7349 C

Urinary Excretion of Vitamin C.

ALFRED F. HESS* AND HELEN RIVKIN BENJAMIN.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

Many authors have demonstrated that vitamin C exists in various organs and fluids of the body. Moreover, it has been shown that in the guinea pig the concentration of vitamin C in the body is dependent upon the intake,[1-4] and that in the tissues of the guinea pig the vitamin C concentration is lower than in species which are not dependent upon a dietary source of this vitamin.[3] In view of such evidence, it was thought worth while to investigate the urinary excretion of vitamin C. The experiments were planned to throw light upon the following questions: 1. Is vitamin C excreted by the urinary route? 2. Is the level of urinary excretion proportional to the intake or is it a measure of excess vitamin ingestion? 3. Is there a difference in urinary excretion between species which do and those which do not require a dietary source of the vitamin?

As far as we are aware, very little work on this subject has been reported. von Euler and Malm[5] state that they were unable to detect vitamin C in normal, febrile or diabetic urine. On the other hand, van Eekelen and his associates[6] found that urinary excretion ran parallel to intake, but since these authors do not give any figures, no comparison between species can be made on the basis of their work.

METHOD

The method employed was that of Birch, Harris and Ray,[7] based on the titration of ascorbic acid against 2:6 dichlorophenol-indophenol. Since urine is known to contain only traces of the compounds (glutathione and other sulphydryl compounds) which

* This work was completed after the death of Dr. Alfred F. Hess.

1 Svirbely, L. J., and Szent-Györgyi, A., *Biochem. J.*, 1933, **27**, 279.

2 Harris, L. J., and Ray, S. N., *Biochem. J.*, 1933, **27**, 303.

3 Bessey, O. A., and King, C. G., *J. Biol. Chem.*, 1933, **103**, 687.

4 Siehrs, A. E., and Miller, C. O., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 696.

5 von Euler, H., and Malm, M., *Arch. f. Kemi. Min. Geol.*, 1932, IIB, 6, 7, 8, 9.

6 van Eekelen, M., Emmerie, A., Josephy, B., and Wolff, L. K., *Nature*, 1933, **132**, 315.

7 Birch, L. W., Harris, L. J., and Ray, S. N. *Biochem. J.*, 1933, **27**, 590.

form the chief source of error in the application of this method, it seemed safe to consider any reducing substance found in urine to be ascorbic acid.

The dye solution was freshly prepared every second day and was standardized against a CCl_3COOH solution of ascorbic acid. Through the kindness of Dr. Szent-Györgyi, the latter was supplied to us in the form of the commercial preparation "cevita". This was found to be an exceedingly convenient source, since it consists of tablets, each of which contains 5 mg. ascorbic acid. The accuracy of these was checked periodically against some pure crystalline ascorbic acid which Dr. King was good enough to give to us.

Since vitamin C was found to be exceedingly unstable in alkaline solution† the urine was collected in bottles containing several drops of glacial CH_3COOH. The urine was then mixed with 20% CCl_3COOH in proportions to make the final concentration of the latter about 5%, filtered and titrated, usually against 0.02 cc. dye solution. Since the urine solution was always colored, it was necessary, in carrying out the titration, to use 2 similar tubes, one containing dye and the other a blank, containing only urine, to the color of which the end point was matched.

This method proved to be highly satisfactory for urines containing large amounts of vitamin C, but was very much less accurate for the more dilute specimens. Attempts to concentrate the vitamin by evaporation of the urine or by precipitation of the ascorbic acid with basic lead acetate, led to losses in potency and were abandoned. All of the urine, CCl_3COOH filtrates, including the dilute ones, were, therefore, used directly for titration. The average was taken of 3 to 6 titrations on each urine. Since the differences here reported, which were produced by administration of vitamin C, are of the order of 1000%, the relative lack of accuracy, even in the most dilute urines (a maximum of about 50%), is not sufficient to interfere with the interpretation.

When ascorbic acid was added *in vitro* to CCl_3COOH urine filtrates, it could be recovered to the extent of 78 to 105% (av. 87%). The more concentrated the solution, the more complete was the recovery.

EXPERIMENTS ON CHILDREN

The experiments were divided into several periods representing

† If the solution was made faintly alkaline, its reducing power was completely lost after standing overnight in the icebox. This was true of urine as well as of solutions of pure ascorbic acid and of orange juice. Reacidification at this point did not restore the reducing power.

different levels of vitamin C intake. The urine was collected in 24-hour samples and examined daily.

In Table I, a typical record is presented on one of the 7 children studied. It is at once apparent that under ordinary dietary conditions, the amount of vitamin C present in human urine is so small as to be considered entirely negligible. The concentration was about one-fortieth of the lowest value found by Bessey and King[3] in a human organ (heart). This was true whether the vitamin was entirely excluded from the diet or whether it was fed in adequate amounts, either in natural foods (1 oz. orange juice daily and the usual amounts of fruits and vegetables) or as pure ascorbic acid (5 "cevita" tablets daily, containing 25 mg. ascorbic acid or the equivalent of about 1½ oz. orange juice). The level of urinary excretion of vitamin C, therefore, appears not to be proportional to the dietary supply. Only when excessively large doses of vitamin C were fed (1 pt. orange juice daily) did appreciable urinary excretion occur. Two characteristic features appear in the table in connection with the rise. It did not occur until the fourth day of high vitamin feeding, and when it did occur, the increase was abrupt and very marked. The highest concentration was usually reached on the fifth day, after which a slight reduction often occurred in spite of

TABLE I.

Effect of Vitamin C Feeding on Urinary Excretion in a Child.

S. B.—3 years old—31½ lb.

Date	Vitamin C Fed*	Ascorbic Acid in Urine	
		per cc.	per 24 hr.
		mg.	mg.
1-12	Normal amount	.001	—
13	" "	.002	.55
14	" "	.002	.84
15	None	.002	1.08
16	"	.002	1.15
17	"	.001	—
18	1 pt. orange juice	.003	2.18
19	" " "	.001	—
20	" " "	.003	2.13
21	" " "	**.023**	**13.31**
22	" " "	.101	58.90
23 } 24 }	" " "	.032	15.50
25	" " "	.050	30.70
26	None	**.002**	**1.18**
27	1 pt. orange juice	**.045**	**18.38**
28	" " "	.031	14.05
29	" " "	.021	16.01
31	" " "	.062	49.40
2-1	" " "	.085	60.80

* Fed during 24 hrs. preceding collection of urine.

continued feeding. The highest value which we observed in this study was 0.188 mg. ascorbic acid per cc. urine or a total excretion of 115 mg. in 24 hours. The intake, in a pint of orange juice, was approximately 275 mg. ascorbic acid.

When the vitamin C feeding was stopped, an immediate reduction in urinary excretion must have occurred, since in the sample for which the collection was begun 24 hours after the last feeding, the concentration of ascorbic acid had already returned to the original negligible level.‡

This observation, coupled with that of the initial lag in urinary excretion following high dosage, and with the finding of negligible amounts of vitamin C in the urine when smaller, but adequate amounts of the vitamin were fed, suggest the explanation that vitamin C in the urine represents an excess which appears only after complete saturation of the tissues has occurred. This hypothesis is also supported by further data obtained on 3 children. In these, after the level of urinary excretion of vitamin C had reached a high value, the orange juice feeding was stopped for only one day and then resumed. The result may be seen in the table (2/26 and 2/27). An immediate rise in urinary excretion followed the resumption of the orange juice feeding. It would appear, therefore, that when the tissues are thoroughly saturated with vitamin C, no lag occurs in the urinary response to feeding of large amounts of the vitamin.

Of the 7 children studied, 2 deviated from the typical course outlined above. In one of these, after a pint of orange juice had been fed daily for 7 days, the urinary content of vitamin C was still not increased. The experiment was stopped at this point because the child became ill with an infection. In the second atypical case, the level of urinary excretion rose as usual, 4 days after the orange juice feeding was begun. When the orange juice was stopped, the drop in urinary excretion was gradual, requiring 3 days to return completely to the "normal" level. Orange juice feeding was resumed at this point and the rise in urinary excretion of the vitamin was similarly gradual, taking 2 days to appear and continuing to mount for the next 3 days. In this instance, it is probable that the continued excretion of the vitamin, after the feeding was discontinued, led to a depletion of the tissue stores, which were first replenished before the excess vitamin appeared in the urine.

‡ In instances in which this period of the experiment was prolonged, the urinary vitamin C remained at the same low level.

EXPERIMENTS ON OTHER SPECIES

Similar studies were also made on guinea pigs, rabbits and rats. The orange juice was fed by pipette to the guinea pigs, and rats and was given by stomach tube to the rabbits. These 3 species showed identical reactions, as regards urinary excretion of vitamin C, which, however, differed from those exhibited in the human series. The data are summarized in Table II. When no vitamin C was fed, the initial concentration in the urine of guinea pigs, rabbits and rats was approximately the same (about 5 to 10 times as high as that in human urine) and the total excretion in 24 hours was proportional to the total volume of urine. Moreover, even when very large doses of vitamin C were fed, no corresponding increase in urinary concentration resulted. This was true when the vitamin C was fed as orange juice; as orange juice + HCl (to insure acidity of the urinary tract); or as ascorbic acid ("cevita" in H_2O or in acid). As regards the urinary excretion of vitamin C, it would, therefore, appear that the human species is different from the others studied, but that no distinction exists between species (guinea pig and human) which do, and those (rabbit and rat) which do not, require a dietary source of the vitamin.

In the case of the guinea pig, no difference was observed between normal animals and those which had moderate scurvy, either as to initial vitamin C concentration in the urine, or urinary response to vitamin C ingestion.

TABLE II.
Effect of Vitamin C Feeding on Urinary Excretion in Various Species.

Species	Daily Intake Vitamin C	Aver. Ascorbic Acid in Urine per cc.	Aver. Ascorbic Acid in Urine per 24 hr.	No. Determinations
		mg.	mg.	
Guinea pig	None (oats)	.014 ± .0010*	.146 ± .0400*	13
	Normal (carrots, lettuce)	.007 ± .0006*	.300 ± .0237*	16
	Excessive (10-60 cc. orange juice)	.005 ± .0005*	.167 ± .0579*	23
Rabbit	None ("Purina Chow")	.021 ± .0023*	1.950 ± .0607*	18
	Excessive (10-80 cc. orange juice)	.034 ± .0033*	2.670 ± .2630*	18
Rat	None (McCollum Diet)	.012 ± .0026*	.147 ± .0427*	7
	Excessive (10-20 cc. orange juice)	.010 ± .0009*	.062 ± .0084*	8
Human (Children)	None (No fruits or vegetables)	.002 ± .0002*	1.27 ± .1300*	29
	Normal (1 oz. orange juice)	.002 ± .0003*	.984 ± .1017*	21
	Excessive (1 pt. orange juice)	.058 ± .0066*	26.23 ± 2.84*	41

* Probable error.

When vitamin C in large quantities similar to those which had been fed (6 to 8 "cevita" tablets in H_2O) was injected intraperitoneally into guinea pigs, or rabbits, a sharp rise (3 to 20 fold) in urinary excretion occurred, usually on the second or third day of injection. When the injections were stopped, the reduction in urinary concentration was often quite gradual. These experiments suggest that in these species a certain amount of destruction of vitamin C occurs in the digestive tract, or that the degree of intestinal absorption of the vitamin is limited.

Conclusions. It would appear that under ordinary nutritional conditions, vitamin C is not excreted in appreciable amounts in human urine and is excreted only in very low concentration in the urine of guinea pigs, rabbits and rats. In the human species (children) ingestion of excessively large amounts of vitamin C leads to urinary excretion after the body stores have become completely saturated. In the other species, urinary excretion can be raised only by injection of the vitamin. There is no difference in urinary excretion of vitamin C between species which do and those which do not require a dietary source of the vitamin.§

7350 P

Studies in the Repair of Bone.

WILBERT H. McGAW. (Introduced by Claude S. Beck.)

From the Laboratory of Surgical Research, the School of Medicine of Western Reserve University and the Lakeside Hospital.

The supremacy accorded the periosteum in osteogenesis has led to an almost complete neglect of the rôle played by other bone elements. Work on epiphyseal tissue has indeed been very scant, but there is both clinical and experimental evidence that cancellous bone plays an important rôle in bone repair, while the osteogenic properties of bone marrow and endosteum though often mentioned have been little studied.

Epiphyseal Tissue Transplants. A set of experiments were car-

§ Since the completion of this paper, an article has appeared by L. J. Harris, S. N. Ray, and A. Ward in the *Biochemical Journal* (Volume 27, page 2011) entitled "The Excretion of Vitamin C in Human Urine and Its Dependence on the Dietary Intake". The authors conclude that in adult urine after the ingestion of a single very large dose of orange juice, the urinary excretion of vitamin C rises to a maximum which is reached after three hours.