Effect of Ascorbic Acid on Calcium Elimination in Humans

C. S. TSAO, Koichi MIYASHITA,¹ and Ping Y. LEUNG

Linus Pauling Institute of Science and Medicine,
440 Page Mill Road, Palo Alto,
California 94306, U.S.A.

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Summary The long-term and short-term influence of large oral doses of ascorbic acid on the urinary excretion of calcium has been investigated. In the first experiment, daily doses of a total of 10 g of ascorbic acid were administered to healthy human subjects. Daily urinary samples of these subjects were collected before and during the treatment, and calcium contents of these samples were measured. Among the 22 subjects, 19 experienced no significant changes in urinary calcium levels during the ingestion of ascorbic acid, one subject experienced an increase, two had a decline. These changes in urinary calcium levels were relatively small and were within the changes from consuming normal diets.

In the second experiment, urinary samples of 46 healthy subjects were collected during a period of 8 hours after the ingestion of 2 g of ascorbic acid (33 times the U.S. RDA). A significant increase in mean urinary calcium excretion from $48.2 \pm 25.1\, \text{mg}$ to $58.3 \pm 28.0\, \text{mg}$ in the 8-h time period was observed. Mean urinary volume and phosphorus were unchanged. Calcium levels of the initially low excretors were significantly elevated while the change in urinary calcium levels of the initially high excretors was not statistically significant following the administration of ascorbic acid. The results suggest that ascorbic acid has a short-term effect on the regulation of the absorption and metabolism of calcium in humans.

Key Words ascorbic acid, vitamin C, calcium, urine, excretion

It has been known that ascorbic acid (vitamin C) chelates calcium ion (1, 2) and thus, a high intake of ascorbic acid may affect calcium absorption, metabolism and elimination. Large doses of ascorbic acid were able to reduce the mineralization of poult femurs in turkeys (3) and to increase the intestinal absorption and urinary excretion of calcium in rats (4, 5). High levels of dietary ascorbic acid caused a

¹ 宫下幸一
reduction in bone density and percent ash in tibia of very young guinea pigs (6), but no such effect was observed when older guinea pigs were used. Ascorbic acid had no effect on urinary excretion, bone ash mineral content, tibia breaking strength or percent tibia ash in hens, chicks and quails (7, 8). There was no significant effect of ascorbic acid on calcium content in femur ash of rabbits (9) in blood calcium levels of chicks, laying hens (7, 10, 11) and guinea pigs (12) or in bone and brain calcium contents of guinea pigs (13).

Human studies show that long-term large intake of ascorbic acid did not affect urinary calcium excretion (14–18). The addition of ascorbic acid to the diet increased calcium absorption in women (19) and in children (20). Ascorbic acid administration decreased calcium excretion in humans whose initial leucocyte ascorbic acid levels were below normal, but did not affect calcium excretion in subjects with normal leucocyte ascorbic acid levels (21).

Calcium oxalate is a major constituent of renal stones (22–25), and most investigators suggest that the calcium and oxalate in the renal stones are derived directly from the diet. There have been many studies on the factors influencing the urinary excretion of calcium. Since oxalate is known to be one of the metabolic products of ascorbic acid (26), the possible increase in calcium excretion due to a high intake of ascorbic acid may enhance renal stone formation. Therefore, one of the objectives of this investigation was to examine the effect of long-term large intake of ascorbic acid on calcium elimination in healthy individuals. In a preliminary experiment, we have found that the changes in the amount of daily calcium excretion in the urine of five healthy human subjects following the daily intake of 10 g of ascorbic acid were not statistically significant (18). In the present study, we report on the effect of dietary ascorbic acid (10 g per day) on the levels of urinary calcium of 22 healthy individuals.

A second objective was to examine the short-term effects of ascorbic acid on calcium elimination in humans. Animal experiments have shown that ascorbic acid administration caused a mobilization of calcium from cultured embryonic chick tibias (17–29) and an increase in urinary calcium excretion from chicks (30) during the initial time of treatment. After 24 hours this effect was lost and eventually normal levels were attained. In the present communication an experiment is reported that was designed to detect whether there was any change in calcium and phosphorus excretion in the urine shortly after the ingestion of ascorbic acid. Urine samples from 46 healthy subjects were collected over a period of 8 hours after the ingestion of 2 g of ascorbic acid (33 times the U. S. RDA), and the urinary calcium and phosphorus content of the test samples were compared with that of the basal values.

METHODS

1. Subjects. The subjects were employees or friends of the Institute. Throughout the experiment, the subjects were instructed to maintain their nor-
mal activities and diet. Calcium and ascorbic acid intakes in the diet averaged 820 ± 460 and 91 ± 46 mg/day/person, respectively for subjects in the long-term effect studies. In the short-term effect studies, these values are 807 ± 492 and 64 ± 37 mg/day/person. Since diet can significantly influence calcium output, the subjects were asked to consume meals similar in composition during the experimental period. Drugs and all vitamin and mineral supplements were discontinued one week before the test or the control period.

Long-term effect studies. Twenty-two healthy subjects (10 males and 12 females, mean age: 45.4 ± 14.5 years) participated in the study. During the test period the acid form of crystalline ascorbic acid (2 × 5 g per day) dissolved in fruit juice, was ingested by each subject with meals. After a period of equilibration of at least one week on the regimen, daily urine samples were collected from each individual. Each urine volume voided in a 24-h period was collected in graduated beakers and acidified with concentrated hydrochloric acid (2 ml HCl per 100 ml urine). The acidified urine was transferred into a glass bottle and stored immediately in a freezer. At the end of the 24-h period, the samples were thawed and mixed together. After the total volume had been recorded, a 50-ml aliquot of the well-mixed urine was stored at −76°C until the time of analysis. The 24-h urine collection was carried out for a total of five days during the test period. During and one week before the control period, the subjects received no ascorbic acid supplementation, and the daily urine samples were collected in the same manner for the determination of the basal levels.

Short-term effect studies. Forty-six healthy subjects (30 men, 16 women, mean age: 42.3 ± 23.7 years, median: 44, range: 24–72) participated in this experiment. On the first three days (control period), urinary specimens were collected each day from each individual during the same 8-h time period of the day for the determination of basal values. On the fourth day, after an oral dose of 2 g of ascorbic acid the Test urine sample was collected from each subject during the same 8-h time period of the day. The urinary specimens were treated with hydrochloric acid and stored as described above.

2. Assay for ascorbic acid. Total ascorbic acid (ascorbic acid plus dehydroascorbic acid) of the vitamin C supplements and of the urine samples was measured colorimetrically by the 2,4-dinitrophenylhydrazine method of Roe and Kuether with modification (31, 32).

3. Analysis of calcium. Total calcium in the urine was determined using an atomic absorption spectrophotometer (Perkin-Elmer Corp., Model 2380). A standard procedure established by Perkin-Elmer was used. Lanthanum oxide was added to the samples and standards. Calcium standard was purchased from Sigma Chemical Co.

4. Analysis of creatinine. Creatinine content in the urine was measured by the Jaffe reaction method. The reagents were obtained from Sigma Chemical Co. (Kit No. 555-A).

5. Analysis of phosphorus. Urinary phosphorus was measured using the
method of Gindler and Ishizaki (33, 34). The reagents were obtained from Pierce Chemical Co. (Phosphorus Auto/Stat Kit No. 48000).

6. Statistical calculation. Levels of probability were determined by an analysis of variance of the data using standard statistical methods. For evaluation of the statistical tests, tables in a standard text were used. Differences were considered to be significant at \( p < 0.05 \).

RESULTS AND DISCUSSION

Long-term effect

Table 1 shows the Test and basal values of ascorbate and calcium levels in the

| Subject | Age | Ascorbic acid (g/day) | Calcium (mg/day) | Calcium (mg/g creatinine) |
|---------|-----|-----------------------|------------------|--------------------------|
|         |     | Basal | Test | Basal | Test | Basal | Test |
| Male    |     |       |      |       |      |       |      |
| 1       | 18  | 0.10±0.05 1.97±0.55  | 145±25 167±32  | 190±15 199±24  |
| 2       | 23  | 0.09±0.03 1.44±0.46  | 137±33 122±23  | 162±24 157±33  |
| 3*      | 28  | 0.11±0.10 1.99±0.28  | 167±22 157±28  | 168±34 179±20  |
| 4       | 29  | 0.12±0.05 1.71±0.49  | 149±73 188±42  | 176±12 154±33  |
| 5*      | 34  | 0.04±0.03 2.22±0.51  | 159±52 187±40  | 155±41 123±31  |
| 6       | 38  | 0.11±0.07 1.85±0.23  | 163±44 192±57  | 134±22 151±24  |
| 7       | 45  | 0.10±0.05 1.32±0.14  | 173±12 123±42* | 163±21 134±30  |
| 8       | 47  | 0.12±0.04 2.25±0.40  | 192±20 180±14  | 198±23 172±41  |
| 9       | 56  | 0.08±0.04 2.03±0.62  | 169±20 172±15  | 134±25 142±19  |
| 10      | 59  | 0.07±0.12 2.62±0.85  | 123±23 108±18  | 104±43 115±32  |

| Female  |     |       |      |       |      |       |      |
|---------|-----|       |      |       |      |       |      |
| 11      | 33  | 0.08±0.02 1.92±0.58  | 122±32 109±21  | 123±30 155±33  |
| 12      | 37  | 0.09±0.04 1.23±0.19  | 122±42 152±27  | 176±24 182±40  |
| 13      | 42  | 0.10±0.09 2.25±0.17  | 132±26 125±18  | 156±33 128±29  |
| 14      | 46  | 0.11±0.08 1.59±0.47  | 164±36 113±33* | 129±24 99±16*  |
| 15      | 46  | 0.10±0.07 1.98±0.84  | 124±25 156±31  | 132±26 157±55  |
| 16      | 49  | 0.15±0.10 1.95±0.54  | 165±23 163±26  | 145±27 130±44  |
| 17      | 53  | 0.08±0.05 1.98±0.33  | 139±39 172±37  | 143±20 136±31  |
| 18      | 55  | 0.08±0.08 1.87±0.22  | 138±31 158±23  | 133±39 129±24  |
| 19      | 59  | 0.10±0.08 1.55±0.55  | 149±12 116±54  | 129±10 130±30  |
| 20      | 60  | 0.04±0.02 2.02±0.19  | 114±34 106±37  | 128±46 151±21  |
| 21      | 69  | 0.05±0.04 1.62±0.65  | 147±33 127±27  | 160±35 124±39  |
| 22      | 73  | 0.06±0.07 1.78±0.23  | 105±25 134±19  | 90±18 123±23*  |

*a All means are for \( n=5 \). b \( n=4 \) for Test samples. *Significance of the difference between Basal and Test values, \( p < 0.05 \).

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24-h urine samples of the twenty-two subjects. During the time of the daily oral administration of 10 g of ascorbic acid, there was a large increase in ascorbate level in the urine of all subjects. The large intake of ascorbic acid produced no statistically significant difference in calcium elimination in 19 of the 22 subjects. Urinary calcium levels of Subject 7 and 14 were significantly decreased during the large intake of ascorbic acid. However, when the calcium excretion is expressed as mg per g creatinine the changes in urinary calcium of Subject 7 is no longer statistically significant. In Subject 22, urinary calcium level was significantly increased when calcium is expressed as mg/g creatinine. Furthermore, although these changes in the 3 subjects are statistically significant, they are probably not physiologically or pathologically significant because these changes are relatively small and are within the range of urinary calcium content from consuming normal diets (35). In addition, it appears that healthy individuals can ingest large doses of ascorbic acid with relatively small changes in oxalate excretion (36) and are probably not at risk for calcium oxalate formation.

Short-term effect

Data for the levels of ascorbic acid, phosphorus and calcium as well as the volume of the urine of the 46 subjects are summarized in Table 2 to determine changes shortly after the ingestion of vitamin C. Mean urinary ascorbic acid excretion in 8 h was 29 times higher in the Test samples than in the basal controls. The changes in mean urinary volume and in phosphorus contents were not statistically significant. The mean urinary calcium level of these subjects was 48 ± 25 mg/8 h or 124 ± 57 mg/g creatinine in the basal controls, and 58 ± 28 mg/8 h or 158 ± 67 mg/g creatinine in the Test samples. The difference between the Test and basal values is statistically significant when calcium level is expressed as mg/g creatinine (p < 0.05), but it is not significant when calcium level is expressed as mg/8 h. Then a paired t-test was carried out. The mean of the individual difference between Test and basal values was calculated to be 10.13 ± 30.32 mg/8 h. This indicates that urinary calcium level is significantly increased following ascorbic acid ingestion (t = 2.26, p < 0.05, n = 46). Comparative studies in men and women revealed that men excreted more calcium than women in the 8-h time period, but the difference is not statistically significant.

The correlation between the levels of calcium (for both Test and basal values) and the amounts of ascorbate excreted was not statistically significant. There was a significant correlation between the calcium level in the Test samples and in the controls (r = 0.351, p < 0.02). The change in calcium level (Test minus basal) in the urine was inversely correlated with the basal value (r = -0.503, p < 0.01). The inverse correlation implies that urinary calcium of the initially low excretors had elevated to a higher level, while calcium of the initially high excretors had declined to a lower level.

Thus a detailed analysis was carried out as follows. Figure 1 is a representation of the changes in urinary calcium of each of the 46 subjects 8 hours after the
Table 2. Levels of urinary excretion (mean ± SD and range) of ascorbic acid, phosphorus and calcium of 46 healthy subjects (30 men, 16 women) 8 hours after the oral dose of 2 g of ascorbic acid.\(^a\)

|                | Basal                     | Test                      |
|----------------|---------------------------|---------------------------|
| Volume, ml     | 383 ± 202 (72–1,320)      | 425 ± 198 (58–976)        |
| Ascorbic acid  |                           |                           |
| µg/ml          | 55 ± 52 (4–295)           | 1,450 ± 976*** (207–3,240) |
| mg/g creatinine| 69 ± 107 (5–623)          | 892 ± 52*** (62–2,782)    |
| mg/8 h         | 15 ± 23 (6–201)           | 433 ± 124*** (28–834)     |
| Phosphorus     |                           |                           |
| µg/ml          | 532 ± 290 (115–1,050)     | 543 ± 256 (152–987)       |
| mg/g creatinine| 479 ± 485 (55–1,732)      | 501 ± 207 (79–1,983)      |
| mg/8 h         | 225 ± 98 (57–267)         | 242 ± 112 (26–480)        |
| Calcium        |                           |                           |
| µg/ml          | 125 ± 82 (8–562)          | 159 ± 98 (9–402)          |
| mg/g creatinine| 124 ± 57 (15–586)         | 158 ± 67* (20–492)        |
| mg/8 h         | 48 ± 25 (7–127)           | 58 ± 28 (9–128)           |
| men, n = 30    | 53 ± 27 (7–127)           | 63 ± 31 (9–128)           |
| women, n = 16  | 39 ± 19 (9–72)            | 50 ± 20 (18–82)           |
| low excretors,\(^b\) n = 26 | 31 ± 10 (7–47) | 53 ± 24** (9–124) |
| high excretors,\(^b\) n = 20 | 71 ± 21 (49–127) | 66 ± 32 (18–128) |

\(^a\) All means are for n = 46. \(^b\) The low excretors had basal urinary calcium levels below the average value of 48 mg/8 h whereas the high excretors had basal levels above this value. Significance of the difference between Basal and Test values: * p < 0.01, ** p < 0.001, *** p < 0.0001.

Ingestion of 2 g of ascorbic acid. Upon completion of the experiment, 32 (21 men, 11 women) of the 46 subjects experienced an increased urinary calcium level, 14 (9 men, 5 women) had a decline. When the 46 subjects are divided into two groups based on

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the basal calcium level in the control samples, we see that 26 subjects had initial calcium levels below the average excretion of 48 mg/8 h. After the ingestion of ascorbic acid, 23 (88%) of the 26 low excretors had increased calcium excretion; whereas among the 20 subjects who had initial urinary calcium levels above 48 mg, only 9 subjects (45%) had an increase. The mean basal calcium content of the 26 low excretors was 31.0 ± 10.4 mg/8 h. After the ingestion of ascorbic acid, the mean urinary calcium content of these subjects had increased to 52.6 ± 23.8 mg, and the difference is statistically significant (p < 0.001). On the other hand, following ascorbic acid ingestion, the mean urinary calcium content of the 20 high excretors had slightly decreased (basal: 70.5 ± 20.5 mg; Test: 65.8 ± 31.7 mg), but the difference is not statistically significant.

Results for the 46 subjects strongly suggest that there is an association between ascorbic acid intake and calcium excretion. From the data of this study we do not know whether the increase in urinary calcium excretion is due to bone calcium mobilization such as that observed in animal studies (20, 27–29). It has been shown that an increase in net acid excretion can increase the content of urinary calcium in humans (37). However, the relatively small amount of ascorbic acid (2 g) used in this study is probably not sufficient to affect the change in calcium excretion. The overall increase in calcium excretion is probably due to the increase in intestinal absorption of calcium because of the chelating action of ascorbate with calcium ion since ascorbic acid is capable of increasing calcium absorption in humans (19, 20) and in rats (4). The increase in calcium excretion is probably transient because results from

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the first experiment showed that large doses of ascorbic acid (10 g daily) did not affect long-term calcium elimination. The fact that the ingestion of ascorbic acid is associated with an increase in calcium excretion of the initially low excretors but not the initially high excretors suggests that ascorbic acid has enhanced calcium absorption in the low excretors. This in turn suggests that ascorbic acid affected the regulation of calcium absorption and perhaps calcium metabolism in these subjects. It has been reported in the literature that ascorbic acid is capable of regulating calcium metabolism in normal and osteoporotic human subjects (21).

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REFERENCES

1) Schubert, J., and Lindenbaum, A. (1952): Stability of alkaline earth-organic acid complexes measured by ion exchange. J. Am. Chem. Soc., 74, 3529–3532.
2) Tsao, C. S. (1984): Equilibrium constant for calcium ion and ascorbate ion. Experientia, 40, 168–170.
3) Dorr, P., and Balloun, S. L. (1976): Effect of dietary vitamin A, ascorbic acid, and their interaction on turkey bone mineralization. Br. Poult. Sci., 17, 581–599.
4) Mocos, S. R., El-Shobaki, F. A., El-Hawary, Z., and Saleh, N. (1976): Effect of vitamin C and carotene on the absorption of calcium from the intestine. Z. Ernährungswiss., 15, 387–390.
5) Keith, M. O., Shah, B. G., Nera, E. A., and Pelletier, O. (1974): The effects of high ascorbic acid and iron intake on the renal excretion of oxalate, calcium, and iron on the kidney of rats. Nutr. Rep. Int., 10, 357–369.
6) Bray, D. L., and Briggs, G. M. (1984): Decrease in bone density in young male guinea pigs fed high levels of ascorbic acid. J. Nutr., 114, 920–928.
7) Rowland, L. O., Roland, D. A., and Harms, R. H. (1973): Ascorbic acid as related to tibia strength in spent hens. Poult. Sci., 52, 347–350.
8) Sifri, M., Kratzer, F. H., and Norris, L. C. (1977): Lack of effect of ascorbic and citric acids on calcium metabolism of chickens. J. Nutr., 107, 1484–1492.
9) Hunt, C. E., Carlton, W. W., and Newberne, P. M. (1970): Interrelationships between copper deficiency and dietary ascorbic acid in the rabbit. Br. J. Nutr., 24, 61–69.
10) Pepper, W. F., Winget, C. M., and Slinger, S. J. (1961): Influence of calcium and ascorbic acid on egg quality. Poult. Sci., 40, 657–662.
11) Sullivan, T. W., and Gehle, M. H. (1962): Effect of ascorbic acid on the serum calcium level in laying hens fed graded levels of dietary calcium. Poult. Sci., 41, 1016–1017.
12) Tsao, C. S., Young, M., Rose, S. M., Leung, P. Y., Davies, M., and Andrews, V. (1985): Effect of ascorbic acid on plasma calcium in guinea pigs. Int. J. Vitam. Nutr. Res., 55, 309–314.
13) Kassounty, M. E., Coen, C. H., and Bebok, S. T. (1985): Influence of vitamin C and magnesium on calcium, magnesium and copper contents of guinea pig tissues. Int. J. Vitam. Nutr. Res., 55, 295–300.
14) Fituri, N., Allawi, N., Bentley, M., and Costello, J. (1983): Urinary and plasma oxalate during ingestion of pure ascorbic acid: a re-evaluation. Eur. Urol., 9, 312–314.
15) Hanck, A. B. (1972): The influence of 1000 mg of vitamin C on renal excretion of electrolyte in healthy human subjects. *Int. J. Vitam. Nutr. Res.*, 43, 34–41.

16) Hanck, A. B. (1974): The behavior of urine parameters in healthy human subjects during intake of 4 g L(+)-ascorbic acid. *Int. J. Vitam. Nutr. Res.*, 44, 302–308.

17) Schmidt, K.-H., Haginaier, V., Hornig, D. H., Vuilleumier, J.-P., and Rutishauser, G. (1981): Urinary oxalate excretion after large intake of ascorbic acid in man. *Am. J. Clin. Nutr.*, 34, 305–311.

18) Tsao, C. S., Salimi, S. L., and Pauling, L. (1982): Lack of effect of ascorbic acid on calcium excretion. *IRCS Med. Sci.*, 10, 736.

19) Leichsenring, J. M., Norris, L. M., and Halbert, M. L. (1957): Effect of ascorbic acid and of orange juice on calcium and phosphorus metabolism of women. *J. Nutr.*, 63, 425–435.

20) Voroboeva, A. M. (1967): Effect of vitamin C on the food calcium and phosphorus assimilation by children of preschool age. *Vop. Pitan.*, 26 (3), 78–83; *Chem. Abstr.*, 67, 3874.

21) Lynch, S. R., Seftel, H. C., Wapnick, A. A., Charlton, R. W., and Bothwell, T. H. (1970): Some aspects of calcium metabolism in normal and osteoporotic Bantu subjects with special reference to effects of iron overload and ascorbic acid depletion. *S. Africa J. Med. Sci.*, 35, 45–56.

22) Pak, C.Y.C. (1978): Calcium Urolithiasis, Chapter 3, Plenum Press, New York.

23) Rofe, A. M., Conyers, R.A.J., and Thomas, D. W. (1981): Renal stone disease in South Australia. *Med. J. Aust.*, 2, 158.

24) Robertson, W. G., Peacock, M. (1980): The cause of idiopathic calcium stone disease: Hypercalcemia or hyperoxaluria? *Nephron*, 26, 105–110.

25) Hodgkinson, A. (1977): Oxalic Acid in Biology and Medicine, Academic Press, New York, p. 325.

26) Lewin, S. (1976): Vitamin C: Its Molecular Biology and Medical Potential, Academic Press, New York, pp. 166–167.

27) Ramp, W. K., and Thornton, P. A. (1971): Ascorbic acid and the calcium metabolism of embryonic check tibias. *Proc. Soc. Exp. Biol. Med.*, 137 (1), 273–276.

28) Thornton, P. A. (1968): Bone salt mobilization effected by ascorbic acid. *Proc. Soc. Exp. Biol. Med.*, 127, 1096–1099.

29) Ramp, W. K., and Thornton, P. A. (1971): Ascorbic acid and bone remodeling in tissue culture. *J. Dent. Res.*, 50, 1504–1550.

30) Thornton, P. A. (1970): Influence of exogenous ascorbic acid on calcium and phosphorus metabolism in the chick. *J. Nutr.*, 100, 1479–1485.

31) Roe, J. H., and Kuether, C. A. (1943): The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. *J. Biol. Chem.*, 147, 399–407.

32) Schaffert, R. R., and Kingsley, G. R. (1955): A rapid, simple method for the determination of reduced, dehydro and total ascorbic acid in biological material. *J. Biol. Chem.*, 212, 59–68.

33) Raabe, S. (1955): *Rec. Trav. Chim. Pays-Bas.*, 74, 652; in Klinische Chemie (1968): "Theorie une Praxis" (2nd Ed.), ed. by Richterich, R., S. Karger, Basel and New York, pp. 307–309.

34) Gindler, E. M., and Ishizaki, R. T. (1969): Rapid semimicro colorimetric determination of phosphorus in serum and nonionic surfactants. *Clin. Chem.*, 15, 807.

35) Weast, R. C. (ed.) (1968): in Handbook of Clinical Laboratory Data (2 Ed.), The Chemical Rubber Co., Cleveland.
36) Tsao, C. S., and Salimi, S. L. (1984): Effect of large intake of ascorbic acid on urinary and plasma oxalic acid levels. *Int. J. Vitam. Nutr. Res.*, 54, 245–249.

37) Lemann, J. L., Adams, N. D., and Gray, R. W. (1979): Urinary calcium excretion in human beings. *N. Eng. J. Med.*, 301 (10), 535–541.