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A RAPID METHOD OF DIFFERENTIATING CHILDREN WITH LARGE OR SMALL RESERVES OF VITAMIN C*

BY

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Previous tests of adequacy of vitamin C storage based on urine analysis have in general been unsuitable for wide use on subjects other than hospital patients owing to the desirability of testing a twenty-four-hour specimen. Abbasy and Harris (1937a) suggested a modification of their original method in which three-hour specimens were tested on two successive days. On the third day, and the fourth if necessary, they gave a test dose of ascorbic acid, and the urine passed four to seven hours after was analysed. The present experiments were carried out to discover if the measurement of the concentration of vitamin C in a single specimen of urine passed four hours after a test dose of ascorbic acid would reveal reliable information regarding the vitamin C reserves of the subject tested.

Experiment on Concentration of Vitamin C in Urine of Normal Adult

The first of the two experiments was designed to show the variations in concentration of vitamin C in the urine from specimen to specimen and from day to day in a normal healthy adult male before and after a test dose of ascorbic acid. This subject (J. P.) had a diet which included fresh fruit at least once a day and which contained on the average approximately 70 mg. of ascorbic acid daily. This figure was derived from an analysis of a detailed record of the diet for one week. Each specimen of urine passed during a period of seventeen days was measured and its concentration of vitamin C determined. On the ninth day a test dose of ascorbic acid—700 mg.—was taken by mouth. The data derived from this experiment are summarized in Tables I and II and in Chart 1. It will be seen that the average concentration of vitamin C in the specimens before the test dose was 1.72 mg. per 100 ml. In 76 per cent. of these specimens the concentration lay between 0.76 and 2 mg. per 100 ml. When the concentration rose to levels of 3 to 6 mg. per 100 ml. extra quantities of fruit had been consumed. That the concentration of vitamin C is not directly dependent on the volume of the specimens is shown in Table II. The effect of a large test dose of ascorbic acid (70 mg. per stone of body weight) on the concentration of vitamin C in subsequent specimens of urine is shown in Chart 1.

* This research was carried out in connexion with the Carnegie United Kingdom Trust dietary and nutritional survey.

TABLE I.—The Excretion of Vitamin C by an Adult Male Weighing 10 st. on a Daily Intake of Approximately 70 mg. and the Response to a Test Dose of 700 mg. Ascorbic Acid

Duration of observation period	17 days
Number of specimens of urine passed	114
.. before test dose (8 days)	55
Average daily urinary excretion	1,495 ml.
Range in volume of specimens	40 to 520 ml.
Average concentration of vitamin C in specimens before test dose of ascorbic acid	1.72 mg. per 100 ml.
Range in concentration of vitamin C in specimens before test dose	0.76 to 6.5 mg. per 100 ml.
Total urinary excretion, showing the diuretic effect of ascorbic acid:	
On day of test dose	2,140 ml.
On day following test dose	1,980 ml.
Average total daily excretion of vitamin C before test dose (8 days)	22.8 mg.
Range of total daily excretion of vitamin C before test dose	13.8 to 29.1 mg.
Maximum concentration reached 6 hours after test dose	27.7 mg. per 100 ml.
Total amount of vitamin C excreted in 12 hours following test dose	245.1 mg.
Percentage of test dose excreted in 12 hours following administration	35%

TABLE II.—Showing Lack of Relation between Volume of Specimen and Concentration of Vitamin C

Time	Volume of Specimen (ml.)	Concentration of Vitamin C (mg. per 100 ml.)
1st day:		
8.30 a.m.	485	1.23
9.45 a.m.	55	1.21
12.30 p.m.	155	1.49
1.45 p.m.	75	1.63
4.30 p.m.	175	1.84
6.0 p.m.	110	1.41
8.0 p.m.	90	2.83
11.15 p.m.	580	1.43
2nd day:		
8.30 a.m.	400	1.94
12.30 p.m.	110	2.11
2.0 p.m.	90	1.85
4.50 p.m.	215	1.85
6.0 p.m.	175	0.89
7.0 p.m.	145	0.77
9.30 p.m.	460	1.91
10.0 p.m.	95	4.67

CHART 1.—Showing the variations in concentration of vitamin C in successive specimens of urine in a male adult before and after a test dose of ascorbic acid.

Following the marked increase in concentration of vitamin C in the urine on the day of the test dose the concentration fell rapidly, and on the third day after the test dose the concentration in five out of six specimens was less than 2.3 mg. per 100 ml. By the eighth day all the concentrations were less than 2 mg. per 100 ml., and the total daily excretion was 19.9 mg. During the week following the test dose increases up to 7 mg. per 100 ml. occurred on several occasions after the consumption of small amounts of fruit, showing a high degree of saturation in the tissues.

Method of Analysis Used

The method of analysis employed in determining the concentration of vitamin C in the urine was the same as that described by Harris and Ray (1935). Each specimen

of urine was acidified with glacial acetic acid to 10 per cent. by volume and was stored in the dark. The urine was titrated into a freshly prepared solution of 2:6-dichlorophenol-indophenol, 5 ml. = 0.1 mg. ascorbic acid, and the end point, decoloration, was reached within two minutes. All specimens were analysed within twenty-four hours and the average of two titrations was taken in each case.

The method has been criticized by Van Eekelen and Heinemann (1938) on account of the presence of substances other than vitamin C in the urine which reduce the dye and which are not removed by acidification. These substances—cysteine, ergothionine, and thiosulphate—amount to a daily total of approximately 3 to 6 mg. in the urine of a normal adult, whereas the total reducing substances, including vitamin C, amount to about 20 mg. on the average (Abbasy, Harris, Ray, and Marrack, 1935). Thus, reducing substances other than vitamin C may account for up to 30 per cent. of the reduction process in a normal subject; but following a test dose of ascorbic acid to which there is a marked response the proportion of these other substances to the total will fall to about 1 per cent. In subjects on a high protein diet and in sufferers from tuberculosis, peptic ulcer, or diabetes, the relation of vitamin C in the urine to the total reducing substances is very variable (Van Eekelen and Heinemann, 1938), but in other subjects the relation is fairly constant, vitamin C accounting for more than 60 per cent. of the reducing substances. The method may therefore be considered reliable enough for the routine testing of subjects other than sufferers from organic disease.

Conclusions from Experiment

The first experiment showed that the average concentration of vitamin C in the urine of a subject on a moderately high intake (approximately 70 mg. per day) was 1.76 mg. per 100 ml., and that the concentration in a given specimen bore little relation to its volume but a definite relation to previously eaten fruit. In specimens passed between four and eight hours after the test dose of ascorbic acid there was a great increase in the concentration. The maximum concentration of 27.7 mg. per 100 ml. was reached six hours after the test dose. In the twelve hours following the test dose 245.1 mg. of ascorbic acid was excreted—that is, 35 per cent. of the test dose.

It was thought likely, on the basis of the above data, that a subject whose diet contained moderate amounts of fresh fruit and vegetables would respond to a test dose of ascorbic acid by yielding a high concentration of vitamin C in a specimen of urine passed four to eight hours after a test dose. Further, if this overflow of ascorbic acid from the tissues into the urine occurred it might be presumed that the tissue requirements of vitamin C were being met. If, on the other hand, the administration of a massive dose of ascorbic acid produced no increase in concentration of vitamin C in the urine it would suggest that the tissues were depleted of vitamin C.

Tests in Two Groups of Boys

To verify this hypothesis a test dose of ascorbic acid was given to a group of sixty-two boys between the ages of 10 and 14 years, living in an institution for orphans and destitute children where the diet was known. During the year immediately preceding the experiment half of these boys had been receiving additional dietary supplements, including a daily orange, as part of the Carnegie United Kingdom dietary and nutritional survey. The diet of the supplemented group contained on the average approximately 63 mg. of vitamin C per head per day; while that of the group on the basic orphanage diet contained approximately 35 mg.

The estimates of vitamin C in the diets are derived from analyses of a detailed record of these diets for one week. This record refers to the gross quantities of uncooked food before it was supplied to the children. The actual quantities of vitamin C consumed by the children were probably somewhat less than the figures given.

In the experiment groups of six boys were tested at a time. They were instructed to pass their urine at 9.15 a.m. After they had micturated they were given a test dose of ascorbic acid (50 mg. per stone of body weight). They were told to pass their urine at the break (11.15 a.m.), and then not again until they returned at 1.15 p.m. They were also told not to drink more than a cupful of water before they returned. These precautions were taken to ensure that the 1.15 p.m. specimens would not be unduly diluted by pre-test-dose urine or by water drunk in the meantime. Estimations of the concentration of vitamin C in the 9.15 a.m. and 1.15 p.m. specimens were made. At the time of testing it was not known which boys belonged to the supplemented group and which to the unsupplemented. The estimations of the concentration of vitamin C in the post-test-dose urine, however, with one exception clearly differentiated the two groups (Table III and

TABLE III.—*The Response to a Test Dose of Ascorbic Acid of Boys on Low and Moderately High Intakes of Vitamin C*

Basic Diet				Supplemented Diet			
Boy	Concen- tration of 9.15 a.m. Specimen	Concen- tration of 1.15 p.m. Specimen	Vol. ml. 1.15 p.m. specimen	Boy	Concen- tration of 9.15 a.m. Specimen	Concen- tration of 1.15 p.m. Specimen	Vol. ml. 1.15 p.m. specimen
	mg. per 100 ml.	mg. per 100 ml.			mg. per 100 ml.	mg. per 100 ml.	
A. W.	1.2	1.1	—	A. Mc.	1.8	44.4	—
G. G.	2.3	1.5	—	M. Mc.	1.5	44.4	—
R. R.	1.1	1.3	200	A. Mc.	2.8	55.5	—
D. H.	0.7	0.9	340	A. M.	2.4	22.2	—
A. F.	1.1	0.7	135	W. R.	1.4	37.0	100
N. G.	0.9	0.7	70	E. M.	0.8	24.7	120
A. D.	0.7	1.9	30	A. Mc.	1.6	27.7	210
A. W.	1.4	0.7	400	G. V.	1.1	55.5	130
I. K.	0.8	0.7	100	J. C.	1.3	13.1	70
A. M.	1.0	1.3	265	J. S.	1.6	20.2	365
E. W.	1.2	0.8	30	W. G.	—	22.2	255
T. Mc.	0.7	0.5	200	J. W.	—	37.0	50
R. R.	1.3	0.7	30	J. L.	1.6	24.7	55
F. B.	0.7	1.0	195	D. D.	1.9	44.4	235
G. P.	1.0	0.8	240	W. J.	1.9	22.3	130
J. D.	(1.8)	(20.2)*	215	J. H.	1.5	14.8	500
N. T.	1.8	2.6	30	R. G.	0.8	44.4	40
H. R.	0.8	1.2	100	J. S.	1.0	44.4	120
P. F.	1.0	1.1	350	T. M.	1.6	27.7	160
O. D.	1.4	2.0	90	D. W.	—	44.4	20
		(3 p.m.)					
T. C.	1.2	2.1	95	W. Mc.	2.4	44.4	30
		(3 p.m.)					
R. G.	1.5	1.2	50	J. R.	1.8	17.1	270
R. Mc.	1.8	1.5	90	H. F.	1.3	20.2	160
C. B.	—	0.6	30	A. B.	—	19.8	50
R. S.	—	1.2	30	R. G.	0.7	55.5	130
S. G.	1.0	0.6	80	W. Mc.	—	74.0	30
D. S.	0.7	0.8	16	R. R.	—	15.8	140
C. S.	0.4	0.6	340	P. S.	—	22.2	80
T. G.	0.7	0.5	100	M. D.	3.2	44.4	80
A. R.	0.5	0.5	290	G. L.	2.1	37.0	110
J. T.	1.2	1.0	230	F. C.	—	31.7	30
Avg.	1.00	1.07			1.66	34.0	

* This boy was later found to have an unofficial source of dietary supplements.

Chart 2). The exception, J. D., gave a result consistent with that of the supplemented boys, though he was on the basic orphanage diet. It was found later, however, that he had a special privilege which gave him access to unofficial dietary supplements.

The average concentration of the 1.15 p.m. specimens in the basic group, excluding J. D., was 1.07 mg. per 100 ml., with a range of from 0.5 to 2.6, and was not significantly different from that of the 9.15 a.m. specimens (1.00 mg. per 100 ml., with a range of 0.4 to 2.3). In the supplemented group the average concentration of the 1.15 p.m. specimens were 34.0 mg. per 100 ml., with a range of from 13.1 to 74.0; while in the 9.15 a.m. specimens the average was 1.66 mg. per 100 ml., with a range of 0.7 to 3.2.

Although the average concentration of vitamin C in the 9.15 a.m. specimens was slightly higher in the supplemented group than in the basic group the difference was clearly not sufficient to differentiate the two groups. The test dose of ascorbic acid, however, made the distinction

CHART 2.—Specimens of urine passed four hours after a test dose of ascorbic acid. □ = boys on high vitamin C intake. ■ = boys on low vitamin C intake.

clear. The table of results also shows that variations in the volume of the specimen passed four hours after the test dose do not affect the general validity of the test. The lowest concentration observed in the post-test-dose specimens of the supplemented group was five times as high as the highest concentration in the post-test-dose specimens of the basic group.

Vitamin C Storage

Further observations were made to determine the difference in regard to vitamin C storage in the supplemented and basic groups. Six of the boys in the latter group were given successive daily test doses. After three test doses (900 mg.) two had reached the "supplemented" level of response, and after four doses (1,200 mg.) three of the remaining four had reached this level. This indicates that if a child is on a low vitamin C intake a considerable amount of the vitamin would be required to bring it up to an optimal or near optimal level of response. An occasional extra allowance of fruit, even if continued for several days, would not produce a good response to the test.

Therefore, if a child responds to a test dose of ascorbic acid by yielding a marked increase in the concentration of vitamin C in the urine, it may be assumed that he has been on an adequate intake of vitamin C for a considerable time.

Discussion

The optimal daily requirement of vitamin C for children and adults is not known. Approximate minimum daily intakes below which signs of scurvy appear have, however, been determined. Göthlin (1931) found that in schizophrenics who had existed for some time on a diet devoid of vitamin C the capillaries were abnormally fragile and that it required an intake of orange juice equivalent to 21 to 30 mg. of ascorbic acid to restore the capillary fragility to normal. Recalculating these requirements and using additional data from animal experiments, Göthlin (1937) obtained values of 19 to 27 mg. for an individual weighing 60 kg. Abbasy and others (1935) suggest a daily intake of 25 mg. as the

"minimal-optimum" requirement. This amount is also said to correspond to the ration of lemon juice that was required to prevent scurvy in sailors (Harris and others, 1936). Smith (1938), reviewing the literature on the human requirements of vitamin C, concludes that "values of 40 mg. for children may be considered the middle or barely adequate consumption level with but little margin of safety or allowance for individual variations in requirement."

Conclusions

The vitamin C intake of the boys in the basic group, which was estimated to be 35 mg. daily before cooking, approached the minimum physiological requirements as determined by Göthlin for adults. The intake of the boys in the supplemented group, estimated at 63 mg. daily, was above Smith's estimate of adequacy (40 mg. for children). The two groups may be considered to represent a low and an adequate level of consumption. It was found that the boys on the lower intake did not respond to a test dose of ascorbic acid. The boys on the higher intake, however, yielded a high concentration of vitamin C in the urine following a test dose of ascorbic acid. It may be presumed, therefore, that there was no marked lack of vitamin C in the tissues of the latter. Further, it may be concluded that a daily orange added to a diet containing approximately 35 mg. of vitamin C will in time produce a concentration in the tissues which is near saturation point. It is suggested that a child which responds to a test dose of ascorbic acid (50 mg. per stone of body weight) by yielding a marked increase in the concentration of vitamin C in the urine passed four hours after is receiving an adequate amount of vitamin C in its diet. If there is no response it may be inferred that the vitamin is being taken up to be stored and/or utilized, and that there is a lack of vitamin C in the tissues. Whether this degree of lack is harmful it is impossible at this stage to say. It is known, however, that in certain infective illnesses—for example, acute rheumatism, osteomyelitis, and tuberculosis—the utilization of vitamin C is increased (Abbasy, Harris, and others, 1936, 1937b, 1937c). Vitamin C is also believed to be necessary for the maturation of the erythrocyte (Witts, 1932). A child should not be considered adequately nourished, therefore, unless there is an abundance of vitamin C in the tissues, as judged by the overflow after a test dose of ascorbic acid.

Summary

Observations are recorded, before and after a test dose of ascorbic acid, on the variation in concentration of vitamin C in the urine of an adult male whose daily intake of vitamin C was approximately 73 mg.

A simplified test, based on the estimation of vitamin C in a single specimen of urine following a test dose of ascorbic acid, is described for assessing the past vitamin C intake.

The test clearly differentiated two groups of boys whose daily vitamin C intakes were approximately 35 mg. and 63 mg.

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