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RATE OF EXCRETION OF VITAMIN C IN HUMAN URINE

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ABSTRACT

The dosage of vitamin C necessary to maintain a level in the urine which could be detected using the 2,6-dichlorophenolindophenol assay was determined with undergraduate students. Students taking 250 mg daily did not excrete significant levels of vitamin C in their urine, while excretion increased at doses from 0.5 to 2 g. A 2 g daily dose caused detectable excretion from about 4 until 16 hr later, on both the first and eighth day. A dose of 500 mg taken every 12 hr led to continuously-detectable levels of vitamin C in the urine. The conclusion is that two conditions are necessary to elevate vitamin c excretion continuously: a dose of at least 500 mg and a dose every 12 hr. This is substantially higher than the U.S. recommended daily allowance and more frequent than administration being used in clinical trials.

INTRODUCTION

Vitamin C, ascorbic acid, has long been known to prevent scurvy, and more recently has been shown to have an effect on the healing of wounds, the state of gums, and the strength of bones through the stabilization of collagen (1-3). However, other possible health benefits, including prevention of cancer (3-6), prevention of heart attacks and reduction of cholesterol (7-9), and as a boost to the immune system to prevent colds (10-13), remain controversial.

The "free radical theory of aging" may explain how an optimal dose of vitamin C might provide the above health benefits (14-15). The "free radical" disease include cancer, heart disease, emphysema, and other diseases associated with aging. By trapping free radicals generated during metabolic activity, vitamin C, in conjunction with another anti-oxidant, vitamin E, may reduce age-related cell damage in animals and plants (16-17). If vitamin C does have this effect, taking an optimal dose of vitamin C could be extremely beneficial to health (4, 18). With around 20 million people in the U.S. taking daily supplements of vitamin C (19), it is appropriate to ask what an optimal dose is.

Unfortunately, the optimal dose of vitamin C is not established. The recommended daily allowance sufficient to prevent scurvy varies from 30 mg in the United Kingdom to 60 mg in the U.S. to 90 mg in the former Soviet Union (1). In contrast, mega-doses up to 16 g per day are suggested to provide additional health benefits (20).

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The optimal dose of vitamin C depends on many factors including absorption into, metabolism by, and excretion from the body. Absorption of large doses into the body is limited by the intestine, metabolism by the liver and tissues may be influenced by factors such as smoking, colds, and stress, and excretion is controlled by the kidney (1,22-23). For example, it has been reported that only 60% of a 500 mg dose is absorbed into the body with 40% excreted unchanged within the first 12 hr (24). It has been found that a certain percentage of any dose is excreted, increasing with the dose (25).

Storage in the body also plays a role. Vitamin C is water soluble and cannot be stored in the body to any great extent (21). However, when vitamin C is regularly ingested, a body pool develops. This pool can become large even though some vitamin C is being excreted in the urine, and it can take three months of ascorbic acid deprivation for the body pool to be depleted and symptoms of scurvy to appear (1).

Without a clear physiological endpoint to measure the optimal dose or a complete understanding of the role of vitamin C, it is unclear how much vitamin C one should take. Because large doses are not entirely absorbed and may irritate the stomach, and small doses may be insufficient to provide sufficient anti-oxidant protection, we decided to determine a dose and rate sufficient to insure that excess vitamin C was always excreted in the urine. The purpose of this study was to determine the minimal dose of vitamin C which could be detected in the urine in significant amounts, and the frequency that the dose must be taken to maintain a continuously elevated level.

DISCUSSION

Because vitamin C has been implicated in a variety of diseases, it has been the subject of frequent clinical studies. Often those studies compare subjects taking vitamin C on a "regular daily basis" with those not taking vitamin C. However, because vitamin C is a water-soluble vitamin, high doses may be readily excreted in the urine. Our study was undertaken to determine what daily dose of vitamin C is necessary to elevate the rate of vitamin C excretion in the urine, whether a single dose is sufficient to maintain elevated excretion for an entire 24-hr period, and whether taking a regular daily dose of vitamin C leads to any noticeable change in vitamin C excretion over time.

A dose of 500 mg is necessary for detectable excretion in most individuals. Figure 1 shows that doses less than 0.5 g per day caused no detectable change in urine

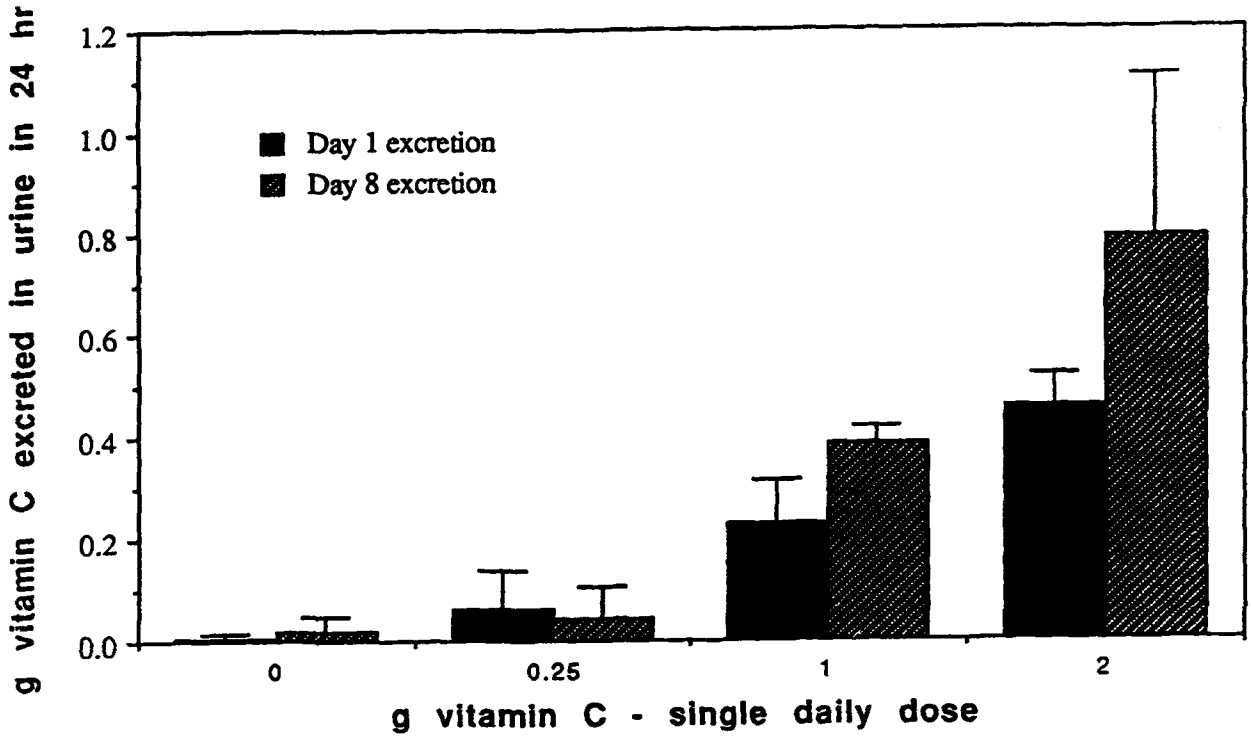

Figure 1: Relationship of Dose of Vitamin C to Total Urinary Excretion. Individuals took vitamin C daily at 8 am for eight days. All urine was collected during the first and last 24 hr and the total amount of vitamin C excreted in the urine was determined by DCIP assay. Each bar represents the average for 5 individuals. Error bars represent one standard deviation.

levels, even when lower doses were taken for 8 consecutive days. Doses of 500 mg to 2 g elevated urinary vitamin C to readily detectable levels but only for approximately 12 hr. Despite individual variation seen in figure 2, taking 2 g daily for 8 days did not lead to any noticeable difference in the pattern of excretion from day to day. A single daily dose is inadequate to elevate urinary excretion continuously.

To do so, it is necessary to take vitamin C twice daily as shown in figure 3. Taking 500 mg twice daily led to continuously elevated levels in 7 individuals tested, and the level of excretion was similar during the first and eighth day.

These results have important implications for the design of clinical studies involving vitamin C. First, small doses of vitamin C are unlikely to provide elevated vitamin C levels in the body, particularly in the blood. At this point, it is unclear whether doses below 500 mg/day are absorbed or metabolized, but the evidence clearly supports that at least 500 mg/day are necessary to saturate the blood sufficiently that vitamin C appears in the urine. Second, although 500 mg/day is sufficient to elevate vitamin C excretion, even a dose of 2 g/day is insufficient to maintain excretion over a 24 hr period. If a study is intended to determine the protective effects of vitamin C, it is necessary that the vitamin be administered every 12 hr.

The precise role of vitamin C in maintaining good health remains to be understood, but a large body of evidence is consistent with the hypothesis that individuals taking doses of vitamin C substantially greater that the USDA Recommended Daily Allowance may have a substantial health benefit. Until the role of vitamin C becomes clear, it may be prudent to take a dose which is large enough that there is continuously detectable excretion. Our results support the conclusion that in order to maintain continuously elevated levels of vitamin C in the urine, at least 500 mg of vitamin C should be taken twice daily.

RESULTS

I. Stability of vitamin C in Water and Urine

To determine whether vitamin C is stable in urine prior to assay, stock solutions of vitamin C in water and in urine were prepared 48, 24, 12, and 0 hr before running standard curves using the DCIP procedure. Each stock solution contained 125 μg Vitamin C/ml of 5% phosphoric acid in water or urine. Samples were stored in the dark at RT. Vitamin C degraded when stored in aqueous solutions of 5% phosphoric acid, but was stable in solutions containing urine in 5% phosphoric acid (results not shown).

II. Effect of Dose of Vitamin C on Total Urinary Excretion

In order to determine how daily dose is related to total excretion and whether high daily doses will alter the rate of excretion after a week, groups of students took either 0, 0.25, 1, or 2 g doses of vitamin C at 8 am daily for 8

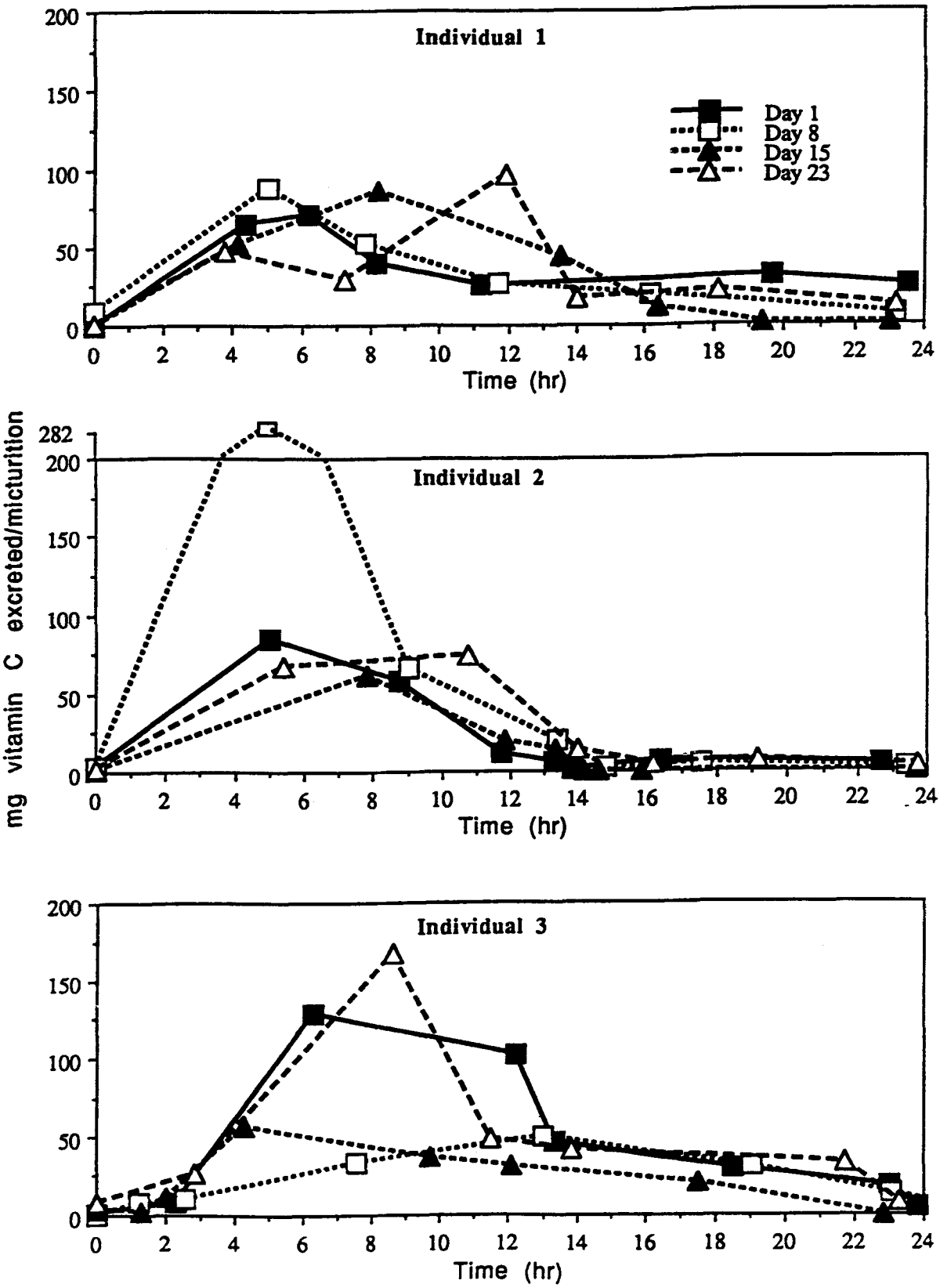

Figure 2: Rate of Excretion of Vitamin C over 24 hr
Two g of vitamin C in pills were taken daily for 8 days, then no pills were taken for 6 days, and then the same individual took 2 g daily for another 8 days. Each micturition during the first and last 24 hr of each 8 day period was collected and assayed to determine the total mass of vitamin C excreted. Each figure (2a-c) represents excretion from different male individuals weighing 63 to 73 kg.

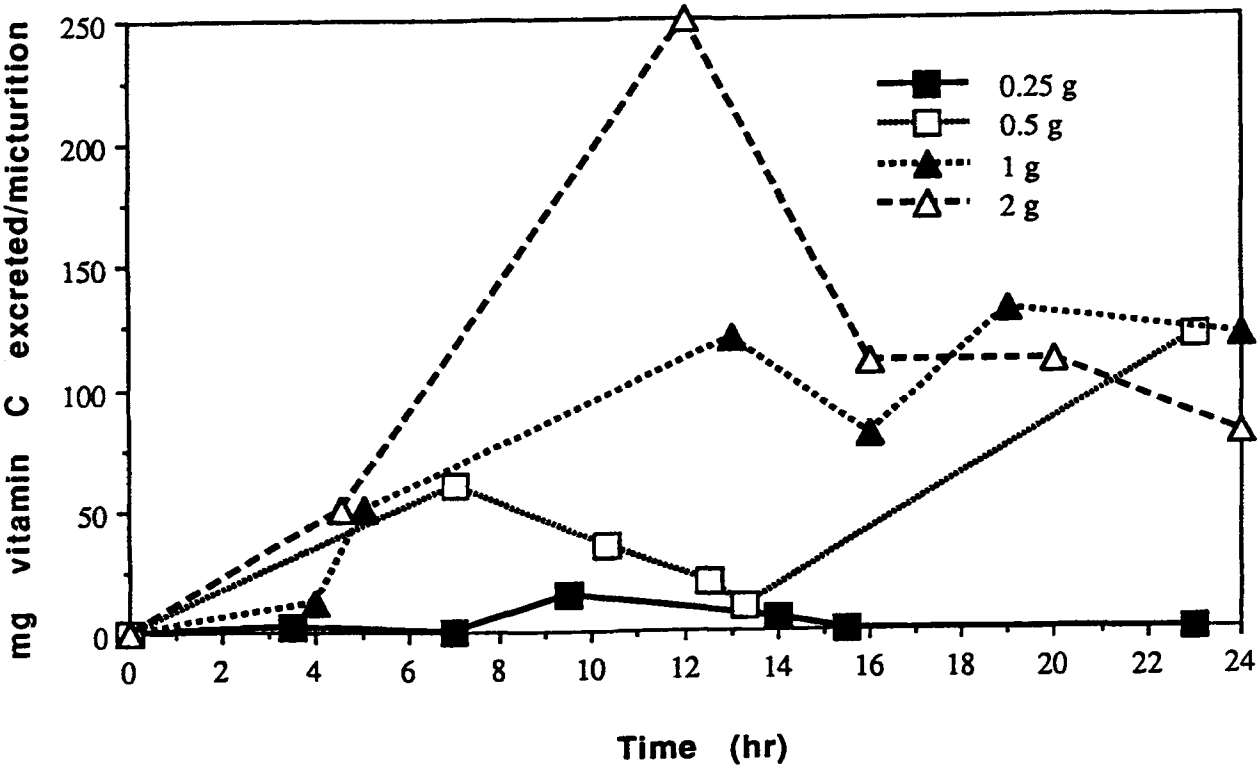

Figure 3: Effect of Dose on Excretion of Vitamin C with Two Daily Doses
Vitamin C was taken at times 0 and 12 hr and the mass of vitamin C was measured in every micturition. Data represent excretion for a 70 kg, 19 year old male who was tested one day each week for four consecutive weeks.

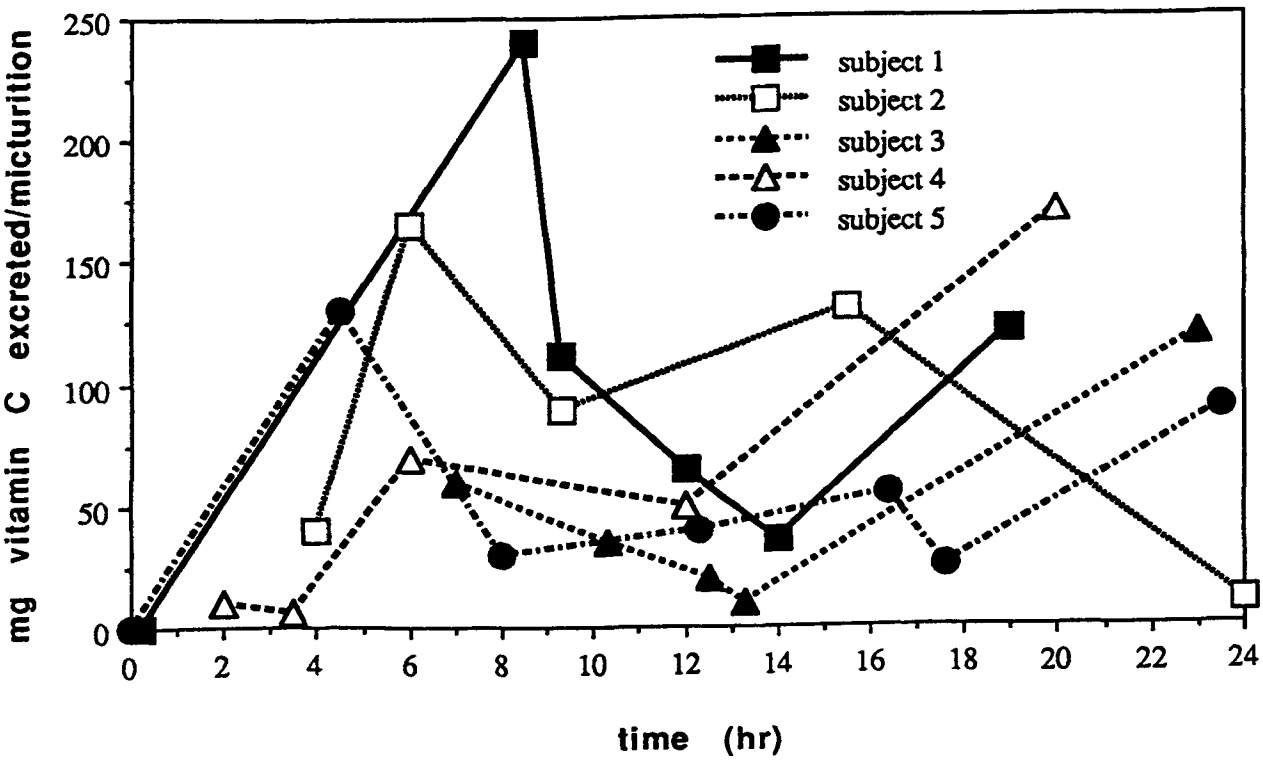

Figure 4: Two Daily Doses of 500 mg Vitamin C Maintain Excess
Data are represented for five individuals, ages 19-21, weighing 50 to 73 kg, each took 500 mg vitamin C at time 0 and 12 hrs. The total mass of vitamin C excreted during each micturition was measured by DCIP assay.

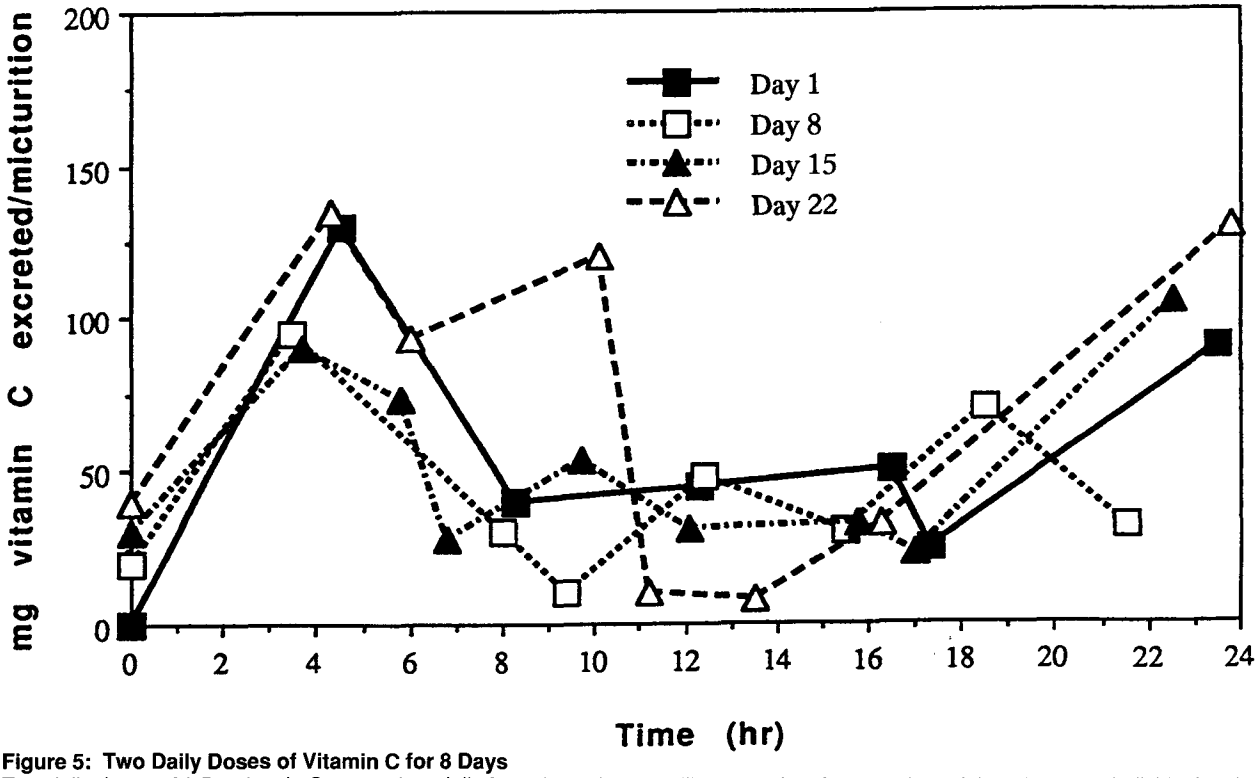

Figure 5: Two Daily Doses of Vitamin C for 8 Days
Two daily doses of 0.5 g vitamin C were taken daily for 8 days, then no pills were taken for a week, and then the same individual took 0.5 g daily for another 8 days. Data are presented for a 19 year old, 60 kg male. Each micturition during the first and last 24 hr of each 8 day period was collected and assayed to determine the total mass of vitamin C excreted.

consecutive days. All urine was collected and pooled on day 1 and on day 8. The concentration was assayed and the total amount of vitamin C excreted during the 24-hr period was determined as shown in Figure 1. The rate of urinary excretion of vitamin C increased with increasing daily doses of vitamin C, and the amount excreted was similar even after taking it for 8 consecutive days.

III. Rate of Excretion of a Single Dose of Vitamin C

In order to determine how rapidly vitamin C is excreted, individuals took 2 g of vitamin C daily for 8 consecutive days, measured the volume, and collected samples of every micturition during the first and last day. Each sample was analyzed to determine the time period, concentration range, and total urinary excretion of vitamin C. Results are shown in Figure 2 for three individuals. Vitamin C concentration in the urine increased after 4 hr and remained elevated for a total of 12 hr, consistent with the hypothesis that vitamin C levels in the body are not elevated for an entire 24 hr period by a single daily dose. However, only a small percentage of the ingested vitamin C was detected in the urine. No noticeable difference in the excretion pattern during the first and last days was observed.

IV. Effect of Twice-daily Doses of Vitamin C on Urinary Output

Because a single dose of vitamin C elevates urine levels for only 12 hr and a 500 mg dose is sufficient to provide detectable levels in the urine, one individual took doses from 250 to 2000 mg of vitamin C every 12 hr to determine whether vitamin C levels would remain continuously elevated. Figure 3 shows that a dose of 250 mg did not cause detectable excretion, but doses of 500 mg or higher provided continuously detectable excretion, with higher levels of excretion at higher doses. Twice-daily doses of 500 mg were sufficient to elevate vitamin C in the urine continuously as shown for 5 individuals in figure 4. When 500 mg are taken twice daily for a week, vitamin C remains continuously elevated on the first and last day, as shown in figure 5.

EXPERIMENTAL PROCEDURES

Vitamin C was assayed by the 2,6-dichlorophenolindophenol (DCIP) assay (26). Samples diluted to 0-40 µg ascorbic acid with 5% phosphoric acid were assayed in a citrate/acetate buffer by reaction with DCIP, and the absorbance at 520 mm was compared with standards. Samples were assayed in duplicate and averaged and each experiment was repeated. The ascorbic acid used in standard curves was from Gibco Laboratories. The 500 mg vitamin C pills ingested by subjects were "Your Life" brand. Sample pills were assayed for vitamin C content by extraction by grinding in 5% phosphoric acid and were shown to contain the appropriate concentration (results not shown). Subjects were healthy 19-23 year old volunteers and a 46 year old male professor from an introductory college chemistry class. Subjects ate three meals daily, avoiding vitamin C-enriched drinks. Informed consent was obtained after the nature and risks of the procedure, taking various doses of vitamin C, had been fully explained.

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ABBREVIATIONS

DCIP - 2,6-dichlorophenolindophenol

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