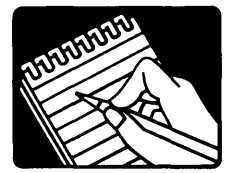
Brief Communication



Noneffect of Oral Urinary Copper Ascorbic Acid on Reduction Glucose Test

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References and texts in the fields of diabetes and clinical chemistry commonly report that ascorbic acid when given orally or parenterally gives a false-positive reaction to the copper reduction glucose test (Clinitest). This impression is based on a study in which ascorbic acid (250 mg./dl.) was added to urine in vitro, with a resultant positive-test reading in the absence of glucose. Ascorbic acid is a reducing agent, and theoretically it could interfere with the copper reduction method of glucose detection. In the current study 10 nondiabetic men were ingesting 4 and 6 gm. ascorbic acid per day. A total of 360 glucose detection tests with the copper reduction method were undertaken. In no instance was there a positive reaction to the glucose test. DIABETES CARE *1*: 34–35, JANUARY–FEBRUARY, 1978.

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Mayson et al.[1] have reported that ascorbic acid, when added to urine in vitro, gives a false-positive reaction to the copper reduction glucose test (Clinitest). They found that a urine concentration of ascorbic acid of 250 mg./dl., with no glucose present, resulted in a trace false-positive reaction to the copper reduction test and that a combination of ascorbic acid 100 mg./dl. and glucose 100 mg./dl. resulted in a similar trace reaction. They also reported that ascorbic acid added to urine in vitro in a concentration of 25 mg./dl. completely inhibits the glucose oxidase (Tes-Tape) reaction.

This reaction is of possible clinical significance since many people ingest rather large quantities of ascorbic acid for various reasons. Pauling[2] has recommended daily supplement doses of 1–5 gm. of ascorbic acid and doses up to 15 gm. daily to prevent or treat the common cold. Many physicians have prescribed ascorbic acid in doses of 2–6 gm. daily to acidify the urine (although this has been shown to be ineffective in a controlled study[3]).

It cannot be assumed, however, that oral ingestion of ascorbic acid will result in the same effect on the copper reduction reaction as the in-vitro addition of ascorbic acid to urine. The current experiment was conducted to determine the effects of oral ingestion of ascorbic acid on the copper reduction test.

METHOD

Twelve normal, nondiabetic male volunteers (age 22–30 years) participated in the study. The subjects took no medication and did not alter in any unusual way their diet patterns during the study period. One of the 12 volunteers was eliminated because of drug therapy initiated during the study and another because of poor compliance with urine collection.

Ascorbic acid (tablets 500-mg. control no. 4J527 and 100-mg. control no. 1K762, McKesson Labs) was given to each subject at two dosage levels and intervals (4 and 6 gm. per day divided into four and five doses). The study was conducted by use of a crossover design in which each of the four dosing regimens was in simultaneous use in one of four subgroups. The 4-gm. dose was given as 1 gm. at 8 a.m., 1 p.m., 6 p.m., and 11 p.m. or as 700 mg. at 7 a.m., 11 a.m., 3 p.m., and 7 p.m. and 1.2 gm. at 11 p.m. The 6-gm. dose was given as 1.5 gm. at 8 a.m., 1 p.m., 6 p.m., and 11 p.m. or as 1 gm. at 7 a.m., 11 a.m., 3 p.m., and 7 p.m. and 2 gm. at 11 p.m.

After two days of ascorbic acid therapy on the assigned regimen, each voided urine was collected in a separate polyethylene bag and immediately refrigerated. Each day during the three-day urine collection period, random urine samples from the preceding 24 hours were tested with the two-drop copper reduction method of glucose detection (Clinitest). The weekend served as a "washout" period before institution of the next five-day therapy period on another ascorbic acid regimen.

In each of the three days of urine collection in each treatment period, urine samples from each subject were tested with the copper reduction method of glucose detec-

tion. Thus each subject's urine was tested 36 times for glucose, nine times on each of the four dosage regimens. A total of 360 glucose detection tests with the copper reduction method were undertaken.

RESULTS

Of the 360 urine samples from the 10 nondiabetic subjects receiving either 4 or 6 gm. of ascorbic acid daily, none gave a positive reaction to the copper reduction method of glucose detection (Clinitest). None of the subjects experienced gastrointestinal upset, cramping, diarrhea, or other side effects to the ascorbic acid therapy.

DISCUSSION

Ascorbic acid is a reducing agent, and theoretically it could interfere with the copper reduction method of glucose detection. This has been demonstrated when ascorbic acid was added to voided urine so that the final ascorbic acid concentration was 250 mg./dl.

The metabolism of ascorbic acid at doses $\leq$100 mg. per day has been studied, although investigation of larger doses has not been reported.[4,5] At this low dosage level, ascorbic acid is metabolized to oxalic acid and ascorbate-3-sulfate.[6–10] Oxalic acid, with a pK_1 of 1.26 and pK_2 of 4.28,[11] is largely ionized at the pH range found with urine, and thus exists as a conjugated base. The pK_a of ascorbate-3-sulfate has not been reported. Angel et al.[5] have reported that at a dose of 3 gm. per day, 50 per cent of the ascorbic acid dose is recovered unchanged in the urine, whereas only 30 per cent of the drug is recovered when the dose is increased to 5 gm. per day. The reason for this lower recovery could be increased metabolism or altered disposition but may simply be due to the absorption saturation phenomenon reported with ascorbic acid.[5]

Because of variable ascorbic acid absorption, variable retention, depending on saturation of body stores, and partial metabolism of ascorbic acid prior to urinary excretion, it is not realistic to assume that an in-vitro ascorbic acid interference with the copper reduction test will hold true in vivo.

In the doses tested (4 and 6 gm. daily) in this study, oral ascorbic acid did not interfere with the copper reduction method of glucose detection. It is possible that oral doses of ascorbic acid higher than 6 gm. daily would result in a false-positive glucose reading, but this is unlikely, due to the decreased absorption with larger doses. Thus, it seems that ascorbic acid should be removed from the list of agents that routinely interfere with the copper reduction method of glucose detection. In cases of injection of more than 6 gm. daily of ascorbic acid, a positive reaction could be suspect and further testing indicated.

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