II. OBSERVATIONS ON THE EXCRETION OF VITAMIN C IN HUMAN URINE.

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SINCE the chemical identification of vitamin C, and the development of a chemical method for its estimation, the excretion of this vitamin in human urine has attracted attention [Harris *et al.*, 1933; Johnson and Zilva, 1934; Harris and Ray, 1935]. The constant daily loss of 30–33 mg. of ascorbic acid in the urine of normal individuals as estimated by Harris and his colleagues did not appear to us quite in accord with accepted notions about the function and requirements of vitamins. It was conceivable that a biological fluid of such complex composition as urine should contain besides ascorbic acid, substances capable of reducing the indicator used in this estimation. Our doubts were further strengthened when a preliminary investigation, covering a period of a week, showed only half as much vitamin C in the daily food of an individual who was found to be excreting in his urine an amount similar to the estimate of Harris and his co-workers. We were, therefore, led to study the excretion of ascorbic acid in the urine and to explore the possibility of the presence of other reducing substances.

The daily output of the reducing substance or substances in human urine under normal conditions was estimated by the usual titration technique for a number of individuals. The results are expressed as mg. of ascorbic acid and are shown in Table I. For different persons the total daily excretion was found to be variable, but for the same person the day to day excretion was strikingly uniform. The level of output stood between 23 and 35 mg. in terms of ascorbic acid. These findings are in conformity with the results of Harris and his colleagues.

Table I. *The reducing capacity of normal urine.*

(Expressed as mg. ascorbic acid.)

Name of the subject	Days	Total volume of urine passed (ml.)	Conc. of reducing substance (mg./100 ml.)	Total reducing substance excreted (mg.)
B. A.	1	512	4.62	23.67
	2	594	4.26	25.39
	3	649	3.91	25.28
S. C. N.	1	549	4.41	24.11
	2	710	3.31	23.50
D. N. M.	1	980	3.47	34.03
	2	903	3.83	34.65
H. E. C. W.	1	641	4.64	29.77
H. D. A.	1	892	3.67	32.78
	2	1510	2.34	35.36
M. D. A.	1	1216	2.13	26.00
	2	1736	1.41	24.53

One of the subjects (B. A.) was then given large daily doses of ascorbic acid in the form of fresh juice of sweet limes and the reducing capacity of the urine was examined at short intervals. The results (Table II) mainly confirm the observations of Harris and his colleagues and of Johnson and Zilva [1934].

Table II. *The reducing capacity of urine after ingestion of large doses of ascorbic acid.*

(Expressed as mg. ascorbic acid.)

Days	Dose of ascorbic acid (mg.)	Total reducing substance excreted in 24 hours (mg.)	Days	Dose of ascorbic acid (mg.)	Total reducing substance excreted in 24 hours (mg.)
1	0	19.65	8	0	33.03
2	200	24.34	9	0	35.95
3	460	41.37	10	0	27.39
4	660	351.17	11	50	72.16
5	0	74.24	12	50	50.54
6	0	27.66	13	50	48.68
7	0	30.25			

The reducing capacity of urine did not increase until the so-called "saturation point" for the tissues was reached after repeated or increased doses of ascorbic acid. The excretion of ascorbic acid was then quite rapid, beginning within 2 hours after the dose, attaining a maximum rate of output after 4-7 hours and then slowly returning to the normal level at the end of 40 hours. Even after the "saturation point", the total excretion by no means approximates to the total intake. The reducing power of urine accounts for only about 50% of the total quantity taken. A week after attaining the "saturation point", a small dose of 50 mg. ascorbic acid was repeated. The response as increased excretion of reducing substances was immediate, showing that the tissues of the subject were still "saturated".

The excretion of reducing substances was now studied under different dietary conditions. Three different diets were first used.

I. A low vitamin C diet consisting of a preponderatingly large proportion of cereal and bread, and containing only small amounts of meat, milk, eggs and vegetables. This was the usual diet of the subject.

II. Low protein diet. A relatively large proportion of vegetables was included with moderate amounts of bread and cereal. Meat, eggs and milk were altogether eliminated from the diet. This diet contained nearly three times as much vitamin C as diet I.

III. High protein diet. This consisted largely of meat and fish together with commercial meat extracts (Lemco), and some bread.

The examination of the urine was begun after the subject had been on the diet for 3 days. The results are summarised in Table III.

During the course of each diet the daily output of the reducing substance was quite constant. On diet I it was about 25 mg. On diet II which had three times more vitamin C than diet I, the reducing capacity of the urine represented only 19-20 mg. ascorbic acid. On the high meat diet the daily excretion increased to 45-65 mg.

Since diet III included beef extracts which generally cause a greater excretion of purines, we suspected that substances of a purine nature might possibly be responsible for the increased reducing action of urine, although the possibility

Table III. *The reducing capacity of urine excreted under different dietary conditions.*

(Expressed as mg. ascorbic acid.)

Diet	Days	Vol. of urine passed (ml.)	Conc. of reducing substance (mg./100 ml.)	Total reducing substance excreted (mg.)	Total nitrogen (g.)	Approx. ascorbic acid ingested in food (mg.)
I	1	512	4.62	23.67	—	13.2
	2	479	4.38	21.00	9.05	13.4
	3	594	4.27	25.38	10.03	11.0
	4	655	3.90	25.53	9.94	14.1
	5	592	4.23	25.05	8.58	52.3
	6	649	3.89	25.26	9.36	—
	7	756	3.88	29.36	9.98	—
II	1	814	2.49	20.29	9.00	44.5
	2	787	2.50	19.71	6.87	113.4*
	3	643	3.08	19.83	6.92	40.6
	4	599	3.08	18.48	6.08	43.2
	5	544	3.61	19.65	5.87	—
III	1	670	7.27	48.71	10.84	19.0
	2	614	7.29	44.79	11.05	18.5
	3	572	11.29	64.62	10.70	18.5
	4	642	9.63	61.68	9.56	20.8
	5	620	7.99	49.57	10.55	18.5
	6	911	6.31	57.49	11.93	20.0
	7	675	6.62	44.73	10.36	20.0

* These high figures are due to the ingestion of a vitamin C-rich fruit.

that the metabolism of proteins by themselves may demand increased metabolism of vitamin C, could not be ignored.

A further extended study of the urine excreted during different diets was continued. In addition to the three diets mentioned above a fourth diet was also included.

IV. Purine-free, high protein diet, containing larger quantities of eggs and milk with small amounts of bread, vegetables and fruit. The results are shown in Table IV.

The results are essentially similar to those of previous experiments. The reducing value of the urine is low on diets I, II, and IV. It increases with a change to the high meat diet III. There is no increase with diet IV.

Table IV. *Analyses of urine excreted during different diets.*

(Reducing substances expressed as mg. ascorbic acid excreted in 24 hours.)

No. of days	Diet	Total volume of urine (ml.)	Total reducing substances (mg.)	No. of days	Diet	Total volume of urine (ml.)	Total reducing substances (mg.)
1	I	659	40.50	13	I	635	37.50
2	I	518	18.87	14	I	710	33.84
3	I	524	18.30	15	I	636	25.46
4	II	608	17.64	16	I	637	33.33
5	II	445	16.83	17	I	879	28.60
6	II	485	16.10	18	I	655	25.65
7	IV	720	15.46	19	I	585	21.20
8	IV	336	14.00	20	I	771	27.10
9	IV	512	16.25	21	III	331	62.10
10	III	721	151.15	22	III	801	66.37
11	III	802	88.21	23	III	806	81.34
12	III	804	266.37	24	III	795	64.34

The exceedingly high values of the reducing substance obtained on the 10th, 11th, and 12th days are not altogether due to a high meat diet. From the 8th to 11th days the subject took one "langra" mango every day. The very high values obtained aroused our suspicions about the mango and on examination it was found that this particular variety of the mango was exceedingly rich in vitamin C. The subject went over to diet I again and changed to diet III on the 21st day. The usual increase in the reducing capacity of urine was found to take place.

Biological test of urine.

It was decided to test the antiscorbutic value of urine excreted on diet III. A group of eight guinea pigs was put on the usual scorbutic diet. Four animals received every day two different doses of fresh urine equivalent in reducing capacity to 0.5 and 1.0 mg. ascorbic acid. The remaining four animals acted as controls. The results of the tests are summarised in Table V.

Table V. *The antiscorbutic value of urine.*

No. of animal	Dose of urine (ml.)	Ascorbic acid equivalent of the dose (mg.)	Total time of test (days)	Total change in weight (g.)	Average growth per week (g.)	Remarks
1	3.2-8.0	0.5	32	- 40	- 8.7	Died, p.m. scurvy
2	3.2-8.0	0.5	37	- 195	- 36.8	"
3	6.4-16.0	1.0	29	- 135	- 32.6	"
4	6.4-16.0	1.0	27	- 119	- 30.8	"
5	0	0	38	- 165	- 30.3	"
6	0	0	38	- 210	- 38.6	"
7	3 ml. of lime juice	1.0	38	- 10	- 1.8	Healthy, killed p.m. no scurvy
8	"	1.0	42	- 60	- 10.0	Healthy

All the four animals receiving a dose of urine died between the 27th and 37th days of the experiment, and the *post mortem* examination showed evidence of scurvy. The negative controls died of scurvy after somewhat longer time as compared with the animals receiving urine, which indicated a possible additional toxic effect of urine. The controls which were fed 3 ml. of lime juice remained quite healthy.

These experiments may appear to support the view that probably the whole of the reducing action of urine excreted during high protein diet, is not due to ascorbic acid. The toxicity of urine, however, is a factor which must be taken into consideration in these experiments. Even after a week's feeding of urine the animals began rapidly to lose weight and looked ill. It is more than likely that under these conditions the absorption and metabolism of ascorbic acid of the urine is interfered with.

The reducing substances in the urine were next studied from the point of view of their stability to heat and their reaction with lead acetate. A striking similarity in behaviour was revealed between the reducing substances of the urine and pure ascorbic acid under both these treatments. The results of one of these experiments are summarised in Table VI.

The rates of destruction of natural reducing substances in urine and of pure ascorbic acid in solutions of urine are evidently very close.

The experiments described above show that there is a definite rise in the reducing capacity of urine when the subject changes from a low or a moderate

Table VI. *The relative heat-stability of the reducing substances in urine and of ascorbic acid.*

(Reducing value expressed as mg. ascorbic acid.)

	Treatment	Reducing substance mg./100 ml.	% loss of reducing substance
I. Urine acidified with acetic acid Urine of diet III	Nil	8.88	0
	Boiled 5 min.	8.23	7.32
	Boiled 15 min.	6.35	28.48
	Boiled $\frac{1}{2}$ hour	5.55	37.49
II. A mixture of urine and pure ascorbic acid acidified with acetic acid	Nil	22.20	0
	Boiled 5 min.	20.40	8.60
	Boiled 15 min.	16.68	23.94
	Boiled 30 min.	14.28	35.64

meat diet to a high meat one. The quantity of meat consumed during high meat diet was 1–2 lb. per day supplemented with 0.5–1.0 oz. of lemco. The increase in the total daily excretion of the reducing substance is as much as two to three times the ordinary output.

The evidence taken as a whole appears to favour the view that the increased reducing action of urine is to a large extent due to increased excretion of ascorbic acid. The biological experiments on the antiscorbutic value of urine are not conclusive on account of the complicating effect of the toxicity of the urine. On the other hand the general behaviour of the reducing substance resembles very much that of ascorbic acid.

If a high meat diet favours a rapid elimination of ascorbic acid by way of urine, we have here a possible explanation of the high incidence of scurvy among sailors during the earlier days. Their diet was chiefly salted meat and ship biscuits, which would quickly deplete their tissues of their stores of vitamin C.

SUMMARY.

The reducing capacity of human urine excreted under different dietary conditions has been studied. There is a considerable amount of increase in the reducing action of urine on high meat diets without any increase in the vitamin C content of the food.

The possibility that urine under these conditions may contain besides ascorbic acid other substances capable of reducing dichlorophenolindophenol in acid solution has been examined. The evidence presented is generally in favour of the view that even on high meat diets the greater part of the reducing substance of urine is ascorbic acid. It appears that high meat diets favour the excretion of vitamin C by way of urine.

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REFERENCES.

- Harris and Ray (1935). *Lancet*, i, 71.
 ——— and Ward (1933). *Biochem. J.* **27**, 2011.
 Johnson and Zilva (1934). *Biochem. J.* **28**, 1393.