from 78·2 to 87·7. With shorter periods, from the 2nd to the 4th and from the 4th to the 7th hr. after the test dose, however, this percentage varies considerably, especially during the initial response, and as often as not more was excreted in the first than in the second period. But, with one exception, both these periods together

Table 2. *Excretion of ascorbic acid in the urine of five subjects at certain intervals after the test dose (see Table 1), expressed as a percentage of the total output in 23 or 24 hr.*

Subject	Total output: mg.	Total output: As percentage of dose	Percentage of total output excreted in interval after dose: 2–4 hr.	4–7 hr.	0–7 hr.	0–13 hr.
			2nd day, 24 hr.			
J.A.	20·1	2·5	12·4	15·9	42·8	75·1
G.I.	10·3	1·4	7·8	16·5	38·8	82·5
H.G.	87·7	13·5	40·5	10·1	79·9	97·0
K.S.	80·6	11·7	39·6	32·5	84·1	95·8
V.P.	73·4	9·5	66·2	8·7	78·2	96·0
			3rd day, 23 hr.			
J.A.	213·7	26·7	30·1	53·2	83·7	94·1
G.I.	128·4	17·4	25·2	53·5	80·1	97·8
H.G.	311·2	47·9	26·2	49·6	80·4	97·6
K.S.	368·6	53·4	37·8	34·3	79·1	95·6
V.P.	365·2	47·4	45·4	38·1	87·7	97·3

(i.e. from the 2nd to the 7th hr. after the test dose) represent a fairly constant proportion of the total output.

From this limited number of observations it would appear that the excretion during the period from 4 to 7 hr. after the test dose is not a good criterion of the magnitude of the response, as too often it does not cover the peak of excretion. Thus in two subjects (H.G. and V.P., Table 1 and Fig. 1) an initial response occurred on the 2nd day, but would hardly have been recognized if only the 4th to 7th hr. specimen had been tested. It should, however, be noted here that, although, as mentioned above, this period was originally recommended by Harris & Abbasy for the collection of the test specimen, Harris (1940, 1943) later preferred a $2\frac{1}{4}$ hr. sample taken between $3\frac{1}{2}$ and $5\frac{3}{4}$ hr. after the test dose (i.e. around the 4th and 5th hr.), when he found the excretion to be at its peak.

The data contained in Table 1 relating to thirteen instances of marked response do not allow an exact timing of the peak of excretion, but obviously it has (with possibly one exception, H.G. on the 2nd day) occurred well within the range of the 2nd to the 7th hr. after the test dose and probably in all but two instances (H.G., 2nd day and J.A., 4th day) within the period from 3 to 6 hr., or even from 3 to $5\frac{1}{2}$ hr., after the test dose.

Only two of the students showed continued increase in excretion up to the 4th day; the other three apparently excreted slightly less on the 4th day than on the 3rd. Unfortunately circumstances did not allow the experiment to be prolonged for some days more so as to see whether this apparent fall in excretion was of any significance. Shortly afterwards, however, similar tests were carried out with one adult male receiving a test dose of 750 mg. (10 mg./kg. body-weight) for 8 consecutive days. Fig. 2 shows the amounts of vitamin C excreted on each of these days, except the 6th when only the night specimen of urine was available. Judging from this specimen it appeared that on the 6th day the excretion was already diminishing. Anyhow, there

was an appreciable, progressive fall for the next 2 days, in spite of continued test dosing as before. After the administration of test doses was discontinued the expected abrupt fall to a still lower level of excretion took place.

A corresponding fluctuation in the test-period excretion, in spite of continued dosage after saturation has apparently been reached, has often been observed by earlier authors (e.g. Harris, 1940, 1943). The most reasonable explanation seems to be that repeated intake of excessive amounts of ascorbic acid leads to an increased rate of destruction.

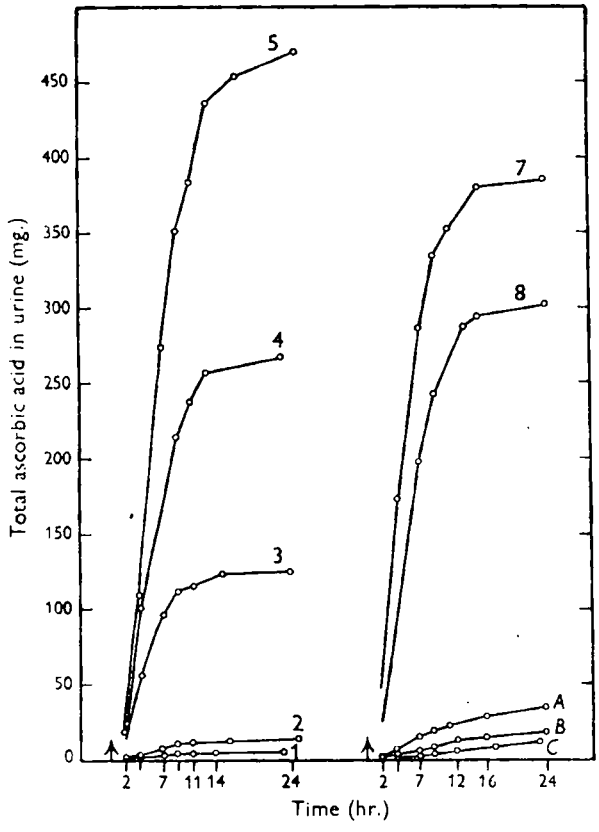

Fig. 2. Cumulative daily excretion of ascorbic acid in the urine of one adult male (weight 75 kg.) following the administration of a 750 mg. test dose on each of 8 consecutive days. (The curve for the 6th day is not shown.) The curves *A*, *B* and *C* represent the excretion on the first 3 days after discontinuing the test dosing.

The maximum output, as observed in these experiments (Tables 1 and 2, Fig. 1) when saturation is reached, is roughly about 50–60 % of the test dose, and—as far as can be judged from the 2–3 hr. afternoon samples—this seems to be in good accord with the findings of Harris (1940, 1942), and others who have used test doses of similar size. When smaller doses are given the percentage excreted may, however, be higher (Spellberg & Keeton, 1939).

Apart from losses through destruction in the tissues, it is possible that appreciable amounts of these unphysiologically large test doses are destroyed before absorption in

the intestine. Whether or not destruction in the tissues is as great before as after saturation has been reached, it may be expected that the total losses are considerable. It therefore appears obvious that the vitamin C deficit cannot be exactly expressed quantitatively in terms of the ascorbic acid required to induce urinary response.

SUMMARY

1. Five male students were given big test doses of ascorbic acid (10 mg./kg. body-weight) on 4 consecutive days. Specimens of urine were collected at frequent intervals and analysed for ascorbic acid.

2. When a distinct response had been obtained, it was found that 94–98 % of the 23–24 hr. output of ascorbic acid were excreted in the first 13 hr., and 78–88 % in the first 7 hr. after administration of the test dose. In shorter periods within the first 7 hr. the relative amount excreted was more variable. As often as not, more was excreted in the 2 hr. period between 2 and 4 hr. after the dosage than in the following 3 hr. period. A distinct response shown by two of the subjects on the 2nd day would hardly have been noticed if the 3 hr. specimen only, collected between the 4th and 7th hr. after the test dose, had been examined.

3. Not more than about 50–60 % of the ascorbic acid administered could be recovered in the urine when full saturation was reached. This confirms previous findings that considerable losses occur through destruction or possibly through incomplete absorption of the large doses.

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