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Testicular and plasma ascorbic acid levels in mice following dietary intake: a high-performance liquid chromatographic analysis

Ravi S. Harapanhalli, Roger W. Howell and Dandamudi V. Rao*

Department of Radiology, Division of Radiation Research, University of Medicine and Dentistry of New Jersey, 185 South Orange Avenue, Newark, NJ 07103 (USA)

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ABSTRACT

A modified buffer system is reported for the analysis of vitamin C in mouse plasma and testes, on a reversed-phase high-performance liquid chromatographic column with ultraviolet detection. The buffer, consisting of 0.1 *M* NaH_2PO_4 and 0.2 m*M* Na_2EDTA adjusted to pH 3.1 with orthophosphoric acid, resolved the ascorbic acid (AA) peak allowing clear quantitation of the chemical. The method is also applicable to the assay of dehydroascorbic acid after its reduction to ascorbic acid, and overcomes problems of AA stability encountered in previously reported procedures. Using the present technique, variations in the vitamin levels of plasma and testes are studied from 3 to 29 days after the commencement of feeding a vitamin C-rich diet (1%, w/w) in mice. The plasma AA levels were elevated above the controls by a factor of 2.5 by day 8. Contrary to this, testicular AA levels increased marginally (1.2-fold) by day 12 and were maintained at levels close to the control values thereafter. It appears that the feedback inhibition mechanism which is effective in plasma is not operational in the testes. The findings are of clinical significance in that testicular AA levels do not change significantly as a result of dietary intake of vitamin C, whereas plasma AA levels do show an increase.

INTRODUCTION

The role of vitamin C in metabolism is complex. Its protective action against the oxidizing effects of free radicals, such as lipid peroxidations, is of crucial importance [1–4]. Its presence is necessary for the activity of dopamine β-hydroxylase [5], as well as for the synthesis of collagen [6,7]. The vitamin is water-soluble and consists of *l*-ascorbic acid (AA) and its oxidized form dehydro-*l*-ascorbic acid (DHAA). The concentrations of AA and DHAA, and their ratio, is an indication of the redox processes taking place in the organism. Recent reports point out that dietary supplementation of AA has proven beneficial in delaying the appearance of tumors and other lesions in experimental mice [8–10].

It is known that many chemicals with antioxidant properties also protect against radiation damage *in vitro* and *in vivo*. However, the role of vitamin C as a radioprotector remains a matter of controversy. O'Conner *et al.* [11] reported protection of Chinese hamster ovary cells by a factor of 1.4 against external X-rays, whereas Naslund *et al.* [12] showed an antagonizing effect of this vitamin in *Escherichia coli* cells. Its potential as an *in vivo* protector is also controversial. Ala-Ketola *et al.* [13] showed it to be a protector in Wistar rats against a lethal dose of X-rays, but others found it to be either devoid of protective properties [14] or a radiosensitizer in mice [15,16]. In all

* Corresponding author.

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these early experiments, the tissues were irradiated with acute external beams. With tissue-incorporated radionuclides, however, the radiation dose to the tissue is delivered in a chronic manner, dictated by the biological and physical half-lives of the radiochemical. Accordingly, the focus of our research has been to assess both the biological damage caused by various radionuclides and the capacity of different radioprotectors and vitamins [17–19] to mitigate such damage. Our experiments, using spermatogenesis in mice as an experimental model, have shown [19] that dietary vitamin C protected the highly sensitive spermatogonial cells in the testes against the radionuclides ^{131}I, and ^{125}I with a dose modification factor (DMF) of nearly 2. Similarly, vitamin C protected the bone marrow from the damaging effects of acute external X-rays [20] (DMF *ca.* 2). In view of these results it is essential to ascertain the effects of dietary intake of this vitamin on its endogenous levels in the testes and bone marrow of mice. Recently Tsao *et al.* [21] reported the AA levels in mice following a long-term dietary supplementation of vitamin C (4 months) at graded doses. Others have reported the effects of one month of dietary supplementation in mice [22]. However, data on variations in vitamin C levels in mouse testes were not provided. In this paper we report on the plasma and testicular AA levels from 3 to 29 days after initiation of dietary intake of vitamin C (1%, w/w) in mice, using a modified reversed-phase HPLC assay with UV detection described below.

There are numerous methods of analysis of AA and DHAA in biological samples. These include the indicator dye reduction method with dichlorophenolindophenol [23,24], the ketone derivatization method with dinitrophenylhydrazine [25,26], an enzymatic method with ascorbic acid oxidase [27] and HPLC with electrochemical [28,29], conductometric [30], UV [31–34] and fluorescence detections [35]. However, the indicator dye reduction and ketone derivatization methods are not very specific, and have the drawback that blank values have to be determined by chemical interference in the color-inducing reaction. The enzymatic method lacks sensitivity for the analysis of low concentrations of vitamin C observed in whole blood. Owing to a very high specificity of the assay, HPLC is by far the preferred procedure for vitamin C analysis. HPLC in conjunction with UV detection does suffer from very low absorbance of DHAA, however, a pre-column derivatization with *o*-phenylenediamine is reported to increase the sensitivity [33,34]. It is reported that the reaction conditions required for this derivatization are not mild enough to prevent some loss of AA due to decomposition. Attempts by choosing a suitable buffer from the above reported systems for the analysis of AA and DHAA have failed to satisfactorily resolve AA in testicular samples, although they were adequate for plasma analysis. A modified buffer system was therefore developed for the reversed-phase HPLC analysis of AA and DHAA in plasma and testicular samples of mice using UV detection. The assay of DHAA is based on its prior reduction to AA by dithioerythritol (DTT) rather than on the pre-column derivatization method. Since, under the present reaction conditions, AA is stable over 3–4 h, this procedure overcomes the problem of loss of AA encountered in the pre-column derivatization reaction.

EXPERIMENTAL

Chemicals

l-Dehydroascorbic acid, *dl*-dithioerythritol, *l*-ascorbic acid and all other chemicals were obtained from Aldrich (Milwaukee, WI, USA). Water for HPLC, from J. T. Baker (Phillipsburg, NJ, USA), was filtered through a Millipore HA 0.45-μm filter (Millipore, Bedford, MA, USA) and degassed under vacuum prior to use.

Animals

Male Swiss Webster mice, 9–10 weeks of age (Taconic Farms, Germantown, NY, USA), average weight 35 g, were maintained in the University animal care facility. The control group was given a regular rodent diet and the vitamin C group was fed a vitamin C-rich diet (1%, w/w, Dyets, Bethlehem, PA, USA). Both groups were provided water *ad libitum*. At the designated days

post-initiation of the feeding experiment (days 3, 6, 8, 12, 29), mice in groups of five were sacrificed by diethyl ether anesthesia and the plasma and testes assayed for AA and DHAA.

Chromatography

Isocratic analyses were carried out with a Waters Series 501 HPLC pump equipped with Model U6K Universal liquid chromatograph injector and Model 440 absorbance detector operating at 254 nm at a sensitivity of 0.016 a.u.f.s. A Model 740 data module was used for peak integration. All these systems were from Waters Assoc. (Milford, MA, USA). The separations were carried out on a μBondapak C_{18} column (average particle size 10 μm, 30 cm × 3.9 mm I.D.) from Phenomenex (Ranchopalos Verdes, CA, USA). Alternatively, the separations were also carried out on a Waters Radial-Pak liquid chromatography cartridge (average particle size 10 μm, 10 cm × 8 mm I.D.) mounted on a Z-module radial compression separation system. A C_{18} Bondapak guard column was used in all the HPLC runs. The mobile phase was a buffer consisting of 0.1 *M* sodium dihydrogenphosphate (NaH_2PO_4) and 0.2 m*M* Na_2EDTA, adjusted to pH 3.1 with orthophosphoric acid. The buffer was filtered through a Millipore HA 0.45-μm filter and degassed each day prior to use. The column was maintained at ambient temperature and the mobile phase was used at a constant flow-rate of 1.0 ml/min.

Standard preparation

A sample buffer of 5 m*M* each of metaphosphoric acid and Na_2EDTA was prepared in water and used for preparing standard AA as well as biological samples. This buffer was found to stabilize the AA over the entire period of assay (3–4 h). A standard stock solution of ascorbic acid was prepared in this buffer (1 mg/ml) and was further diluted in the same buffer to various concentrations ranging from 75 to 8000 ng/ml. Samples were injected in a 10-μl volume using a precision-crafted Hamilton syringe (Microliter No. 901, Hamilton, Reno, NV, USA). A standard calibration plot was obtained for AA concentrations (ng/ml) *versus* peak area (numerical units on 740 data module). Multiple plots were constructed on different days using freshly prepared samples.

Plasma sample preparation

Using a heparinized syringe, about 200 μl of blood were collected via cardiac puncture and dispersed into a 1.5-ml capped vial containing 1 mg of potassium metabisulphite and a drop of heparin. After centrifuging at 1000 *g* (Model TJ-6, Beckman Instruments, Palo Alto, CA, USA), 50 μl of plasma were added to 150 μl of sample buffer. This mixture was centrifuged at 1000 *g* for 10 min at 0°C. The resulting supernatant was stored at −20°C, and a 10-μl aliquot was injected for analysis within 3 h. For the determination of DHAA, 50 μl of plasma were treated in a similar way, except that the sample buffer contained 5 m*M* DTT as a reducing agent.

Testis sample preparation

Two testes from each mouse were excised, blotted dry of blood and weighed immediately. They were transferred to a 20-ml test tube, and 3 mg of potassium metabisulphite and 5 ml of sample buffer were added. The testes were homogenized (Type PT 10/35 Kinematica, Switzerland) at a speed setting of 7.5 for 1 min. The homogenate was thoroughly vortex-mixed and centrifuged at 1000 *g* for 10 min at 0°C. A 50 μl aliquot of the clear supernatant was processed in the manner described above for plasma AA and DHAA assays.

AA content of vitamin C-rich diet

A known amount of finely ground vitamin C-enriched rodent feed was homogenized in the sample buffer and the homogenate was treated in a manner similar to plasma and testis samples. Assays were carried out periodically over a period of 29 days and revealed no significant variation in the AA content (1%, w/w).

Statistical analysis

Testes and plasma homogenates were prepared and kept at 0°C for 24 h to allow the AA of endo-

genous origin to decay (sample staling). Two standard solutions (500 and 4000 ng/ml) were prepared with these homogenates and were used to evaluate the precision and accuracy of the assay method. The assays were repeated five times and accuracy was calculated as the percentage difference between the mean calculated concentration and the actual concentration of AA in the solutions. Precision was estimated from the relative standard deviation expressed as a percentage of the mean.

The method detection limits (MDL) were generated as described by Inman and Rickard [36] by varying n replicates in the equation

$$\text{MDL} = \frac{k\sqrt{2}}{\sqrt{n}} \times S_m$$

where k is a proportionality constant, corresponding to the z value of the normal distribution which corresponds to >95% confidence (*i.e.*, $k = 2$), and S_m is the standard deviation of the method. The S_m was determined as follows. Spiked homogenates were prepared as described above to a concentration approximately two times the detection limit (300 ng/ml). Samples were prepared on five separate days, the concentration was measured, and S_m calculated.

To compare the AA levels between the control and the vitamin C diet groups, the standard errors were calculated for each measurement and the Student's t-test was used as a test of significance. The difference was considered significant when the p value was <0.05. The intra- and inter-assay variations were checked in a similar manner used in checking the accuracy and precision of the method.

RESULTS AND DISCUSSION

Assay of AA and DHAA

Development of assay buffer. A few closely related buffer systems are reported for the analysis of vitamin C in biological samples. Nagy and Degrell [29] made use of a buffer system of 0.1 m*M* Na_2HPO_4, 0.07 m*M* EDTA and 0.15 m*M* sodium octyl hydrogensulphate at pH 3.1 with orthophosphoric acid for reversed-phase HPLC of vitamin C. Although this system is reportedly useful for electrochemical detection, it was not suited for UV detection of AA owing to interference of the sample buffer. Use of organic modifiers was also not helpful. Hallstrom *et al.* [37] employed a reversed-phase system consisting of 0.1 *M* phosphate at pH 2.4; however, the biological samples had to be dialyzed prior to their injections. Based on these two systems, we developed a new buffer system consisting of 0.1 *M* di-

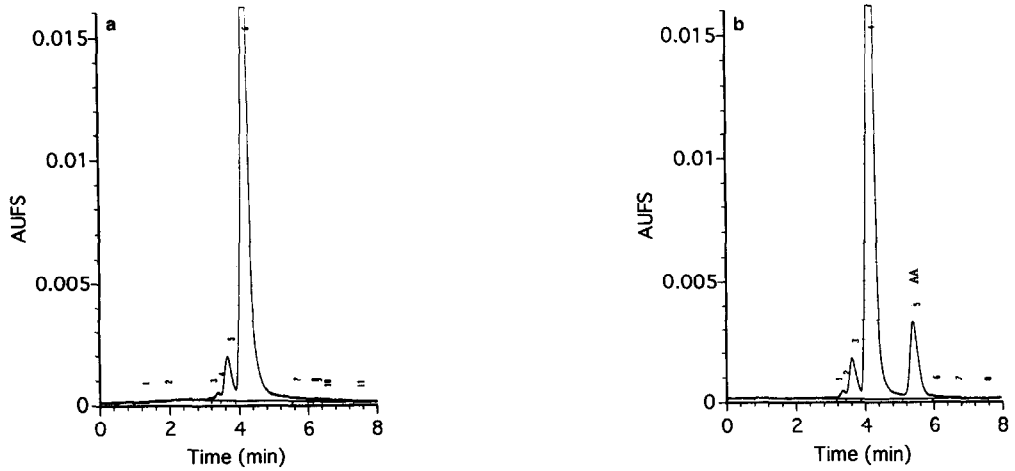

Fig. 1. (a) HPLC elution profile of the sample buffer (5 m*M* metaphosphoric acid, 5 m*M* Na_2EDTA). Note the main buffer peak at 4.25 min and a return to baseline thereafter. (b) Elution profile of authentic ascorbic acid (AA) dissolved in the sample buffer. Note the clear separation between the AA (5.75 min) and buffer peaks.

hydrogen phosphate and 0.2 m*M* Na_2EDTA adjusted to pH 3.1 with orthophosphoric acid. With this system a baseline resolution of the AA peak from all interfering peaks could be achieved in undialyzed plasma as well as testicular samples, when monitored at 254 nm. Quantitation of DHAA could also be done by this method. DHAA has no significant absorption at 254 nm and, in this system, does not interfere with the AA peak. Thus, after the AA assay, samples were reduced with DTT to convert all DHAA to AA and were analyzed for total AA. The advantages of this method are the direct assay of AA and DHAA under UV detection without a need for pre-column derivatization.

Calibration plot of standard AA solutions. Fig. 1 shows the HPLC profiles of authentic AA preparations. In Fig. 1a the elution profile of the blank sample buffer is shown. In Fig. 1b the peak due to authentic AA is seen at 5.75 min and is clearly separated from the peak of the sample buffer. Addition of an equal quantity of DHAA to this solution did not change the area of the AA peak. However, when DTT was added to reduce DHAA to AA, a proportionate increase in the AA peak area was noticed (data not shown). A

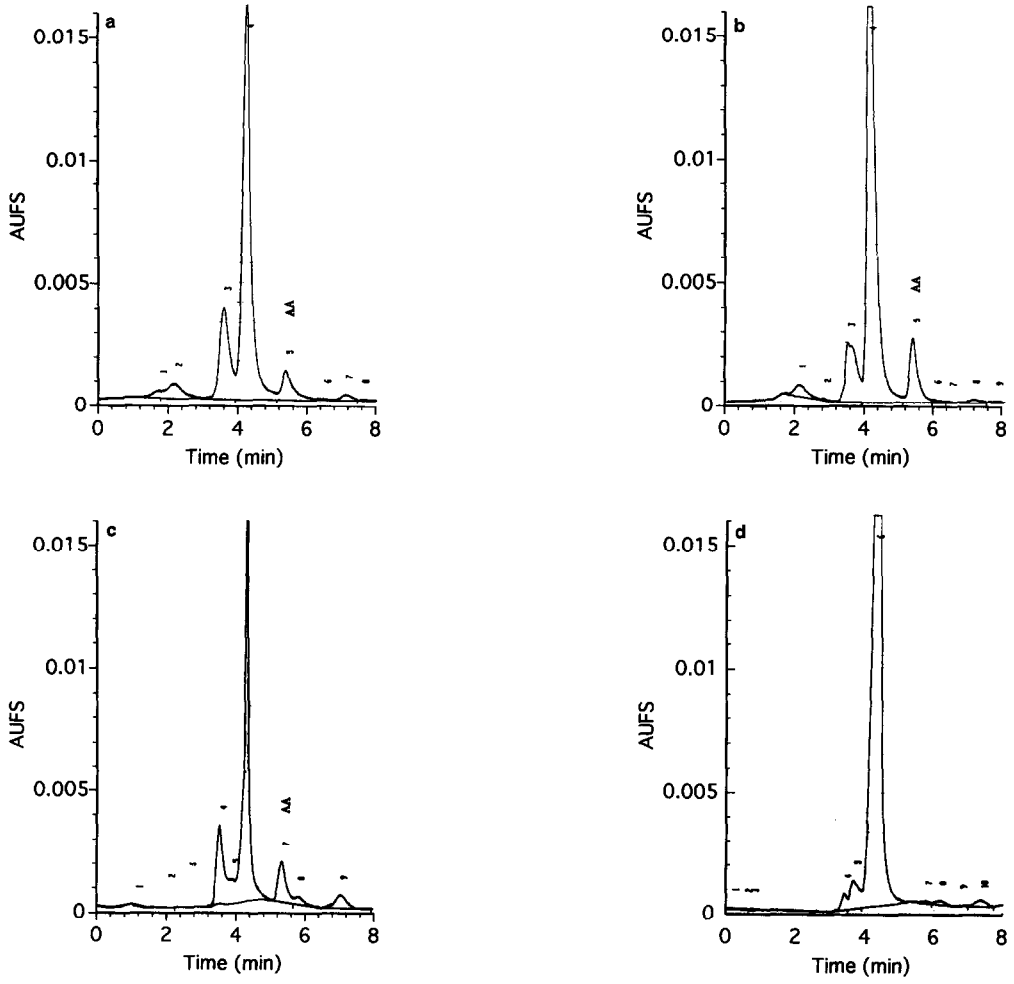

Fig. 2. HPLC elution profiles of mouse plasma samples prepared in the sample buffer. (a) Normal processed plasma. Note the fully resolved AA peak at an elution time of 5.75 min. (b) Plasma sample after spiking with a small known amount of AA. A proportionate rise in the peak area of AA is noted. (c) Plasma sample after reduction with DTT for the assay of total AA. A small increase in the peak area of AA compared to that in (a) is noticed. This represents the additional contribution from DHAA. (d) Effect of storing the plasma sample at 0°C for 24 h. Note a complete disappearance of the AA peak.

standard calibration plot of AA was obtained on three different occasions during the entire time span of the experiments using freshly prepared AA samples. A linear response was observed over the concentration range of 75–8000 ng/ml (injection volume of 10 μl) and followed the equation peak area = 27.2 C, where C is the concentration of AA in ng/ml. The sample buffer was found to stabilize AA solutions over 3–4 h and hence all assays were carried out within that period.

Assay of plasma and testicular AA. The chromatograms for the plasma and testis sample preparations are shown in Figs. 2 and 3, respectively. Figs. 2a and 3a represent the chromatograms for normal plasma and testis samples, respectively, whereas Figs. 2b and 3b are the corresponding chromatograms after spiking with a known amount of AA. The increase in peak area corresponded to the amount of added AA. Figs. 2c and 3c are the respective chromatograms for plasma and testis samples processed for total AA (*i.e.* AA + DHAA) after reduction of DHAA

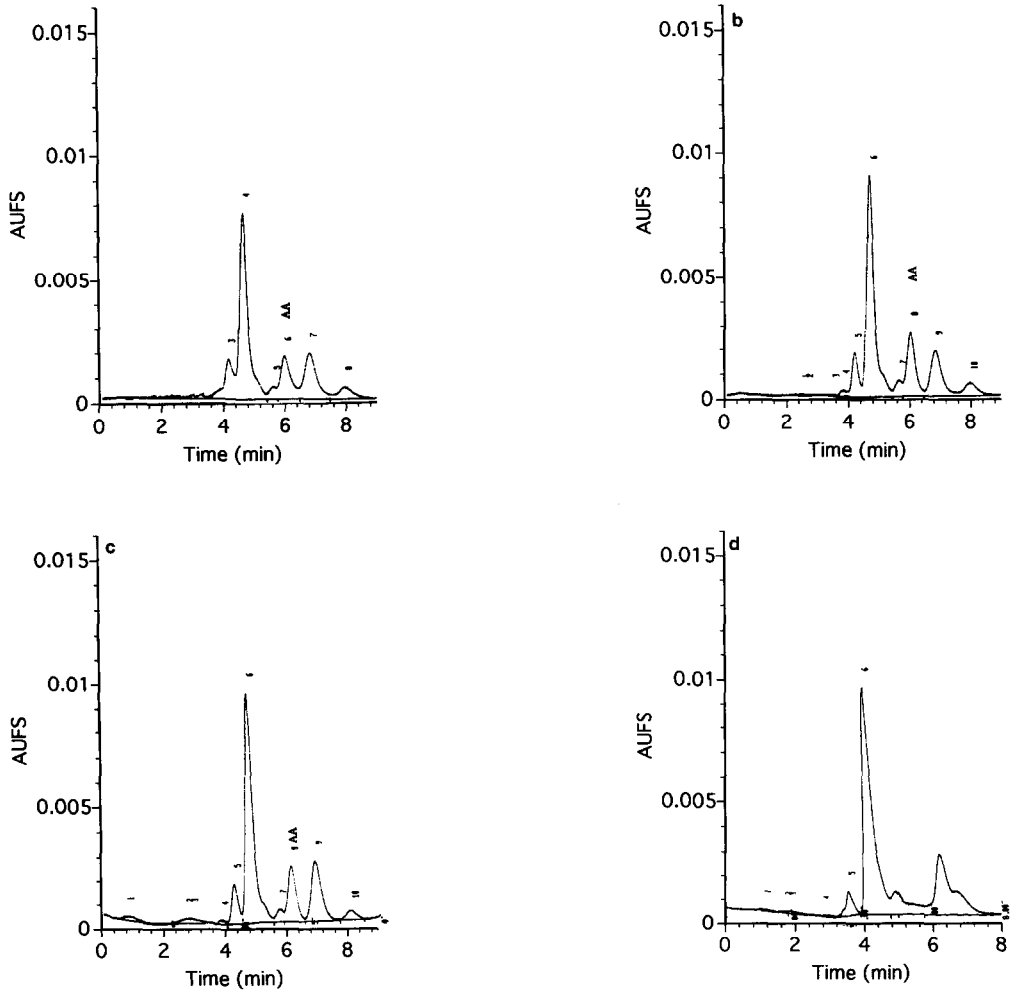

Fig. 3. HPLC elution profiles of testicular samples prepared in the sample buffer. (a) Normal testicular homogenate. Note the clearly resolved AA peak at 5.75 min that is in between two peaks of endogenous origin. (b) Testicular homogenate after spiking with a small known amount of AA. A proportionate rise in the peak area of AA is apparent. (c) Testicular sample after reduction with DTT for the assay of total AA. A small increase in the peak area of AA compared to that in (a) is noticed. (d) Effect of storing the testicular sample at 0°C for 24 h. A complete disappearance of the peak due to AA is evident.

TABLE I

PRECISION AND ACCURACY IN THE ASSAY OF AA IN SPIKED TESTES AND PLASMA HOMOGENATES

Concentration added (ng/ml)	Lot No.	Mean concentration measured (ng/ml)	Precision (% of mean)	Accuracy (%)
Testis samples				
500	1	496	1.13	−0.72
	2	491	0.78	−1.80
	3	501	0.93	+0.24
4000	1	3990	0.12	−0.26
	2	3991	0.13	−0.24
	3	4004	0.11	+0.11
Plasma samples				
500	1	499	0.82	−0.16
	2	499	0.58	−0.28
	3	499	0.85	−0.24
4000	1	3997	0.12	−0.10
	2	3995	0.08	−0.13
	3	4003	0.06	+0.14

with DTT. An increase in the peak area was noticed without a change in peak shape. Storing of the above samples at 0°C for 24 h (sample staling) results in a complete decomposition of AA as indicated by the complete disappearance of the AA peaks in Figs. 2d and 3d. The above observations revealed the following facts about this assay. First, the AA in these preparations is extremely unstable (Fig.4), regardless of the sample buffer used. Second, the chromatograms of plasma samples are free of many of the peaks found in the testicular samples. However, there is no other peak coeluting with the AA peak in either sample. The accuracy and precision data for the plasma and testis samples are presented in Table I and the detection limits for assays performed are presented in Table II . The detection limits for the testis and plasma homogenates were similar.

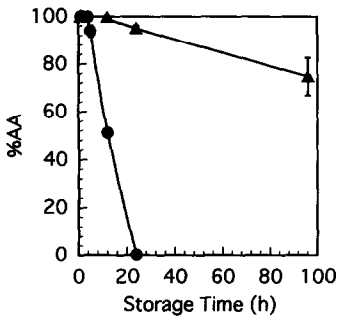

Fig. 4. Stability of ascorbic acid prepared in sample buffer as a function of storage time at −20°C. The triangles represent standard solutions of ascorbic acid whereas the circles are for the plasma and testis samples in the same sample buffer. Note the ascorbic acid in the biological samples is stable for 4 h at −20°C.

Peak authenticity. It is normal practice to em-

TABLE II

METHOD DETECTION LIMITS IN PLASMA SAMPLES

Method detection limits for testis were similar to those for plasma.

$$\text{MDL} = \frac{k\sqrt{2}}{\sqrt{n}} \times S_m$$

where S_m = 61.4 ng/ml (sample strength 300 ng/ml).

n	MDL for >95% confidence (ng/ml)
1	174
2	123
3	100
5	78
7	67
10	55

ploy a suitable internal standard in the assay of biological samples. However, in the case of vitamin C, the peak authenticity is established by sample spiking. In the present study, sample spiking (linear increase in AA peak area), sample reduction by DTT (linear increase in AA peak area) and sample staling (disappearance of AA peak) have clearly established the authenticity of the AA peak.

Sample recovery. In order to determine losses due to decomposition of AA during handling and sample preparations, recovery experiments were carried out. A known amount of AA was added to blood and testicular homogenate of pre-determined AA content. The samples were processed in the usual way and the amount of AA was estimated. The excess AA in these samples corresponded to the added amount of AA and thus sample recoveries ranged from 99 to 102%. However, when samples were processed beyond a delay of 4–5 h, sample recoveries tended to drop significantly (Fig. 4). Therefore, the maximum time the plasma and testis samples could be stored was 4 and 5 h, respectively. Interestingly, the standard AA solutions seemed to be more stable than the biological samples. This is perhaps due to enzymatic oxidation of AA in plasma and testis samples. Hence, all assays were performed immediately after preparation of the samples.

AA and DHAA levels in plasma and testis

Plasma AA levels following dietary intake. The variations in plasma AA levels following dietary intake, are shown in Table III. The control groups maintained nearly constant total AA levels (8.3 ± 0.3 μg/ml of plasma) although the relative amount of the reduced form varied. In the experimental groups, however, the total AA levels doubled by the 3rd day and reached a maximum by day 8. The levels then dropped slightly down to 16.2 μg/ml by day 12.

Following its dietary intake, AA is catabolized to carbon dioxide and the excess vitamin is excreted in urine. However a small amount of exogenous AA is known to equilibrate with the body stores, and hence the dietary intake has to be substantial in order to see a significant change in tissue AA levels [38,39]. In our studies, the plasma levels of AA in the vitamin C diet group were higher (20.2 ± 2.1 μg/ml) than the values reported by Tsao *et al.* [21] (13.9 ± 6.6 μg/ml). However, the present studies consisted of feeding the animals the vitamin C-rich diet (1%, w/w) for only 12 days, whereas the studies of Tsao *et al.* [21] were for a long-term feeding of 4 months. It is probable that such a long-term feeding may have resulted in an equilibrium state where the endogenous synthesis and exogenous intake balanced catabolism and storage of AA in the body.

TABLE III

PLASMA AA LEVELS IN MICE FOLLOWING DIETARY INTAKE

Days 3, 6, 8 and 12 are post-initiation of 1% vitamin C diet.

Compound	Concentration (mean ± S.D., n = 5)[a] (μg/ml)			
	Day 3	Day 6	Day 8	Day 12
Control Group				
AA	8.8 ± 1.4	8.2 ± 1.9	2.9 ± 1.9	–
AA + DHAA	8.9 ± 1.2	8.4 ± 1.5	8.0 ± 1.0	8.1 ± 1.2
Vitamin C Group				
AA	16.0 ± 3.7	14.6 ± 6.3	14.7 ± 3.7	14.6 ± 9.3
AA + DHAA	16.0 ± 2.8	15.1 ± 3.3	20.2 ± 2.1	16.2 ± 3.3

[a] Significance of the difference between control and experimental values $0.01 < p < 0.05$.

TABLE IV

TESTICULAR AA LEVELS IN MICE FOLLOWING DIETARY INTAKE

Days 3, 6, 8, 12 and 29 are post-initiation of 1% vitamin C diet.

Compound	Concentration (mean ± S.D., $n = 5$)[a] (μg per 100 mg)				
	Day 3	Day 6	Day 8	Day 12	Day 29
Control Group					
AA	–	9.5 ± 2.2	10.9 ± 1.9	11.9 ± 1.3	12.4 ± 1.2
AA + DHAA	12.9 ± 1.2	10.8 ± 1.1	13.2 ± 1.0	14.2 ± 1.0	14.9 ± 0.1
Vitamin C Group					
AA	10.8 ± 1.9	14.0 ± 1.2	12.5 ± 1.4	16.0 ± 2.5	15.2 ± 0.1
AA + DHAA	12.9 ± 1.0	14.3 ± 0.2	14.3 ± 0.9	17.5 ± 2.3	15.7 ± 0.1

[a] Significance of the difference between control and experimental values $0.01 < p < 0.05$.

Testicular AA levels following dietary intake. The effect of dietary vitamin C on testicular levels in mice are presented in Table IV. The control groups showed a small increase in AA levels from about 13 μg to 14.9 μg per 100 mg. This is perhaps attributable to their advancing age. The vitamin C diet group also showed a similar trend and reached levels of *ca.* 15.7 μg per 100 mg. However, between days 3 and 12, these animals showed significantly higher AA levels than the controls. Table V provides a comparison of testicular AA levels observed in the present studies with those in the literature. It should be noted that all others have made use of non-HPLC methods of assay. As can be seen from the table, our values closely compare with the data from others. To the best of our knowledge, this is the first report of the use of HPLC in the assay of testicular AA levels.

From the data in Tables III and IV it is clear that the variations in testicular AA levels in mice are far less than the changes in plasma levels as a result of dietary intake of vitamin C. The biochemical regulation of testicular AA is of interest in that the testis do not synthesize AA. However, it is reported that AA is synthesized in the mouse liver [40,41]. All other organs get their required amount of AA through the blood supply. In the present studies, although an increase in plasma AA levels following dietary intake paralleled a rise in testicular levels, the magnitude of the testicular rise was minimal (1.2-fold) compared to the increase in plasma levels (2.5-fold). Previous experiments in mice have shown that the AA lev-

TABLE V

ASCORBIC ACID CONTENTS IN NORMAL RODENT TESTES

Ref.	Rodent studied	AA content (μg per 0.1 g tissue)	Method of assay
44	Swiss albino mice (8–9 weeks)	13.6 ± 0.5	Dichlorophenolindophenol
45	Albino mice	51.7 ± 0.02	DNP derivative
46	Swiss albino mice	11.5 ± 0.6	DNP derivative
Present studies	Swiss Webster	13.2 ± 1.4[a]	RP HPLC UV

[a] The average values (mean ± S.D.) are determined over a 29-day growth period.

els in brain, [42,43], adrenal glands, and white blood cells, [21] vary within relatively narrow limits following dietary intake of AA. Our present studies show that the testicular AA levels also change within a very narrow range, unlike the variations in plasma levels. The relative concentrations of AA in these organs is probably related to its slow turn over.

Vitamin C as a radioprotector. For protection against incorporated radionuclides [19,20], where the radiation dose is delivered to the tissues in a chronic fashion, it is preferable to maintain a fairly steady *in vivo* concentration of the vitamin. When AA is administered repeatedly by injection, organ levels may vary dramatically. Conversely, when it is taken in with the diet, the intake is more uniform over the entire duration of the chronic radiation exposure. Hence, when vitamin C is utilized as a radioprotector, the dietary mode of administration of AA should be preferred over bolus injections. Our present studies clearly indicate that, within the first 12 days of commencement of the vitamin C diet, there is a significant body uptake of AA, as reflected by the plasma levels. Since there is no barrier between blood and bone marrow, plasma levels of AA are a reflection of its levels in bone marrow. Therefore, the elevated AA levels in the plasma (Table III) seem to be responsible for the radioprotection of the bone marrow observed in our earlier studies [20]. Similarly, the protection of spermatogonia by dietary vitamin C also seems to be due to somewhat higher levels of the chemical in the testis (Table IV) during the period of chronic irradiation [19]. Further studies on the radioprotective mechanisms of dietary vitamin C are currently in progress.

CONCLUSIONS

The HPLC assay procedure reported here for AA is facile in nature and provides unambiguous determination of both ascorbic acid and dehydroascorbic acid in mouse plasma and testis. The dietary intake of ascorbic acid seems to have a profound effect on plasma AA levels, resulting in a 2.5-fold rise. This suggests that a feedback inhibition of AA appears to operate in plasma. In the testis, however, the AA levels increase marginally (1.2-fold) as a result of the dietary intake. Thus, besides brain, adrenals, and white blood cells, the testis also maintain steady levels of AA regardless of the dietary intake of vitamin C. The findings may have clinical significance in that testicular AA levels do not change appreciably as a result of dietary intake of vitamin C, where as in plasma, AA levels do show an increase.

ACKNOWLEDGEMENTS

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