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Influence of Large Doses of Ascorbic Acid on Laboratory Test Results

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To determine the effect of large doses of vitamin C on laboratory tests, we administered 3 g of ascorbic acid per day to 10 healthy adults for 18 days. Thirty-four biochemical parameters were measured four days per week during vitamin C administration and the results were compared to those obtained during a four-week control period. Statistically significant changes, similar in all individuals, were seen in alanine aminotransferase, alkaline phosphatase, total bilirubin, carbon dioxide, creatinine, and potassium measured on the SMAC as well as for creatinine measured with the AutoAnalyzer II and for manually determined monoamine oxidase and catechol methyltransferase. The response to vitamin C differed among individuals for a second group of tests: alanine and aspartate aminotransferases measured with the Abbott ABA-100; alanine aminotransferase, creatine kinase, and lactate dehydrogenase measured with Perkin-Elmer KA-150; aspartate aminotransferase, cholesterol, lactate dehydrogenase, and uric acid measured with the AutoAnalyzer II; and manually determined dopamine-β-hydroxylase. For 13 other tests no significant changes were detected during megadoses of vitamin C. Using linear discriminant functions, we identified the presence or absence of vitamin C effect in 60 of 76 randomly chosen SMAC test profiles.

Many drugs alter laboratory test results (*1*). Ascorbic acid is a widely self-administered compound. According to the Market Research Corporation of America (New York, N.Y.), over 2000 tons of vitamin C are incorporated every year into pharmaceutical products, excluding multivitamin preparations. Of 581 urine samples from NIH Clinical Center patients, 76 had detectable ascorbate concentrations by "C-STIX" (Ames Co., Elkhart, Ind. 46514) (*2*).

Recently, several groups have studied the in vitro effect of vitamin C on clinical chemical procedures (*3-6*). This report describes the effect of large doses of vitamin C on 34 laboratory assays in 10 healthy volunteers. These 34 assays include both constituents commonly measured in the clinical laboratory as well as three enzymes of catecholamine metabolism [catechol methyltransferase (EC 2.1.1.6), dopamine-β-hydroxylase (EC 1.14.17.1), and monoamine oxidase (EC 1.4.3.4)]. We studied these three enzymes because vitamin C plays a role in catecholamine metabolism (*7*).

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Materials and Methods

Experimental Design

Blood was collected from 10 apparently healthy adult volunteers four times each week for six weeks. At the end of the fourth week the volunteers began taking ascorbic acid (Vitamin C, 1-g, chewable tablet; Roerig-Pfizer, Pfizer Inc., New York, N.Y. 10017), 1.0 g three times per day.

Erythrocyte lysate was analyzed for catechol methyltransferase the day of specimen collection. Monoamine oxidase activity was determined within one week on serum stored at −20 °C. Serum, stored at −70 °C, was assayed for dopamine-β-hydroxylase within two months of the beginning of the study. Serum stored at −70 °C was analyzed with the SMAC continuous-flow analysis system (Technicon Instruments Corp., Tarrytown, N.Y. 10591) 36 days after the end of specimen collection. The following 19 tests were performed with the SMAC: alanine aminotransferase (EC 2.6.1.2), albumin, alkaline phosphatase (EC 3.1.3.1), aspartate aminotransferase (EC 2.6.1.1), total bilirubin, calcium, carbon dioxide, chloride, cholesterol, creatine kinase (EC 2.7.3.2), creatinine, lactate dehydrogenase (EC 1.1.1.27), inorganic phosphorus, potassium, total protein, sodium, triglycerides, urea nitrogen, and uric acid. On the 25th and 26th days after the end of specimen collection, sera stored at −20 °C were assayed for cholesterol, creatinine, triglycerides, and uric acid with the Technicon AutoAnalyzer II continuous-flow analysis systems; alanine and aspartate aminotransferases and lactate dehydrogenase were analyzed with Abbott Bichromatic Analyzer ABA-100 discrete analyzers (Abbott Laboratories, South Pasadena, Calif. 91030). With the Perkin-Elmer KA-150 Enzyme Analyzer (Perkin-Elmer Corp., Norwalk, Conn. 06856), serum stored at −20 °C was analyzed for alanine and aspartate aminotransferases, alkaline phosphatase, creatine kinase, and lactate dehydrogenase 34 to 38 days after the last specimen was collected. Detailed information about the

volunteers, specimen collection and handling, analytical methods, and data editing appears elsewhere in this issue (*8*).

Statistical Analyses

Specimens for analysis with the ABA-100, the AutoAnalyzer II, the KA-150, and the SMAC were frozen on the day of collection and held for later batch analysis. Thus, individual specimens were stored for different periods of time. To compensate for changes occurring during storage, we did linear regression analyses for each test on the data from the first part of the study (when the volunteers were not receiving ascorbic acid). Analytical result was treated as the dependent variable, subject as a class variable, and serial day of the study as an additional independent variable. Using the regression coefficients for concentration change per day of storage, we normalized the observed analytical results to a common collection date.

The effect of vitamin C on laboratory results was analyzed by using a general linear model (*9*) with test result as the dependent variable and vitamin C status and subject as independent variables. To test for different responses to vitamin C among individual subjects, we repeated these statistical analyses, this time including a term for the interaction of subject with vitamin C as an additional independent variable. For those assays in which we detected a significant effect of ascorbic acid, we estimated the magnitude of the effect by calculating for each patient the difference between the means for his period of increased vitamin C intake and his control period. The mean of these 10 volunteers' differences was also calculated. For each assay for each patient, we prepared a cumulative sum plot (*10*), using the mean of the control period as the reference point.

Specimens for which all 19 SMAC results were available were randomly divided into two groups. Using the first group of 154 specimens, linear discriminant functions were computed (*11*) to classify specimens as belonging to either the control or vitamin C period. These discriminant functions were then applied to the second group of 76 randomly selected specimens, classifying them into the two periods.

Results

Table 1 lists assays for which a statistically significant ($P < 0.01$) change was observed during the period of ascorbic acid administration. This table includes only those tests for which no statistically significant difference was found in the magnitude of the response among the 10 subjects ($P > 0.01$ for interaction between subject and vitamin C status). The 9.4 U/liter decrease in SMAC-measured alanine aminotransferase values was the only consistent change large enough to be of clinical interest.

For several assays, we saw significantly ($P < 0.01$) different responses to vitamin C among the 10 volunteers; interaction between subject and vitamin C status was significant ($P > 0.01$), as summarized in Table 2. Despite the heterogeneity of individual responses, there was a statistically significant overall group change during the large doses of ascorbic acid for alanine aminotransferase and lactate dehydrogenase as measured with the KA-150 and for uric acid. For lactate dehydrogenase the 19 U/liter decrease during vitamin C administration could be of interpretive importance. The 39 U/liter decrease in creatine kinase activity for a single volunteer reflects a dramatic but transitory increase in his creatine kinase during the control period. During the period the volunteers were taking vitamin C the average decrease in uric acid values was 5.0 mg/liter as measured with the AutoAnalyzer II and 2.6 mg/liter as measured with the SMAC. There was significant variation among the individual uric acid responses to ascorbic acid, with one individual showing a marked increase as illustrated in Figure 1.

Table 1. Changes Observed with Large Doses of Vitamin C (Constituents for Which All Individuals Appear to Respond Similarly)

Constituent	Instrument	Concn. change	*P*-value less than
Alanine aminotransferase	SMAC	−9.4 U/liter	0.0001
Alkaline phosphatase	SMAC	0.84 U/liter	0.006
Bilirubin, total	SMAC	0.65 mg/liter	0.005
Carbon dioxide	SMAC	−0.54 mmol/liter	0.004
Catechol methyltransferase	Manual	−0.28 arb. units	0.005
Creatinine	AutoAnalyzer II	0.27 mg/liter	0.0003
Creatinine	SMAC	0.20 mg/liter	0.002
Monoamine oxidase	Manual	−0.45 arb. units	0.002
Potassium	SMAC	−0.16 mmol/liter	0.0001

Table 3 lists the 14 assays for which no significant changes were observed after the large doses of vitamin C.

Using linear discriminant functions derived from 154 randomly chosen SMAC-analyzed specimens, we correctly assigned 79% of a second group of 76 specimens to the vitamin C or control periods on the basis of the pattern of their biochemical test results. This leads us to conclude that large doses of vitamin C have a statistically recognizable effect on the pattern of results from a panel of SMAC tests.

Discussion

The changes we observed could be due to an effect of vitamin C on the analytical procedure, to a concentration change of the measured constituent due to a biological effect of ascorbic acid on the volunteers, or to both. Uric acid appears to illustrate combined analytical and biological effects. Ascorbic acid is known to falsely

Table 2. Changes Observed with Large Doses of Vitamin C (Constituents for Which Different Individuals Have Different Responses)

Constituent (units)	Instrument	Group responses: Concn. Change	Group responses: P-value less than	Individual responses: Range of concn. changes	Individual responses: P-value less than
Alanine aminotransferase	ABA-100		NS[a]	−2.1–+3.4	0.0001
(U/liter)	KA-150	−1.0	0.0001	−2.0–+2.1	0.0001
Aspartate aminotransferase	ABA-100		NS	−1.9–+2.2	0.0001
(U/liter)	SMAC		NS	−7.3–+6.1	0.0001
Cholesterol	AutoAnalyzer II		NS	−190–+160	0.0001
(mg/liter)	SMAC		NS	−210–+130	0.0001
Creatine kinase	KA-150		NS	−39–+8.5	0.001
(U/liter)	SMAC		NS	−110–+37	0.004
Dopamine-β-hydroxylase (U/liter)	Manual		NS	−2.2–+5.7	0.0001
Lactate dehydrogenase	KA-150	−19	0.0001	−34–−3.5	0.0001
(U/liter)	SMAC		NS	−10–+13	0.008
Uric acid	AutoAnalyzer II	−5.0	0.0001	−9.1–+5.0	0.0001
(mg/liter)	SMAC	−2.6	0.002	−8.8–+5.8	0.0007

[a] NS, not significant ($P > 0.01$).

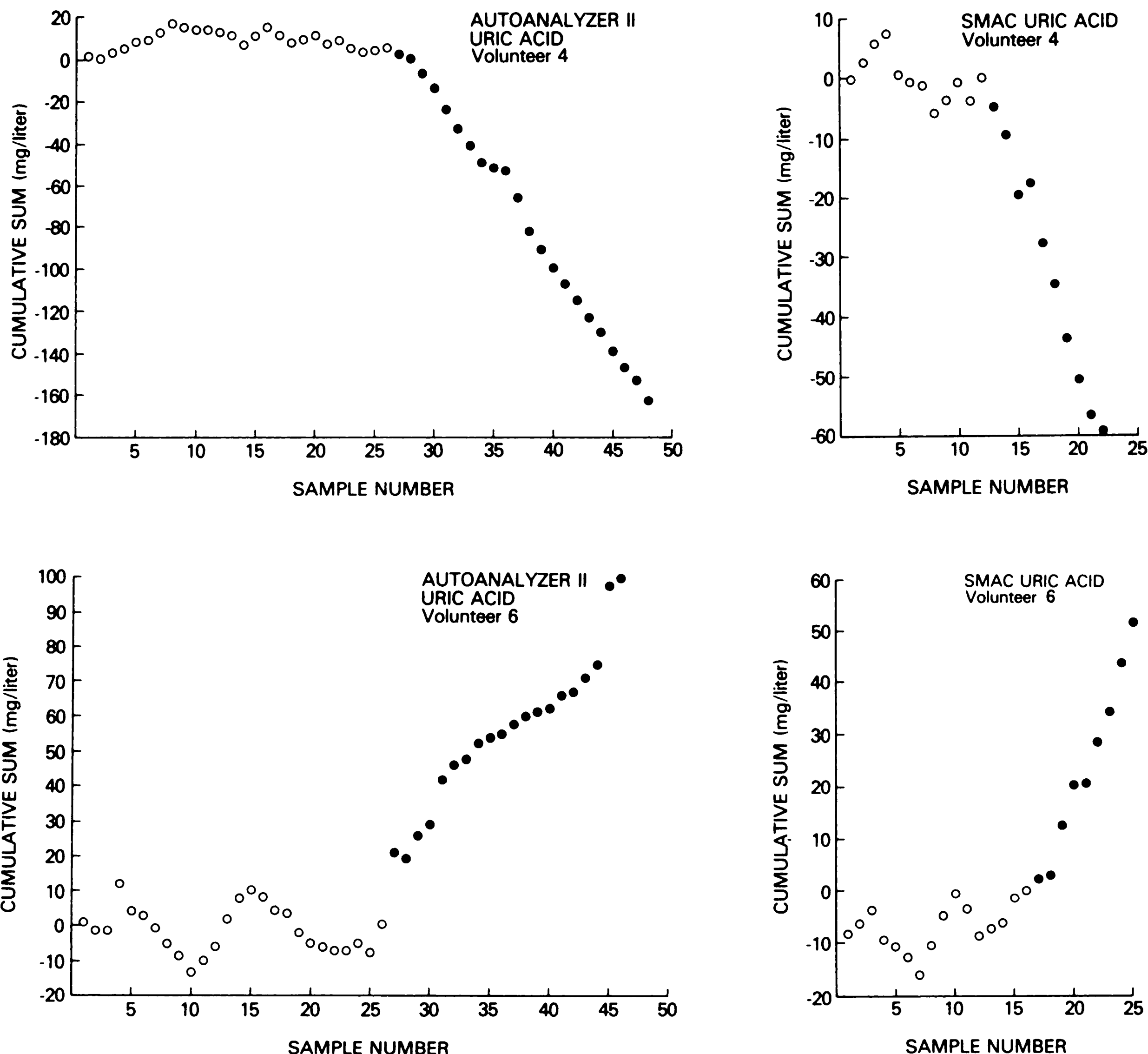

Figure 1. Cumulative sum plots of serum uric acid concentrations for two volunteers before (*open circles*) and during (*solid circles*) ingestion of 3 g of ascorbic acid per day

Table 3. Constituents for Which No Changes Were Detected with Large Doses of Vitamin C [a]

Constituent	Instrument
Albumin	SMAC
Alkaline phosphatase	KA-150
Aspartate aminotransferase	KA-150
Calcium	SMAC
Chloride	SMAC
Lactate dehydrogenase	ABA-100
Phosphorus	SMAC
Protein, total	SMAC
Sodium	SMAC
Triglycerides	AutoAnalyzer II
Triglycerides	SMAC
Urea nitrogen	SMAC

[a] *P*-value >0.01

increase values for apparent uric acid as measured by the phosphotungstate method used with the SMAC (*3*). In contrast, it artifactually decreases uric acid values assayed by the uricase dye oxidation procedure used on our AutoAnalyzer II (*12*). Furthermore, oral administration of ascorbic acid increases the renal excretion of uric acid, producing a decrease in serum uric acid concentration (*13*). In our volunteers, the apparent uric acid concentrations measured by the AutoAnalyzer II declined during ascorbic acid administration. This could have been due to a negative analytical interference combined with a physiological uricosuric effect. We observed a smaller decrease when uric acid was measured by the SMAC phosphotungstate procedure. Apparently the physiological decrease was partly offset by the positive analytical interference of vitamin C in the SMAC uric acid assay. Interestingly, one individual's uric acid concentration increased during vitamin C administration, presumably reflecting a unique physiological response (see Figure 1).

The increase in apparent creatinine concentration during ascorbic acid administration may be attributed, at least in part, to a positive analytical interference, which has been reported in the vitro studies of Vinet and Letellier (*5*). In vitro studies have yielded conflicting information regarding the effect of ascorbic acid on the bilirubin assay: Vinet and Letellier (*5*) reported a small negative interference, but Singh et al. (*3*) found a small positive interference. After oral administration of vitamin C, we observed an inconsequential but statistically significant increase in bilirubin values.

Although some statistically significant changes were observed, only for alanine aminotransferase, lactate dehydrogenase, and uric acid were the changes of sufficient magnitude to be of possible clinical importance.

Several aspects of the experimental design influence the interpretation of our observations. We studied the effect of a specific ascorbic acid dose within the range suggested by Pauling (*14*). Much higher daily and single dosages have been used (*15*). These doses might produce interferences that we did not observe with our lower dosages. Because our specimens were frozen and stored before analysis, some ascorbic acid may have been lost. Thus, our results may underestimate direct analytical interferences or reflect interference by ascorbic acid derivatives such as dehydroascorbic or diketogulonic acids. Published data suggest that vitamin C in serum is stable at −70 °C for several weeks, but slowly deteriorates at −20 °C (*16*). Our volunteers were apparently healthy adults. Administration of vitamin C to a geriatric or sick population with impaired renal or metabolic function might result in additional changes in laboratory profiles.

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