CLXXXVII. THE URINARY EXCRETION OF ASCORBIC AND DEHYDROASCORBIC ACIDS IN MAN.

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The antiscorbutic activity of dehydroascorbic acid (the reversibly oxidised form of ascorbic acid) brings to the forefront the problem of the relative functions of these two active forms in animal and plant metabolism. As a contribution to this main thesis, representative experiments are described in this communication dealing with the relation between the ingestion and urinary excretion of ascorbic and dehydroascorbic acids. The investigation was carried out on four adult subjects.

Van der Walle [1922] found that a daily dose of 20 ml. of fresh normal human urine afforded no significant protection from scurvy to guinea-pigs on a scorbutic diet.

Euler and Klussman [1933] examined some specimens of urine from normal, febrile and diabetic subjects and found that they reduced indophenol to an appreciable extent.

Van Eekelen *et al.* [1933] claim that the indophenol-reducing capacity of human urine increases after the consumption of much fruit or of high doses of decitrated lemon juice. They also state that this capacity gradually disappears from the urine of guinea-pigs on a scorbutic diet. On the other hand when decitrated lemon juice is injected intravenously into rabbits the oxidised form of the vitamin is excreted in the urine in consequence.

Two more communications [Harris *et al.* 1933; Hess and Benjamin, 1934] have appeared in this connection whilst our work was in progress.

Harris *et al.* observed that the urine of normal individuals showed a surprisingly constant indophenol-reducing capacity (equivalent to 0·03 mg. of ascorbic acid per ml.) the daily output being generally in the neighbourhood of 30–33 mg. In the case of an adult subject on a scorbutic diet after ingestion of a high dose, the urinary output of ascorbic acid, as measured by the indophenol titration, rose rapidly, reaching a maximum in 3 hours after ingestion from which it quickly fell to a nearly normal level.

Hess and Benjamin, who made their observations on children, also assessed the ascorbic acid content of the urine by means of the indophenol titration. They found that the urinary output of ascorbic acid under normal conditions was low whether vitamin C was excluded from the diet or whether it was ingested in adequate amounts. Only when 1 pint of orange juice daily was given

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to the children did the capacity for reducing indophenol become manifest in the urine. Even then an appreciable excretion of ascorbic acid in the urine did not appear until the fourth day, when the amount excreted rose rapidly but fell immediately the dose was discontinued.

Experimental methods.

The ascorbic acid in the urine was determined by direct indophenol titration. The figures thus obtained were found by biological tests to be truly representative of ascorbic acid under the conditions of our experiments.

During the day the urine was titrated immediately after being passed. The night urine when pooled with the evening urine was collected over sulphuric acid and titrated on the following morning. Under these conditions the ascorbic acid excreted was found to be stable for several days. Acetic acid had a less stabilising effect and was used occasionally when only 2–3 hours elapsed between the collection and titration, for instance in the urine collected at 8 a.m.

The titrations were carried out with $N/1000$ indophenol at p_H 3. At this reaction either the reduction of the indicator by substances in urine other than ascorbic acid such as phenolic compounds is suppressed, or the speed of reduction is reduced to a rate which does not seriously vitiate the end-point due to the quickly reducing ascorbic acid.

Ascorbic acid was taken in the form of orange juice when small doses (150–350 mg.) were employed and in the form of concentrated decitrated lemon juice when higher doses were required (1000 mg.). The latter was prepared by concentrating decitrated lemon juice to a small volume at low pressure at 40°, precipitating it with 96 % alcohol, filtering and concentrating the filtrate at low temperature and pressure to a suitable volume. The ascorbic acid contents of these preparations and of the orange juice were determined by direct titration with indophenol.

Ascorbic acid in urine.

As will be seen from the observations described below the ingestion of antiscorbutic substances raised the indophenol-reducing capacity of the urine. On the other hand the urine from subjects who subsisted on diets not very rich in vitamin C or on scorbutic diets showed indophenol-reducing properties of a very low order, which at times were not even characteristic of ascorbic acid. These observations would therefore suggest that the quick reduction of the indicator by urine in acid solution was due to ascorbic acid. On reflection, however, one could not exclude the possibility of the conversion in the body of ascorbic acid into inactive or less active isomerides or degradation products which would retain the reducing properties of the original acid. To test this hypothesis the following experiment was instituted. A. E. K. received 875 mg. of ascorbic acid. After 7 hours he passed 450 ml. of urine containing 71 mg. of ascorbic acid per 100 ml. as assessed by direct indophenol titration. Similarly at a later stage of the test S. W. J. received 1000 mg. of ascorbic acid and after 5 hours passed 140 ml. of urine containing 83 mg. of the acid per 100 ml. of urine. These urines were brought to p_H 3 with sulphuric acid and stored in the cold room. A. E. K.'s urine lost during storage only about 12 % of its indophenol-reducing capacity in 5 days whilst S. W. J.'s urine lost 30 % in 10 days. These urines were tested curatively for their antiscorbutic potency on guinea-pigs which had subsisted for 14 days on a scorbutic diet. An amount of urine (titrated daily) which reduced 6 ml. $N/1000$ indophenol (equivalent to

0·5 mg. of ascorbic acid) was administered each day. Fig. 1 which gives a graphic representation of the test shows that the antiscorbutic potency of the urine dose was of the same order as that of 0·5 mg. of ascorbic acid. Its indophenol-reducing capacity can therefore be ascribed mainly to this compound.

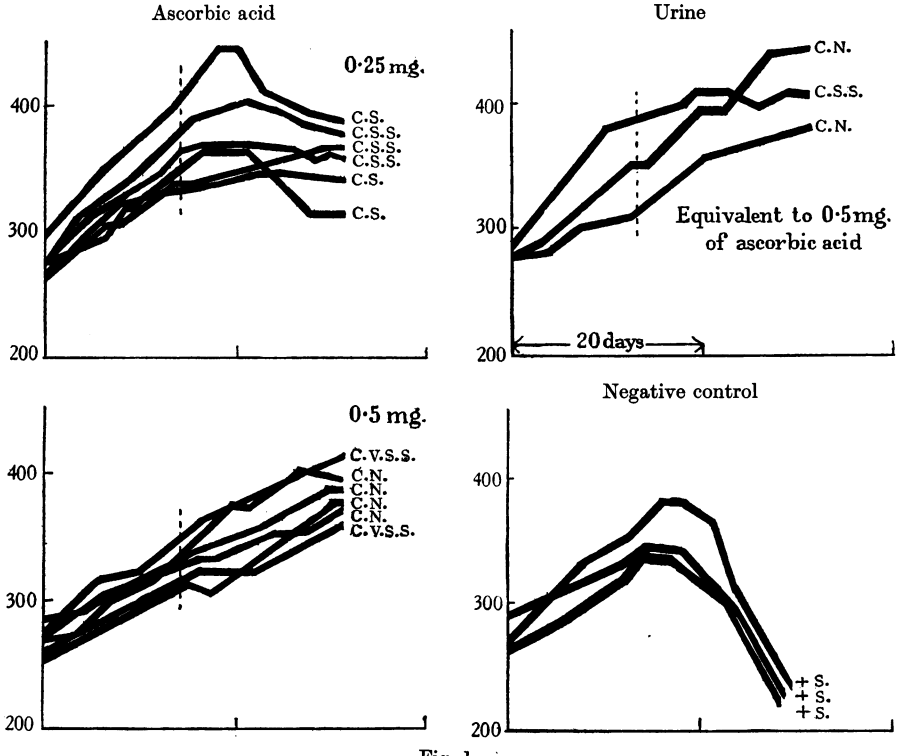

Fig. 1.

| = beginning of dosing.
+ = died.
C = chloroformed.

N = Normal.
V.S.S. = very slight scurvy.
S.S. = slight scurvy.
S. = scurvy.

Preliminary.

Before dealing with any further results, it is necessary to record the "normal" indophenol-reducing properties of the urines of the four experimental subjects concerned in this investigation. Three of these, S. W. J., A. E. K. and H. H., while using ordinary mixed diets on which they usually subsist during the winter passed urine which for days scarcely reduced indophenol. Even when the titrations were significant the output of ascorbic acid per 24 hours was of the order of 10–15 mg. During the summer, as was to be expected, the indophenol-reducing capacity rose to some extent. The fourth subject (S. S. Z.) for reasons not connected with the present inquiry used a diet in which bread and potatoes were replaced by green vegetables; in addition he consumed regularly two oranges a day. In consequence his "normal" urinary output of ascorbic acid was 80–150 mg. per day even during the winter. At no time in the period of 8 months during which the experiments described in this communication were carried out were there any indications even of latent scurvy in any of the four subjects.

Conditions under which ascorbic acid is excreted in the urine.

It soon became evident from exploratory experiments that the appearance of ascorbic acid in the urine after its ingestion was dependent not only on the magnitude of the dose but also on the previous dietetic history of the subject in respect of vitamin C. The subject had to be "saturated" with the vitamin before his level of excretion in the urine became constant. The time taken to attain this was controlled by the degree of "saturation" of the subject at the beginning of the experiment and by the size of the daily dose. This is exemplified by the following experiments. S. W. J. was subsisting on a diet low in vitamin C which consisted mainly of eggs, cheese, fried bacon and bread with small quantities of meat, fish and potatoes. On this regimen his urine did not show any capacity for reducing indophenol for a number of consecutive days. The small amount of vitamin C contained in this diet exercised no influence on the results, since similar ones were obtained in the case of S. S. Z. on a strictly scorbutic diet. On successive days at the beginning of the experiment S. W. J. took, 30, 30, 60, 60, 90 and 90 mg. ascorbic acid respectively. No sample of urine, which was collected at frequent intervals, showed any significant capacity for reducing indophenol. Only after the seventh day were significant figures obtained. Table I gives the data from this day to the eighteenth day of the experiment. The figures show that the amount of ascorbic acid returned in the urine

Table I.

	Intake		Excretion in urine				
Day	Time dose taken	Ascorbic acid mg.	Time urine passed	Volume of urine ml.	Ascorbic acid per 100 ml. mg.	Total amount of ascorbic acid mg.	Ascorbic acid output per hour mg.
7th	10.30 a.m.	120	12.45 p.m.	200	0·4	0·8	0·3
			2.00	200	0·4	0·8	0·6
			4.30	270	0·8	2·3	0·9
			5.30	60	0·8	0·5	0·5
			8.00 a.m.	1010	0·8	8·4	0·6
			10.15	110	0·8	0·9	0·4
			Total	**1850**	—	**13·7**	—
8th	12.15 p.m.	120	1.00 p.m.	180	0·8	1·5	0·5
			2.00	70	1·7	1·2	1·2
			3.45	140	6·3	8·8	5·0
			5.45	100	6·7	6·7	3·4
			8.00 a.m.	880	1·3	11·0	0·8
			10.15	110	0·8	0·9	0·4
			Total	**1480**	—	**30·1**	—
9th	10.30 a.m.	85	11.30 a.m.	100	1·7	1·7	1·4
			12.30 p.m.	200	—	—	—
			2.00	180	1·3	2·3	1·5
			3.00	205	0·8	1·7	1·7
			5.15	170	1·7	2·8	1·2
			6.15	70	1·7	1·0	1·0
			8.00 a.m.	930	0·8	7·8	0·6
			11.30	170	1·7	2·8	0·8
			2.00 p.m.	210	1·7	3·5	1·4
			Total	**2235**	—	**23·6**	—
10th	2.45 p.m.	100	8.00 a.m.	1270	2·5	31·8	1·8
			10.30	80	1·7	1·3	0·5
			Total	**1350**	—	**33·1**	—

Table I (*cont.*).

	Intake		Excretion in urine				
Day	Time dose taken	Ascorbic acid mg.	Time urine passed	Volume of urine ml.	Ascorbic acid per 100 ml. mg.	Total amount of ascorbic acid mg.	Ascorbic acid output per hour mg.
11th	11.00 a.m.	130	11.30 a.m.	130	5·0	6·5	3·3
			12.30 p.m.	630	5·8	36·8	3·3
			8.00 a.m.	480	1·7	8·0	1·0
			Total	**1240**	—	**51·3**	—
12th	8.30 a.m.	120	6.00 p.m.	770	3·3	25·7	2·6
			8.00 a.m.	465	2·5	11·6	0·8
			10.15	60	1·7	1·0	0·4
			Total	**1295**	—	**38·3**	—
13th	11.00 a.m.	90	1.15 p.m.	120	1·7	2·0	0·7
			4.00	140	6·7	9·3	3·4
			5.45	130	2·5	3·3	1·9
			8.00 a.m.	720	1·7	12·0	0·8
			10.30	120	1·7	2·0	0·8
			12.15 p.m.	175	2·5	4·4	2·5
			Total	**1405**	—	**33·0**	—
14th	12.30 p.m.	120	2.15 p.m.	100	7·5	7·5	3·8
			8.00 a.m.	800	5·0	40·0	2·3
			10.30	110	2·5	2·8	1·1
			12.30 p.m.	155	2·5	3·9	2·0
			Total	**1165**	—	**54·2**	—
15th	12.30 p.m.	120	2.15 p.m.	160	4·1	6·7	3·8
			5.00	140	18·3	25·7	9·3
			8.00 a.m.	860	3·3	28·7	1·9
			10.30	90	6·7	6·0	2·4
			Total	**1250**	—	**67·1**	—
16th	12.30 p.m.	120	1.00 p.m.	170	5·0	8·5	3·4
			3.30	220	11·7	25·7	10·3
			4.30	180	11·7	21·0	21·0
			8.00 a.m.	1010	4·1	42·1	2·7
			10.30	160	5·0	8·0	3·1
			Total	**1740**	—	**105·3**	—
17th	12.30 p.m.	150	1.00 p.m.	120	5·0	6·0	2·4
			3.15	220	18·3	40·3	17·9
			5.15	165	21·7	37·8	18·9
			8.00 a.m.	710	7·5	53·3	3·4
			Total	**1215**	—	**137·4**	—
18th	10.30 a.m.	150	11.00 a.m.	80	12·5	10·0	3·3
			12.30 p.m.	70	22·9	15·1	10·0
			8.00 a.m.	1260	8·3	105·1	5·4
			Total	**1410**	—	**130·2**	—

gradually rose to a level which remained constant after the seventeenth day. This suggested that the subject was "saturated" with ascorbic acid, but in order to insure complete "saturation" he took a further 1000 mg. of which 307 mg. was returned during the first, and only 45 mg. during the second, 24 hours. 150 mg. of ascorbic acid were then taken. Full details of the intake and urinary excretion of ascorbic acid *etc.* during this and the subsequent 9 days are given in Table II. It will be seen that in this part of the experiment the return of ascorbic acid in the urine after discontinuing the dose fell to very low values in a few days. In contrast to what happened in the initial stages of the

Table II.

	Intake		Excretion in urine				
Day	Time dose taken	Ascorbic acid mg.	Time urine passed	Volume of urine ml.	Ascorbic acid per 100 ml. mg.	Total amount of ascorbic acid mg.	Ascorbic acid output per hour mg.
1st	12.45 p.m.	150	3.30 p.m.	140	25·0	35·0	12·7
			5.00	85	29·1	24·8	16·5
			8.00 a.m.	800	8·3	66·7	4·5
			10.30	90	8·3	7·5	3·0
			12.00 noon	90	3·3	3·0	2·0
			1.0 p.m.	70	2·5	1·8	1·8
			Total	**1275**	—	**138·8**	—
2nd	12.45 p.m.		5.45 p.m.	180	6·7	12·0	2·5
			8.00 a.m.	620	3·3	20·7	1·4
			10.45	120	4·1	5·0	1·8
			12.15 p.m.	90	1·7	1·5	1·0
			1.15	160	1·7	2·7	2·7
			Total	**1170**	—	**41·9**	—
3rd	12.45 p.m.		4.30 p.m.	150	5·0	7·5	2·3
			8.00 a.m.	900	0·8	7·5	0·5
			10.30	150	0·8	1·3	0·5
			Total	**1200**	—	**16·3**	—
4th	12.45 p.m.		2.30 p.m.	210	0·8	1·8	0·5
			8.00 a.m.	1270	0·4	5·3	0·3
			Total	**1480**	—	**7·1**	—
5th	12.45 p.m.		8.30 a.m.	1300	0·4	5·4	0·2
			10.30	180	0·4	0·8	0·4
			Total	**1480**	—	**6·2**	—
6th	12.45 p.m.		4.30 p.m.	450	0·4	1·8	0·3
			8.00 a.m.	800	0·4	3·2	0·2
			10.30	130	0·4	0·5	0·2
			Total	**1380**	—	**5·5**	—
7th	12.00 noon	180	2.30 p.m.	155	16·0	24·8	6·2
			4.30	130	30·0	39·0	19·5
			5.30	75	11·7	8·8	8·8
			8.00 a.m.	1450	4·1	60·4	4·2
			10.30	110	8·3	9·1	3·6
			11.30	110	3·3	3·6	3·6
			Total	**2030**	—	**145·7**	—
8th	12.00 noon		1.00 p.m.	100	3·3	3·3	2·2
			3.15	110	5·8	6·4	2·8
			5·45	110	5·8	6·4	2·5
			8.00 a.m.	650	1·7	10·8	0·8
			10.00	110	1·7	1·9	0·8
			11.30	130	4·1	5·4	3·6
			Total	**1210**	—	**34·2**	—
9th	12.00 noon		8.00 a.m.	1200	1·7	20·0	1·0
			10.30	110	1·7	1·9	0·8
			Total	**1310**	—	**21·9**	—
10th	12.00 noon		2.30 p.m.	140	2·5	3·5	0·9
			6.00	180	1·7	3·0	0·8
			9.15	210	1·7	3·5	1·0
			8.00 a.m.	800	0·8	6·7	0·6
			11.30	180	1·7	3·0	0·8
			Total	**1510**	—	**19·7**	—

experiment (Table I) however the level of excretion could now be raised by the administration of a single dose of 150 mg. or 180 mg. to that of the previously obtained constant level. In other words when the subject is "saturated" this level is attained at once, but on the other hand many days may elapse in cases where the vitamin C store is depleted. This phenomenon has also been observed in the other three subjects and the results are in agreement with those obtained by Hess and Benjamin on children. Our results further suggest that the maximum rate of excretion of ascorbic acid invariably occurred about the fourth hour after ingestion and that the initial rate was reached again within about 24 hours. Harris *et al.* also observed that the maximum rate of excretion occurred after a similar interval. It may be further noted that the concentration of ascorbic acid in the urine at any time is controlled by the amount of water excreted, but, as will be amplified later, its rate of excretion is independent of diuresis. The concentration of ascorbic acid in urine is therefore by itself of no great significance.

Urinary excretion of ascorbic acid after ingestion of high doses.

It will be remembered that as S. W. J. became "saturated," about 80 % of the ingested ascorbic acid appeared in the urine when the dose taken was of the order of 150 mg. When, on the other hand, the dose was raised to 1000 mg. only about 35 % of the acid was returned in the urine during the subsequent 48 hours. This observed disparity in the relative proportions of ascorbic acid "retained" on high or low doses called for further investigation and it was subsequently shown that the lower percentage of excretion is indeed characteristic of high doses. This is illustrated by the following typical experiments.

S. S. Z. subsisting on his "normal" diet on which, as has already been mentioned, he excreted 70–150 mg. of ascorbic acid, took a daily dose of the order of 1000 mg. on six consecutive days. The urine was titrated at each collection during the 24 hour period. It will be seen from Table III that, even if his basal output of about 100 mg. *per diem* is ignored, only 45 % of the ingested ascorbic acid was accounted for in the urine. Furthermore, during the 4 days immediately following this period of high dosing he excreted 117, 114, 110 and 77·3 mg. of ascorbic acid respectively, thus returning immediately to his basal level of urinary excretion of the vitamin. According to these figures about 400–600 mg. of the acid were not accounted for in this case as against about 25 mg. "retained" by S. W. J. on the low dose.

Whilst the above is illustrative of a "saturated" case, the following experiment deals with a subject who was not previously "saturated." A. E. K. subsisted for a long time on a diet low in vitamin C which was constituted similarly to the one used by S. W. J. in the experiment previously described. He received high doses of ascorbic acid on each of nine successive days. The results which are given in Table IV in an abridged form show that prior to the high dosage he returned only 10·5 mg. of the acid in the urine during 24 hours. The level then rose until the fourth day, after which it became more or less constant. Immediately following this period of high dosing the urinary excretion of ascorbic acid fell rapidly, and on the third day reached the low level of 5·5 mg. for the 24 hours.

This case differs from that of S. S. Z. in that being "unsaturated" he took several days to reach the relatively constant maximum level of output. On the other hand, as might be expected, it took very much less time to "saturate" A. E. K. on high doses than it did S. W. J. on low doses (Table I). Yet the return to the original level in all three cases occurred in approximately the same time.

Table III.

Dose taken at 8.00 a.m. on each day.

Day	Intake. Ascorbic acid mg.	Excretion in urine: Time urine passed	Volume of urine ml.	Ascorbic acid per 100 ml. mg.	Total amount of ascorbic acid mg.	Ascorbic acid output per hour mg.	Ascorbic acid output as percentage of intake
1st	1125	10.30 a.m.	130	58·3	75·8	30·0	
		3.15 p.m.	270	79·0	213·8	45·0	
		5.30	160	29·0	47·0	20·0	
		12.00 midnight	385	20·0	77·0	11·8	
		8.00 a.m.	660	13·3	88·0	11·0	
		Total	**1605**	—	**501·6**	—	**44·5**
2nd	875	11.00 a.m.	120	116·7	140·0	46·7	
		3.15 p.m.	230	54·1	124·4	19·5	
		5.30	90	41·6	37·5	16·7	
		12.00 midnight	430	20·0	86·0	13·0	
		8.00 a.m.	740	11·0	80·0	10·0	
		Total	**1610**	—	**467·9**	—	**48·6**
3rd	1083	8.00 a.m. to 12.00 midnight	930	43·7	407·0	25·4	
		12.00 midnight to 8.00 a.m.	750	10·0	75·0	9·3	
		Total	**1680**	—	**482·0**	—	**44·5**
4th	1083	8.00 a.m. to 12.00 midnight	950	31·2	297·0	18·6	
		12.00 midnight to 8.00 a.m.	450	15·0	67·5	8·4	
		Total	**1400**	—	**364·5**	—	**33·6**
5th	1167	10.15 a.m.	180	33·3	60·0	26·7	
		3.15 p.m.	305	62·5	190·6	38·1	
		12.00 midnight	490	36·7	179·7	20·5	
		8.00 a.m.	750	15·0	112·5	14·0	
		Total	**1725**	—	**542·8**	—	**46·5**
6th	917	10.15 a.m.	140	50·0	70·0	31·2	
		2.30 p.m.	280	62·5	175·0	41·1	
		5.30	250	41·7	104·0	34·7	
		12.00 midnight	275	45·2	126·0	19·4	
		8.00 a.m.	790	10·0	79·0	10·0	
		Total	**1735**	—	**554·0**	—	**60·4**

Table IV.

Day	Intake of ascorbic acid mg.	Excretion in urine: Volume of urine ml.	Total ascorbic acid mg.	Ascorbic acid output as percentage of intake	Maximum output per hour mg.	Minimum output per hour mg.
1st	—	630	10·5	—	0·6	0·2
2nd	916	1715	77·0	8	8·0	0·3
3rd	933	1455	303·0	30	31·5	1·1
4th	1000	1100	402·8	40	55·0	1·2
5th	800	2135	492·8	60	50·3	4·5
6th	867	1395	537·5	61	75·0	3·6
7th	750	1865	391·8	52	48·9	2·6
8th	833	2025	476·8	57	71·1	3·6
9th	875	1050	432·1	50	62·2	2·1
10th	875	1280	529·5	60	111·1	1·7
11th	—	1405	68·2	—	3·7	1·2
12th	—	810	22·8	—	1·7	0·4
13th	—	890	5·5	—	0·5	0·3

It would therefore seem that there is a definite capacity for "saturation" which is independent of the dose. Further, in the case of the high doses, the amount of ascorbic acid not accounted for in the urine is very high and apparently differs with the individual, since in the case of S. S. Z. it was of the order of 400–600 mg. whilst in that of A. E. K. it was only about 300 mg.

The retention of ascorbic acid.

The preceding experiments make it clear that the balance between the amount of ascorbic acid ingested and that returned in the urine cannot be taken as a true index of retention of the acid for the actual physiological function of the vitamin, since this balance depends on the dose taken. Exploratory experiments were nevertheless carried out in order to ascertain whether it was possible to obtain by this technique using low doses a rough estimate of the daily minimum amount of ascorbic acid needed. The experiments to be described show that this is unlikely since it was found that the same individual manifested two levels of urinary excretion of ascorbic acid on the same dose at different times and conversely the same level of output may occur after the ingestion of doses of different magnitude. This is illustrated by the following two experiments.

S. S. Z. was subsisting on a scorbutic diet consisting of eggs, cheese, mushrooms, bacon, chocolate and boiled sweets. Between the morning meal taken about 8 a.m. and the evening meal taken about 7 p.m. no food except tea and milk was consumed. This subject was "saturated," but before beginning the experiment he remained on the scorbutic diet without any vitamin C addition for several days till the urinary excretion fell to a low level. He then ingested 150 mg. of ascorbic acid every 24 hours. It took him 2 days (Table V) to reach the constant level of excretion of about 60 mg. which was maintained for 4 days. At this stage S. S. Z. went on a mixed diet for a few days after which he took in addition 1000 mg. of ascorbic acid to ensure full saturation and returned to the scorbutic diet for another 4 days; the level of urinary excretion reached this time was about 100 mg. for 24 hours (Table VI). After 4 days on a mixed diet the scorbutic diet was resumed for another 10 days; the level of about 100 mg. per 24 hours was consistently maintained.

S. W. J., using the previously mentioned diet low in vitamin C, "saturated" himself with ascorbic acid by ingesting standardised amounts of orange juice. His intake of ascorbic acid per day during the first 5 days of the experiment was 350 mg. of which he returned about 220 mg. daily in the urine (Table VII). On the sixth day his daily dose was reduced to 300 mg. and was taken in two portions of 150 mg. each, at midday and midnight respectively. Under these conditions it will be seen that on the whole the same daily amount was returned in the urine as with the higher dose. It may be noted that in the second part of the experiment, when the daily dose was taken in two portions, with the exception of one day, a higher percentage of the intake was returned in the urine during the period between midday and midnight than in the succeeding 12 hour period.

The fate of ingested dehydroascorbic acid.

The urinary excretion when dehydroascorbic acid was ingested in excess of the possible requirements of the body was next studied. Experiments described below supply definite proof that the dehydrogenated acid is reduced in the body.

Since the oxidation of ascorbic acid with indophenol or iodine would have made the preparations unsuitable for internal consumption and further since

Table V.

	Intake		Excretion in urine				
Day	Time dose taken	Ascorbic acid mg.	Time urine passed	Volume of urine ml.	Ascorbic acid per 100 ml. mg.	Total amount of ascorbic acid mg.	Ascorbic acid output per hour mg.
1st	11.30 a.m.	150	1.00 p.m.	80	4·0	3·2	2·2
			2.00	85	5·7	4·9	4·9
			3.00	135	4·0	5·4	5·4
			4.00	245	2·1	5·3	5·3
			5.00	205	2·5	5·1	5·1
			10.00	315	4·5	14·2	2·5
			8.00 a.m.	900	0·8	7·5	0·9
			10.00	155	1·8	2·8	1·4
			Total	**2120**	—	**48·4**	—
2nd	10.00 a.m.	150	11.00 a.m.	105	3·3	3·5	3·5
			12.00 noon	140	2·7	3·7	3·7
			1.00 p.m.	200	2·7	5·3	5·3
			2.00	430	1·7	7·3	7·3
			3.00	80	4·1	3·3	3·3
			4.00	260	1·0	2·6	2·6
			5.00	210	1·5	3·3	3·3
			10.00	330	2·5	8·3	1·7
			8.00 a.m.	1120	0·8	9·3	0·9
			10.00	170	1·7	2·8	1·4
			Total	**3045**	—	**49·4**	—
3rd	10.00 a.m.	150	11.00 a.m.	135	3·5	4·7	4·7
			12.00 noon	120	4·1	5·0	5·0
			1.00 p.m.	155	4·0	6·2	6·2
			2.00	330	2·7	8·8	8·8
			3.00	315	1·7	5·2	5·2
			4.00	355	1·5	5·6	5·6
			5.00	410	1·7	6·8	6·8
			10.00	375	1·3	5·0	1·0
			8.00 a.m.	1120	0·8	9·3	0·9
			10.00	235	1·8	4·2	2·1
			Total	**3550**	—	**60·8**	—
4th	10.00 a.m.	150	11.00 a.m.	135	3·0	4·0	4·0
			12.00 noon	255	2·0	5·1	5·1
			2.00 p.m.	170	6·7	11·3	5·7
			5.30	235	6·7	15·6	4·5
			10.30	280	3·8	10·7	2·1
			9.30 a.m.	1120	1·5	15·8	1·4
			Total	**2195**	—	**62·5**	—
5th	9.30 a.m.	150	11.00 a.m.	140	5·8	8·2	5·5
			2.00 p.m.	215	5·3	11·4	3·8
			6.00	150	6·7	10·0	2·5
			10.00	255	5·0	12·8	3·2
			8.00 a.m.	875	1·5	13·3	1·3
			10.00	130	3·5	4·5	2·3
			Total	**1765**	—	**60·2**	—
6th	10.00 a.m.	50	11.00 a.m.	130	3·3	4·3	4·3
			12.00 noon	190	2·7	5·0	5·0
			2.00 p.m.	305	2·7	8·1	4·0
			3.00	110	2·5	2·8	2·8
	3.00 p.m.	50	4.00	130	2·3	3·0	3·0
			5.00	335	1·8	6·1	6·1
			8.00	370	3·7	13·5	4·4
	10.00 p.m.	50	8.00 a.m.	700	2·0	14·0	1·4
			10.00	145	3·8	5·7	2·9
	Total	**150**	—	**2415**	—	**62·5**	—

Table VI.

The dose was taken at 10.00 a.m. on each day except the first day when it was taken at noon.

	Intake		Excretion in urine				
Day	Diet	Amount of ascorbic acid mg.	Total amount of urine ml.	Total amount of ascorbic acid mg.	Maximum output of ascorbic acid per hour mg.	Time taken to reach maximum hours	Minimum output of ascorbic acid per hour mg.
1st	Scorbutic	150	2545	111·2	12·8	5	1·7
2nd	,,	150	2470	105·0	17·3	4	1·6
3rd	,,	150	2625	105·7	16·3	4	1·5
4th	,,	150	2510	101·0	11·7	4	1·9
5th	Mixed	No dose taken, no urine collected					
6th	,,						
7th	,,						
8th	,,						
9th	Scorbutic	150	2055	183·7	21·6	4	3·5
10th	,,	150	1535	142·3	13·6	4	2·5
11th	,,	150	1795	109·8	15·2	4	1·8
12th	,,	150	2090	88·9	10·1	4	1·7
13th	,,	150	2155	98·7	9·4	4	1·8
14th	,,	150	2510	103·1	12·7	4	1·5
15th	,,	150	2310	103·8	12·8	4	1·5
16th	,,	150	2215	97·4	11·7	3	1·9
17th	,,	150	2360	101·6	14·4	4	1·4
18th	,,	150	2150	98·8	16·7	4	2·0

Table VII.

	Intake		Excretion in urine				
Day	Time dose taken	Ascorbic acid mg.	Time urine passed	Volume of urine ml.	Ascorbic acid per 100 ml. mg.	Total amount of ascorbic acid mg.	Ascorbic acid output per hour mg.
1st	5.15 p.m.	350	8.00 a.m.	780	20·8	162·5	12·2
			10.00	120	8·3	10·0	5·0
			12.00 noon	110	11·0	11·9	6·0
			2.30 p.m.	130	10·0	13·0	5·0
			4.30	70	21·7	15·0	7·5
			Total	**1210**	—	**212·4**	—
2nd	5.30 p.m.	350	6.30 p.m.	120	6·7	8·0	4·0
			8.00 a.m.	710	23·3	165·7	14·4
			10.00	100	13·3	13·3	6·0
			12.00 noon	120	11·7	14·0	7·0
			3.00 p.m.	190	6·7	12·7	4·2
			4.30	60	16·7	10·0	6·7
			Total	**1300**	—	**223·7**	—
3rd	5.00 p.m.	350	6.15 p.m.	120	21·7	26·0	14·8
			8.00 a.m.	780	21·7	169·0	12·3
			10.30	90	13·3	12·0	5·0
			1.30 p.m.	200	6·7	13·3	4·4
			Total	**1190**	—	**220·3**	—
4th	5.00 p.m.	350	Urine not collected on this day				
5th	4.30 p.m.	350	8.00 a.m.	920	16·7	153·3	10·0
			10.30	100	5·0	5·0	2·0
			12.30 p.m.	120	3·3	4·0	2·0
			Total	**1140**	—	**162·3**	—

Table VII (*cont.*).

	Intake		Excretion in urine				
Day	Time dose taken	Ascorbic acid mg.	Time urine passed	Volume of urine ml.	Ascorbic acid per 100 ml. mg.	Total amount of ascorbic acid mg.	Ascorbic acid output per hour mg.
6th	12.15 p.m.	150	2.30 p.m.	130	11·7	15·0	7·5
			6.15	170	28·3	48·0	12·8
			12.00	220	18·3	43·3	7·0
			Total for 1st 12 hours	**520**	—	**106·3**	—
	12.00 midnight	150	8.00 a.m.	220	43·8	96·3	12·0
			10.45	60	20·8	12·5	4·5
			12.00 noon	40	15·0	6·0	4·5
			Total for 2nd 12 hours	**320**	—	**114·8**	—
			Total for 24 hours	**840**	—	**221·0**	—
7th	12.15 p.m.	150	4.00 p.m.	105	45·8	48·1	12·0
			11.30	240	21·7	52·1	7·0
			Total for 1st 12 hours	**345**	—	**100·2**	—
	11.45 p.m.	150	8.00 a.m.	200	17·5	35·0	4·1
			11.45	130	18·3	24·0	6·4
			Total for 2nd 12 hours	**330**	—	**59·0**	—
			Total for 24 hours	**675**	—	**159·2**	—
8th	12.00 noon	150	3.00 p.m.	140	33·3	46·7	14·3
			5.30	110	29·1	32·0	12·8
			11.30	270	8·3	22·5	3·4
			Total for 1st 12 hours	**520**	—	**101·2**	—
	11.45 p.m.	150	8.00 a.m.	250	13·3	33·3	3·9
			10.00	90	11·7	10·5	5·2
			12.00 noon	165	8·3	13·8	7·0
			Total for 2nd 12 hours	**505**	—	**57·6**	—
			Total for 24 hours	**1025**	—	**158·8**	—
9th	12.15 p.m.	150	1.00 p.m.	100	12·5	12·5	12·5
			2.15	105	25·0	26·3	21·0
			4.15	100	46·7	46·7	46·7
			5.30	90	13·3	12·0	10·0
			11.30	250	8·3	20·8	3·5
			Total for 1st 12 hours	**645**	—	**118·3**	—
	11.45 p.m.	150	8.00 a.m.	300	16·7	50·0	6·0
			10.00	110	11·7	12·8	6·4
			11.30	120	13·3	16·0	10·0
			Total for 2nd 12 hours	**530**	—	**78·8**	—
			Total for 24 hours	**1175**	—	**197·1**	—
10th	11.45 a.m.	150	1.15 p.m.	150	11·7	17·5	10·0
			2.30	130	21·7	28·0	22·4
			4.30	130	30·0	39·0	20·0
			11.45	500	7·5	37·5	5·0
			Total for 1st 12 hours	**910**	—	**122·0**	—
	11.45 p.m.	150	8.00 a.m.	480	15·0	72·0	8·7
			10.00	110	11·7	12·8	6·4
			11.30	120	11·7	14·0	10·0
			Total for 2nd 12 hours	**710**	—	**98·8**	—
			Total for 24 hours	**1620**	—	**220·8**	—
11th	12.00 noon	150	**Total for 1st 12 hours**	**670**	**16·7**	**111·2**	**9·0**
	12.00 midnight	150	**Total for 2nd 12 hours**	**630**	**16·7**	**105·0**	**8·7**
			Total for 24 hours	**1300**	—	**215·2**	—
12th	12.00 noon	150	**Total for 1st 12 hours**	**770**	**15·8**	**122·0**	**10·4**
	12.00 midnight	150	8.00 a.m.	750	10·0	75·0	9·0
			10.00	100	10·0	10·0	5·0
			11.00	70	11·7	8·0	8·0
			12.00 noon	80	8·3	5·3	5·3
			Total for 2nd 12 hours	**1000**	—	**98·3**	—
			Total for 24 hours	**1770**	—	**220·3**	—

oxidation with hydrogen peroxide *per se* is slow and troublesome, a more convenient method, which is incidentally capable of very broad application, was therefore developed. A small quantity of iodine was used as the oxidising agent and this was regenerated from the hydriodic acid formed by the action of hydrogen peroxide, thus making it available for further oxidation. In the presence of excess of hydrogen peroxide this cycle of reaction is continuous. The following are details of the procedure used in the experiment. To the concentrated decitrated lemon juice, brought to p_H 3 with sulphuric acid, were added 1/50 of the amount of $N/10$ iodine necessary to oxidise the ascorbic acid and 1·5 times the theoretical quantity of N hydrogen peroxide. The mixture was allowed to stand at room temperature until the indophenol-reducing capacity of the juice had just disappeared. The doses used in these experiments took 1–1·5 hours to become oxidised.

S. S. Z., who was "saturated" with ascorbic acid, placed himself on a scorbutic diet as described above. The first day he took a dose of 150 mg. of ascorbic

Table VIII.

	Intake		Excretion in urine				
Day	Time dose taken	Dose mg.	Time urine passed	Volume of urine ml.	Ascorbic acid per 100 ml. urine mg.	Total amount of ascorbic acid mg.	Ascorbic acid output per hour mg.
1st	10.00 a.m.	150 Ascorbic acid	11.00 a.m.	235	3·3	7·8	3·9
			1.00 p.m.	300	6·7	20·0	10·0
			2.00	370	4·1	15·4	15·4
			4.00	140	6·7	9·3	4·7
			5.00	85	6·7	5·7	5·7
			10.00	230	5·5	12·7	2·5
			8.00 a.m.	1100	1·3	13·8	1·4
			10.00	280	2·0	5·6	2·8
			12.30 p.m.	305	2·1	6·6	2·6
			Total	**3045**	—	**96·9**	—
2nd	12.00 noon	250 Dehydro-ascorbic acid	2.00 p.m.	270	13·1	35·5	23·7
			3.00	170	10·0	17·0	17·0
			4.00	160	6·5	10·4	10·4
			5.00	300	4·0	12·0	12·0
			10.00	265	8·3	22·0	4·4
			8.00 a.m.	750	1·8	13·8	1·4
			10.00	250	2·7	6·7	3·9
			12.00	190	2·5	4·8	2·4
			Total	**2355**	—	**122·2**	—
3rd	12.30 p.m.	220 Dehydro-ascorbic acid	2.00 p.m.	210	17·0	35·7	17·9
			3.00	160	15·8	25·3	25·3
			4.00	520	3·1	16·4	16·4
			5.00	380	3·7	13·9	13·9
			10.00	260	9·3	24·3	4·9
			8.00 a.m.	860	3·1	27·2	2·7
			10.00	360	3·1	11·1	5·6
			12.00	160	4·3	6·9	3·5
			Total	**2910**	—	**160·8**	—
4th	12.30 p.m.		2.00 p.m.	280	3·3	9·3	4·7
			4.00	225	2·7	6·0	3·0
			5.00	120	2·8	3·4	3·4
			10.00	210	3·8	8·0	1·6
			8.00 a.m.	630	2·0	12·6	1·3
			10.00	310	1·25	3·9	2·0
			12.00	225	1·3	3·0	1·5
			Total	**2000**	—	**46·2**	—

acid, on the second and third days 250 and 220 mg. of dehydroascorbic acid were ingested respectively. No dose was taken on the fourth day. The excretion during the first 24 hours of 90 mg. of the 150 mg. of ascorbic acid ingested on the first day (Table VIII) shows that the subject was "saturated." On the second day, of the 250 mg. of dehydroascorbic acid ingested, 122 mg. were excreted in the reduced form and similarly on the third day an even higher proportion was excreted in the urine as ascorbic acid. On the fourth day, when no dose was taken, the total amount of ascorbic acid in the urine as well as the hourly excretion fell to a low level. Similar results which are given in an abridged form in Table IX were obtained with S. W. J. and A. E. K. on diets very low in vitamin C.

Table IX.

			Excretion in urine				
Subject	Day	Intake mg.	Volume of urine ml.	Total ascorbic acid mg.	Maximum output of ascorbic acid per hour mg.	Time taken hours	Minimum output of ascorbic acid per hour mg.
S. W. J.	1st	300 Ascorbic acid	1770	220·3	—	—	5·0
	2nd	360 Dehydroascorbic acid	1285	289·4	55·7	2·75	3·0
A. E. K.	1st	150 Ascorbic acid	990	96·0	—	—	—
	2nd	370 Dehydroascorbic acid	740	170·0	—	—	2·0
	3rd	320 Dehydroascorbic acid	950	205·7	34·3	3·5	1·1
	4th	—	1425	22·0	—	—	0·6

The indophenol-reducing capacity of several specimens of urine from the above experiments could not be raised by treatment with hydrogen sulphide, showing that no dehydroascorbic acid was present in the urine.

In these experiments also it was found by a biological test that the indophenol-reducing capacity of the urine was mainly due to the presence of ascorbic acid.

Discussion.

At the outset we are faced with the fact that there is no standard of normality as regards the urinary excretion of ascorbic acid by man. At one end of the scale are S. W. J., A. E. K., H. H. and the cases described by Hess and Benjamin, whose urines did not reduce indophenol to any significant extent for a number of days, even when "adequate" amounts of vitamin C were consumed daily. At the other end of the scale there is S. S. Z., who, because his intake of the vitamin was rather high, excreted in the urine as much as 70–150 mg. per day. The cases mentioned by Harris *et al.* apparently occupy an intermediate position. It is of course impossible at this stage of the inquiry to judge which of the three categories is the most prevalent, but that undoubtedly will depend on many factors, such as season of the year, individual habits, availability of antiscorbutic foods, *etc.*

The experiments show definitely that the store of ascorbic acid in the subject plays an important part in its urinary excretion. This fact also emerges from

Hess and Benjamin's work on children. Our results demonstrate that at least two factors are concerned with the appearance of ascorbic acid in the urine of normal individuals, the degree of "saturation" of the subject and the magnitude of the intake. When the store is low many days may elapse before a constant level of excretion, indicating "saturation," is reached if doses of the order of 150 mg. are taken. The time necessary to reach this condition can, however, be markedly shortened by ingestion of high doses (1000 mg.).

It is a striking fact that on discontinuing the dose when the subject is fully "saturated," the daily urinary output of ascorbic acid falls rapidly, reaching the initial low level in a little more than 24 hours. During this period of low output, the subject, however, remains "saturated" for some time. This fact, taken in conjunction with the observation that part of the ascorbic acid dose can be excreted in the urine even long before "saturation" is reached, makes it difficult to judge the degree of "saturation" of the subject from his level of urinary output taken at random. For example, S. W. J., who took daily about 120 mg. and excreted about 30 mg. (Table I) on each of several days, was not "saturated" since he did not reach his constant level of output until some time later. On the other hand, on discontinuing the dose a little later his urinary output fell within a few days even to a lower level, 6 mg. per day, yet he was "saturated" at the time, as is seen from the fact that a single dose of 180 mg. brought his output back to that of the "saturation" level (Table II). Further, when high doses are taken by an "unsaturated" subject, the daily urinary output may be very high while the subject is still in this condition. Such a case is illustrated by A. E. K. who, while still "unsaturated," returned 77 and 303 mg. on the second and third days respectively of the experiment (Table IV).

It must be particularly noted here that S. W. J., A. E. K. and H. H. existed for months on diets containing quantities of vitamin C which were sufficient to keep them in perfect health and apparently quite free from scurvy, yet their store of ascorbic acid was such that it took a number of days to bring about a condition of "saturation" on a daily dose of about 150 mg.

The results obtained with dehydroascorbic acid were so clear cut that they leave little room for comment.

The fate of the large quantities of ascorbic acid not accounted for in the urine when massive doses were ingested calls for further inquiry. At present it may be asserted almost with certainty that none of it was excreted in the urine as dehydroascorbic acid.

The exploratory experiments performed with the object of ascertaining whether under certain conditions such as complete "saturation" and a suitable dose, the balance between the intake and the urinary excretion of ascorbic acid could possibly be utilised as a means of determining the daily requirements of the vitamin were disappointing. Besides the experiments described, results obtained in this connection with other subjects (A. E. K. and H. H.) were equally unsatisfactory. It must, however, be pointed out that a final judgment may only be formed from work carried out on a greater number of subjects than we were able to employ.

During the process of this investigation we studied the effect of diuresis, induced by a high intake of water, on the urinary excretion of ascorbic acid. We found that it did not influence either the rate or the level of output. Although the quantities of liquid consumed are not given here, this fact can be appreciated by comparing the amounts of the ascorbic acid excreted with the corresponding volumes of urine passed in the various experiments.

Another point worthy of comment, which emerges from all the experiments described, is that although the rate of urinary excretion of ascorbic acid during the night was low it invariably increased soon after rising (period 8 a.m. to 10 a.m.).

SUMMARY.

The urinary excretion of ascorbic acid under normal conditions of existence is variable.

The output of ascorbic acid in the urine is conditioned by the amount stored in the body and by the quantity consumed in the diet.

When the store of ascorbic acid is complete, a more or less constant level of urinary excretion can be achieved, and this level varies with the magnitude of the daily quantity of ascorbic acid taken.

The percentage of ingested ascorbic acid excreted in the urine is lower when high doses are taken than when the doses are low. The actual amount of ascorbic acid not accounted for in the urine is therefore very much higher in the first than in the second case and is evidently not indicative of the real vitamin requirements of the individual. No dehydroascorbic acid is found in the urine when high doses are used.

On discontinuing the intake of ascorbic acid, a subject who is "saturated" may yet remain in this condition for some time despite the fact that his urinary excretion drops to a very low level.

When the subject is "saturated" or approaching "saturation" and a dose is taken, the rate of urinary excretion rapidly rises to a maximum in about 4 hours and then gradually returns to its initial rate in about 24 hours.

Diuresis induced by excessive intake of water does not influence either the rate or level of urinary output of ascorbic acid.

The rate of urinary excretion of ascorbic acid is low during the night and increases soon after rising.

When dehydroascorbic acid is ingested it appears in the urine as ascorbic acid and is excreted in the same way as the latter.

It is possible to exist on diets containing sufficient vitamin C to insure good health and freedom from scurvy even when the store of ascorbic acid in the body is maintained low.

It is shown by means of a biological test that the reducing capacity of the urine for indophenol in our experiments is due to ascorbic acid.

We wish to express out thanks to Mr A. E. Kellie and Mr H. Hutchins for kindly placing themselves at our disposal for experimental purposes. One of us (S. W. J.) is indebted to the Medical Research Council for a whole time grant.

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