# EXCRETION OF VITAMIN $B_6$ IN THE URINE OF A HEALTHY INDIVIDUAL

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From our own and from published observations, it would appear that increasing ascorbic acid intake normally causes an increased amount of it to be excreted in urine collected over a definite period of time in the morning, before food is taken.

The object of the present investigation was to study the effect of an increased percentage of ascorbic acid in the food on the excretion of vitamin  $B_6$ , as determined from the amount of 4-pyridoxic acid in the morning urine collected before food was taken.

#### METHOD

The observations were made on 38 men aged 19-25 years. They lived under the same conditions and took the same food.

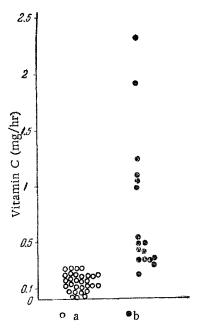


Fig. 1. Excretion of vitamin C in the morning urine before taking food.
a) Before vitamin treatment; b) by the 49-51st day after taking 100 mg of ascorbic acid daily.

During the whole period, they were kept on a standard diet whose constituents were not varied from day to day. The daily ration contained an average of 115 g of protein (including 44 g of animal protein), 79 g of fats, and 614 g of carbohydrates. The calorific value was 3,721 calories. The calculations were made from tables of the chemical composition of the food product [2].

The ascorbic acid content of the prepared food was found to vary from 0.6 to 19.5 mg, and had an average

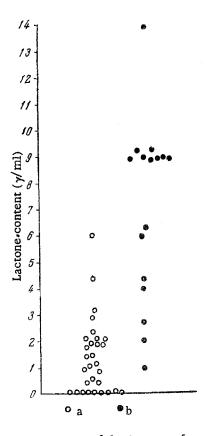


Fig. 2. Amount of the lactone of 4-pyridoxic acid. a) Before taking vitamin C; b) on the 49-51st day after taking 100 mg of ascorbic acid daily.

value of  $8.8 \pm 0.6$  mg per day. Measurements of fruit and vegetable dishes were made by the simplified method using the All-Union State Standard (GOST 7047-55).

The urine was collected at a definite period before food was taken, but after the night urine had been voided. Measurements were made of the amount of ascorbic acid and of the lactone of 4-pyridoxic acid: For the first determination, the urine was titrated in the presence of acetic acid with 2,6-dichlorophenolindophenol and for the second, the fluorometric method [3] of Huff and Perlzweig was used. For this purpose 1 ml of urine was poured into each of two test tubes, and hydrochloric acid was added. One tube was the control and the other was heated for 15 minutes at 100° in order to obtain the lactone of 4-pyridoxic acid. The contents of both tubes were then diluted, and borax was added to bring the pH to 9.0. A comparison was then made of the fluorescence in both test tubes with that of the standard lactone solution. A Bach lamp and a Wood's filter were used for exciting the fluorescence.

To determine the lactone of 4-pyridoxic acid without adding pyridoxin, V. A. Bogdanova [1] recommends diluting the urine further up to 200 times (and if this is not sufficient, up to 400 times) in order to reduce the fluorescence of impurities which may interfere with the determination of lactone. When we had become familiar with the fluorometric method, we were convinced that, when making large numbers of determinations of the lactone of 4-pyridoxic acid in the urine of subjects who had not received additional vitamin B<sub>6</sub>, it was most convenient to use the maximum dilution of 400 times straight away. In many cases this procedure eliminates certain superfluous measures which are unavoidable if the solution has been insufficient. By diluting 400 times at once, we were able to carry out up to 12 determinations per day.

## RESULTS

Thirty subjects were kept on a diet low in ascorbic acid, and one test tube from each was tested. The morning urine contained a small amount of ascorbic acid having a range of from 0.03 to 0.29 mg/hr, and a mean value of 0.18  $\pm$  0.01 mg/hr. At the same time we demonstrated the small amount of the lactone of 4-pyridoxic acid whose range of values was 0-6  $\gamma$ / ml, with a mean value of 1.45  $\pm$  0.23  $\gamma$ / ml. By calculating the amount of the lactone of 4-pyridoxic acid per portion of

urine collected hourly, it was found that the excretion varied from 0 to 161.9  $\gamma$ / hr, and had a mean value of 40.6 + 6.9  $\gamma$ / hr.

Some of the subjects received up to 100 mg ascorbic acid per day as tablets. After 49-51 days, samples of urine from 17 subjects were examined, and it was found that the ascorbic acid excreted in the morning urine ranged from 0.23 to 2.28 mg/hr, with a mean value of  $0.72 \pm 0.13$  mg/hr (Fig. 1). At the same time there was a considerable increase in the amount of the lactone of 4-pyridoxic acid: It varied from 2 to 14  $\gamma$ /ml, and had a mean value of  $6.28 \pm 0.74 \gamma$ /ml. (Fig. 2). The calculated amount of lactone per portion of morning urine ranged from 66.4 to  $456.5 \gamma$ /hr, and had a mean value of  $234.6 \pm 24.4 \gamma$ /hr.

The results obtained for the content of 4-pyridoxic acid in human urine agree with those of V. A. Bogdanova who found that, without added vitamin  $B_6$ , from 0 to  $14\gamma$  of lactone per ml of urine was present.

Mathematical treatment of the results which we obtained showed that, for ascorbic acid and for the lactone of 4-pyridoxic acid (in g per ml and in g per hr), the difference between the excretion rates before and after taking the vitamin was statistically significant.

We also discovered a new phenomenon; namely, that the enhanced excretion of ascorbic acid in the urine caused by a raised ascorbic acid intake brings about a spontaneous increase in the excretion of the lactone of 4-pyridoxic acid.

#### SUMMARY

Morning urine on an empty stomach taken from males aged 19-25 years, fed on a diet deficient in ascorbic acid, was found to contain a small proportion both of this acid and of vitamin B<sub>6</sub>, excreted in the form of lactone of 4-pyridoxic acid. When these subjects were given 100 mg of ascorbic acid daily, there was a statistically significant increase in the amount of ascorbic acid and 4-pyridoxic acid excreted in the morning urine.

### LITERATURE CITED

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