Quantitative Aspects of Ascorbic Acid Metabolism in Man

G. L. Atkins,* Betty M. Dean, Wendy J. Griffin, and R. W. E. Watts†

From the Medical Professorial Unit (Dunn Laboratories), St. Bartholomew's Hospital, London E.C.1, England.

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Ascorbic acid is catabolized to yield oxalate from carbon atoms 1 and 2 (1, 2) and carbon dioxide from carbon atom 1 in man as well as in other species (3–5). It has also been shown that n-glucuronolactone is converted to ascorbic acid in the human (6).

The present studies were designed to determine the l-ascorbic acid metabolic pool size and turnover rate, the fraction of the urinary oxalate which arises from ascorbic acid, and the fractions of the total ascorbic acid turnover which give rise to urinary oxalate and urinary ascorbic acid, respectively. We used l-ascorbic acid-1-13C and hence did not study the incorporation of the isotope into respiratory CO2 because of the extensive dilution of the isotope by CO2 arising from other metabolic pathways. The importance of ascorbic acid metabolism other than by renal excretion or oxalate formation was evaluated, and our mathematical analysis also takes account of ascorbic acid arising from sources other than the prescribed dietary intake. Oxalate is metabolically inert in animals and man (7–9), and our results enable us to compare the order of magnitude of the oxalate metabolic pool and its turnover rate with the corresponding parameters for ascorbic acid. The proportion of the urinary oxalate which was derived from glycine, which is another major precursor of urinary oxalate (7, 10, 11), was also determined in the same subject.

The measurement of a metabolic pool size and turnover rate by isotope dilution analysis involves sampling the metabolic pool. The ascorbic acid metabolic pool cannot be sampled directly in the human, and although the plasma ascorbic acid is presumably in equilibrium with that in the metabolic pool, the plasma ascorbic acid concentration is too low to permit the isolation of sufficient amounts of a derivative for mass spectrometric analysis. The urinary ascorbic acid is separated from the plasma by glomerular filtration and therefore samples the plasma ascorbic acid, and hence the ascorbic acid metabolic pool. Our chemical isolation procedure does not, however, distinguish between L-ascorbic acid, dehydro-L-ascorbic acid, and 2,3-diketo-L-gulonic acid which are present in urine. The use of the uncombined urinary glycine as a sample of the glycine metabolic pool which gives rise to oxalate has been discussed elsewhere (12).

**EXPERIMENTAL PROCEDURE**

*Materials*

Sodium cyanide-13C (50 atom % excess of 13C, supplied by Merck Sharp and Dohme, Canada) was converted to L-ascorbic acid-1-13C (13), which was recrystallized once from 1-butanol and once from glacial acetic acid (27% yield based on NaCN). The product, m.p. 188–190°, [α]D +23.5 ± 1° (c, 0.7 in water), had the following elemental analysis (allowing for the enrichment with 13C).

\[
\text{C}_6\text{H}_8\text{O}_6\text{N}
\]

Calculated: C 41.1%, H 4.6%
Found: C 41.1%, H 4.8%

Analysis by the method of Hughes (14) gave a purity of 99% (±2.5%), confirming that no dehydroascorbic acid was present. The L-ascorbic acid-1-13C contained 48.13 atom % excess of 13C (mean of six determinations by dry combustion) and formed a bis-2,4-dinitrophenylhydrazone in 3 x HCl (red needles, m.p. 283–284°, recrystallized from acetone-ethanol, 1:1) which had the same 13C enrichment as the parent compound, allowing for dilution by 12 additional carbon atoms. Glycine-1-13C was synthesized from Ba13CO3 (12).

\[
\text{C}_2\text{H}_7\text{O}_3\text{N}
\]

Calculated: C 32.4, H 6.7%
Found: C 32.3, H 6.8%

It gave a single spot corresponding to authentic glycine when tested chromatographically, and contained 42.8 atom % excess of 13C (mean of three determinations by dry combustion).

*Experimental Methods*

The subject was a normal, healthy man, aged 26 years; weight, 58 kg; height, 179 cm.

**Experiments 1 and 2**—The subject was shown to be saturated with respect to ascorbic acid (15) on the 6th day before the experiments were begun. Subsequent to this, he took a diet from which oxalate-rich foods (rhubarb, strawberries, beets, cabbage, chocolate, cocoa, spinach) and food containing more than 10 mg of ascorbic acid per 100 g, fresh weight (16), were omitted. Unlabeled L-ascorbic acid (50 mg daily by mouth) was taken during the 5-day equilibration period after the ascorbic acid saturation test, and throughout each study except on Day 1, when the subject took L-ascorbic acid-1-13C (50 mg, 0.86 mg per kg of body weight, 48.1 atom % excess of 13C (single oral dose)) dissolved in water. Urine collections were 24-hour specimens in both experiments except for the first 3 days of Experiment 2, when 12-hour samples were used. Urine was voided directly into a bottle containing 50 ml of 6 N HCl per 24-hour collection. The urinary oxalic acid and ascorbic acid were isolated and their 13C contents were determined. Experiment 2 was begun 15 months after the start of Experiment 1.

**Experiment 3**—The subject took a repetitive diet containing...
overnight. The precipitated 2,4-dinitrophenylhydrazones were washed with 3 to about 400 ml at 35° under 1 to 5 mm of Hg, treated with 10 g of 2,4-dinitrophenylhydrazine for 3 hours at 100°, and cooled. The dried product (Rr 0.65; 50 to 100 mg) was dissolved in ethyl acetate and the orange product, R.P 0.88, was converted to the red form, RF 0.65, by treatment with boiling HCl (2 ml), and water (20 ml) and dried, and the solvent was removed. The residue was dried to constant weight at room temperature. Yield, 10 to 20 mg of ascorbic acid bis-2,4-dinitrophenylhydrazone per urine sample. All the specimens were chromatographically pure (RF 0.65) in the solvent system described above.

Isolation of Urinary Ascorbic Acid—Sufficient concentrated HCl was added to the urine samples to bring the concentration to approximately 2 N, and the acidified urine was extracted with peroxide-free ether for 16 hours (17). Water (5 ml) was added to the extract, the ether was removed at 37°, and the aqueous residue was adjusted to pH 8 with NH4OH (sp. gr. 0.880). Oxalate was isolated as Ca(CO3)2·H2O by the method described previously (11) but without the Li2CO3 wash. The purity of the product was greater than 95% as estimated by reduction of a portion to glycolic acid followed by colorimetric determination with chromatropic acid (18). We confirmed that less than 5% of the l-ascorbic acid and 2,3-diketogulonic acid in the urine were converted to oxalic acid under the conditions used for the oxalate isolations.

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Isolation of Urinary Ascorbic Acid (as Bis-2,4-dinitrophenylhydrazones) in Experiment 1—Bromine (2 ml) was added to the urine aqueous phase remaining after the removal of oxalic acid, and after standing for 1 hour the solution was concentrated to about 400 ml at 35° under 35°. Water (5 ml) was added to the extract, the ether was removed at 37°, and the aqueous residue was adjusted to pH 8 with NH4OH (sp. gr. 0.880). Oxalate was isolated as Ca(CO3)2·H2O by the method described previously (11) but without the Li2CO3 wash. The purity of the product was greater than 95% as estimated by reduction of a portion to glycolic acid followed by colorimetric determination with chromatropic acid (18). We confirmed that less than 5% of the l-ascorbic acid and 2,3-diketogulonic acid in the urine were converted to oxalic acid under the conditions used for the oxalate isolations.

Isolation of Urinary Ascorbic Acid (as Bis-2,4-dinitrophenylhydrazones) in Experiment 2—Formation of the orange product (RF 0.88) was avoided here by recovery of the acidic 2,4-dinitrophenylhydrazones from Al2O3 with cold acetone-concentrated HCl (95:5; five 100-ml volumes), and the Na2CO3 washing process was performed after recovery of the band with RF 0.65 from eight sheets of Whatman No. 17 paper. The final purification on Florisil was omitted and replaced by separation on an acid-washed Al2O3 column (42 cm) essentially by the method of Mapenn (19), with the modification that l-ascorbic acid bis-2,4-dinitrophenylhydrazones, when separated as an orange band 20 to 30 cm below the top of the column, was cut out and recovered with cold acetone-concentrated HCl (95:5; three 50-ml volumes). The washings were evaporated to dryness below 37°, and the residue was partitioned between ethyl acetate (80 ml) and water (80 ml). The ethyl acetate phase was washed with water (50 ml), dried, and evaporated. The final yield of l-ascorbic acid bis-2,4-dinitrophenylhydrazones after recrystallization from ethanol-acetone (1:1) was 5 to 10 mg.

Isolation of Free Urinary Glycine (as 2,4-Dinitrophenylglycine)—This derivative was isolated from the aqueous phase which remained after removal of the oxalic acid (12) and recrystallized from ethyl acetate-cyclohexane (1:9).

Isotope Determinations—Calcium oxalate-13C was oxidized to 13CO2 (11). L-Ascorbic acid-13C bis-2,4-dinitrophenylhydrazone was degraded reductively to yield 13CO2 selectively from carbon atom 1. The derivative (4 to 8 mg), dissolved in acetone, was introduced into a 10-ml pear-shaped flask, the solvent was removed by gentle suction, and a solution of SnCl2·2H2O in constant boiling HCl (10%, w/v; 1 to 2 ml) was added. The flask was connected to a vacuum manifold via a reflux condenser 10 cm long and a horizontal tube (10 cm long) containing a layer of ZnO. The apparatus was flushed with dry helium for 10 minutes, closed to the atmosphere, and opened to the vacuum line (gas flow rate, 20 ml per minute). The flask was heated gently for 3 hours. Traces of HCl were removed by the ZnO trap; water vapor was retained in a solid CO2-ethanol-cooled spiral trap. The 13CO2 was collected in two spiral traps cooled with liquid O2 and its volume was measured (20). The yields of 13CO2 corresponded to 80 to 95% of the volumes obtained by continuing the reaction for 24 hours (100% theoretical). The observed values for the 13C enrichments were multiplied by a factor of 1.045 ± 0.022 (seven determinations) to allow for traces of CO2 derived from sources other than carbon atom 1 of ascorbic acid.

The 2,4-dinitrophenylglycine-13C was degraded to 13CO2 (20). Determinations were duplicated except for some samples of l-ascorbic acid bis-2,4-dinitrophenylhydrazone, of which insufficient material was available. The 13C enrichments were...
EXPERIMENT 1

FIG. 1. Change with time of the isotope contents (atom per cent excess of $^{13}$C plotted as log, $a$) of the urinary ascorbic acid and urinary oxalate following a single oral dose of ascorbic acid-$^{13}$C (50 mg; 48.13 atom % excess of $^{13}$C; Experiments 1 and 2). $\circ$ --- $\circ$, ascorbic acid; $\Delta$ --- $\Delta$, oxalate. In Experiment 1, the changes in isotope content of the urinary ascorbic acid ($S_a$) and oxalate ($S_b$) with time are described by the equations

$$\log S_a = -0.0139 t - 0.0538 \quad (5)$$

$$\log S_b = -0.0110 t - 0.5097 \quad (6)$$

measured with a Consolidated Nier mass spectrometer, model 21-201.

Estimation of Urinary Oxalate and Ascorbic Acid—Urinary oxalic acid was determined by isotope dilution analysis with oxalic acid-$^{14}$C in Experiments 2 and 3. The values for the oxalic acid excretion in Experiment 1 were calculated from the weight of Ca(CO$_3$)$_2$·H$_2$O, which was isolated by applying a correction factor of 1.37 ± 0.11 (14 determinations) derived from data obtained in Experiment 3.

Urinary ascorbic acid was determined on 1- to 2-ml aliquots of undiluted urine by the method of Roe and Kuether (21), which does not distinguish between $L$-ascorbic, dehydro-$L$-ascorbic, and 2,3-diketo-$L$-gulonic acids.

RESULTS

Experiments 1 and 2

The isotope enrichments of the urinary $L$-ascorbic acid and urinary oxalate both decreased as single exponential functions with respect to time (Fig. 1). Each value has been plotted at the midpoint of the corresponding urine collection period, and the lines are the straight lines calculated to give the best fit to the observed points, with the omission of those enclosed in brackets.

The over-all metabolism of $L$-ascorbic acid in man can be represented schematically (Fig. 2). We have assumed that the system was in a steady state during our experiments, and that equilibrium mixing of the ascorbic acid-$^{13}$C in the system was achieved immediately. In the following equations, $\lambda$ = rate constant (day$^{-1}$) for the total turnover of the $L$-ascorbic acid pool = $k_1 + k_2 + k_3$ (Fig. 2); $A_0$ and $A$ = the isotope content of the $L$-ascorbic acid pool at zero time and time $t$ (atom per cent excess × mmole); $B_0$ and $B$ = the isotope content of the oxalate pool at time $t$ (atom per cent excess × mmole); and $S_{ao}$ and $S_{a}$ = $^{13}$C enrichment of the urinary $L$-ascorbic acid at zero time and time $t$ (atom per cent excess). Hence

$$S_{ao} = \frac{A_0}{A}$$

and

$$S_a = \frac{A_t}{A}$$

Similarly

$$S_b = \frac{B_t}{B} = \delta{C} \text{ enrichment of urinary oxalate at time } t$$

The rate of change of the isotope content of the $L$-ascorbic acid metabolic pool can be expressed by the equation

The corresponding equations for Experiment 2 are

$$\log S_{a1} = -0.0103 t - 0.0866 \quad (7)$$

$$\log S_{b1} = -0.0088 t - 0.4215 \quad (8)$$

The values for the slopes in Equations 6 and 8 lie within the 95% confidence limits of the corresponding values in Equations 5 and 7, respectively.
Similarly, for the oxalate metabolic pool, we have

\[ \frac{dA_t^*}{dt} = -\lambda A_t^* \]

The solutions of these equations are

\[ A_t^* = A_0^* e^{-\lambda t} \]

and

\[ B_t^* = \frac{k_2}{k_4} A_0^* e^{-\lambda t} \]

If \( k_t \gg \lambda \), then

\[ B_t^* = \frac{k_2}{k_4} A_0^* e^{-\lambda t} \]

Substituting for \( A_t^* \) and for \( A \) in Equation 1, and since

\[ a = k_t A \]

\[ S_{sa} = \frac{k_1}{a} A_0^* e^{-\lambda t} \] (3)

Substituting for \( B_t^* \) and for \( B \) in Equation 2, and since

\[ b = k_4 B \]

\[ S_{sb} = \frac{k_2}{b} A_0^* e^{-\lambda t} \] (4)

The $^{13}$C enrichment of both urinary oxalate and urinary L-ascorbic acid should thus be exponential functions of time with the same decay constant and with intercepts

\[ S_{sa} = \frac{k_2 A_0^*}{a} - \frac{A_0^*}{A} \]

and

\[ S_{sb} = \frac{k_2 A_0^*}{b} \]

If \( k_t \) were not large compared with \( \lambda \), we would expect the oxalate $^{13}$C enrichments to show an initial increase to a maximum and then decline exponentially. This is not so (Fig. 1); therefore \( k_t \gg \lambda \). Corroborative evidence supporting this conclusion has been obtained in a study of a hyperoxaluric patient.1

Knowledge of \( \lambda, a, b, S_{sa}, \) and \( S_{sb} \) leads to the evaluation of the following quantities: ascorbic acid pool size, \( A = A_t^*/S_{sa} \); ascorbic acid pool turnover rate = \( \lambda A \); half-life \( (t_\frac{1}{2}) \) of ascorbic acid in pool = \( 0.6932/\lambda A \); fraction of the ascorbic acid turnover which gives rise to urinary oxalate =

\[ \frac{k_2}{\lambda} \left[ \frac{S_{sa} \cdot b}{S_{sb} \cdot b} \right] \]

The fraction of the ascorbic acid turnover which is excreted unchanged =

\[ \frac{a}{\lambda A} \]

The fraction of urinary oxalate derived from the ascorbic acid pool =

\[ \frac{k_2 A}{b} = \frac{S_{sb}}{S_{sa}} \]

The oxalate metabolic pool is

\[ \frac{b}{k_4} \ll \frac{b}{k_1} \]

The results obtained by substitution in these expressions with the use of the weighted mean value \( \bar{\lambda} \) derived from Equations 5 and 6 in Experiment 1 and Equations 7 and 8 in Experiment 2 (Fig. 1) for the rate constant, \( \lambda \), are presented in Table I.

**Experiment 3**

The $^{13}$C contents of the urinary oxalate and glycine reached plateau values on the 3rd and 4th days of the study. The fraction of the urinary oxalate which is derived from glycine is given by the mean ratio of $^{13}$C oxalate enrichment to $^{13}$C glycine enrichment on these days, 0.179 ± 0.007 (four determinations).

**DISCUSSION**

Abt, Von Schuching, and Enns (3) found that 1 to 3 mg of ascorbic acid daily fulfill an adult's metabolic requirement for the vitamin, although the recommended dietary intake based on nutritional data is between 30 and 70 mg (22). It has been shown in the guinea pig (23) that the proportion of an intramuscular dose of ascorbic acid which is retained is inversely related to the animal's ascorbic acid intake. Because such variation may influence the values obtained for the metabolic pool size and turnover rate, it should be emphasized that the values which we have obtained for these parameters only refer to normal human subjects under our experimental conditions, which we consider to involve a clinically adequate vitamin C intake.

Our method of sampling the ascorbic acid metabolic pool does not distinguish between ascorbic acid, dehydroascorbic acid, and 2,3-diketogulonic acid, and our results are therefore comparable in this respect with those obtained by other investigators. The values obtained for the ascorbic acid metabolic pool size and turnover rate are compared in Table II with the results obtained by other workers. Hellman and Burns (2) and Baker et al. (6) found no evidence for the conversion of ascorbic acid-1-14C to CO$_2$, and based their calculations on the difference between the dose and urinary excretion of the isotope. The results of Abt et al. (3) were derived in a similar manner, although they allowed for some excretion of the isotope in respiratory carbon dioxide.

Some of the differences shown in Table II may be due to the different dietary histories of the subjects. However, unlike previous workers, we calculated our results directly from the experimental data on the basis of a model system, which provides for removal of ascorbic acid from the metabolic pool by urinary excretion, oxalate formation, and the production of other metabolites, including carbon dioxide from C-1 of ascorbic acid.

About half the daily turnover (Table I) of ascorbic acid occurs by oxalate formation or the excretion of ascorbic acid, dehydroascorbic acid, and 2,3-diketogulonic acid in the urine. It is unlikely that the remainder represents ascorbic acid deposition for replenishment of the body stores of the vitamin, but our re-
TABLE I

Ascorbic acid metabolic pool size, turnover rate, and fractional turnover to urinary ascorbic acid and oxalate

Standard deviations are given for all the experimental values. The numbers in parentheses are the numbers of experimentally determined points for $A^*$, $a$, $b$, $\lambda$, $S_{50}$, $S_{100}$, and $t_i$ and are the numbers of values used in calculating the standard deviation of the factor contributing most to the over-all error for the other parameters.

<table>
<thead>
<tr>
<th>Experimental data</th>
<th>Symbol</th>
<th>Units</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Ascorbic acid-L-[^13]C dose</td>
<td>$A^*$</td>
<td>atom % excess X mmole</td>
<td>$13.7 \pm 0.52$ (6)</td>
<td>$13.7 \pm 0.52$ (6)</td>
</tr>
<tr>
<td>Daily urinary L-ascorbic acid excretion</td>
<td>$a$</td>
<td>mmole</td>
<td>$0.116 \pm 0.041$ (15)</td>
<td>$0.066 \pm 0.049$ (10)</td>
</tr>
<tr>
<td>Daily urinary oxalate excretion</td>
<td>$b$</td>
<td>mmole</td>
<td>$0.212 \pm 0.037$ (15)</td>
<td>$0.323 \pm 0.037$ (19)</td>
</tr>
<tr>
<td>Rate constant for total turnover of ascorbic acid metabolic pool</td>
<td>$\lambda$</td>
<td>day$^{-1}$</td>
<td>$0.028 \pm 0.0028$ (28)</td>
<td>$0.023 \pm 0.0009$ (36)</td>
</tr>
<tr>
<td>$^13$C enrichment of ascorbic acid metabolic pool at zero time</td>
<td>$S_{50}$</td>
<td>atom % excess</td>
<td>$0.884 \pm 0.075$ (12)</td>
<td>$0.819 \pm 0.059$ (15)</td>
</tr>
<tr>
<td>$^13$C enrichment of urinary oxalate at zero time</td>
<td>$S_{100}$</td>
<td>atom % excess</td>
<td>$0.309 \pm 0.054$ (10)</td>
<td>$0.379 \pm 0.017$ (21)</td>
</tr>
<tr>
<td>Ascorbic acid metabolic pool size</td>
<td>$A$</td>
<td>mmol day$^{-1}$</td>
<td>$15.11 \pm 1.46$ (12)</td>
<td>$16.72 \pm 1.38$ (15)</td>
</tr>
<tr>
<td>Ascorbic acid metabolic pool turnover rate</td>
<td>$\lambda$</td>
<td>mmol day$^{-1}$</td>
<td>$0.437 \pm 0.06$ (28)</td>
<td>$0.387 \pm 0.035$ (36)</td>
</tr>
<tr>
<td>Half-life of ascorbic acid in metabolic pool</td>
<td>$t_i$</td>
<td>day</td>
<td>$24.6 \pm 2.4$ (28)</td>
<td>$30.1 \pm 1.2$ (36)</td>
</tr>
<tr>
<td>Fraction of ascorbic acid pool turnover that gives rise to oxalate</td>
<td>$\frac{S_{50}}{S_{100}}$</td>
<td>mmol day$^{-1}$</td>
<td>$0.17 \pm 0.05$ (15)</td>
<td>$0.40 \pm 0.037$ (10)</td>
</tr>
<tr>
<td>Fraction of ascorbic acid turnover excreted unchanged</td>
<td>$\frac{a}{A}$</td>
<td>day</td>
<td>$0.26 \pm 0.10$ (15)</td>
<td>$0.16 \pm 0.05$ (19)</td>
</tr>
<tr>
<td>Fraction of urinary oxalate derived from ascorbic acid metabolic pool</td>
<td>$\frac{S_{50}}{S_{100}}$</td>
<td>day</td>
<td>$0.35 \pm 0.07$ (16)</td>
<td>$0.46 \pm 0.039$ (15)</td>
</tr>
<tr>
<td>Fraction of ascorbic acid turnover metabolized via other pathways</td>
<td>$\frac{b}{\lambda}$</td>
<td>mmol day$^{-1}$</td>
<td>$0.57 \pm 0.11$ (15)</td>
<td>$0.44 \pm 0.084$ (19)</td>
</tr>
<tr>
<td>Size of oxalate pool</td>
<td>$\ll b/\lambda$</td>
<td>mmol day$^{-1}$</td>
<td>$\ll7.4$</td>
<td>$\ll34.4$</td>
</tr>
</tbody>
</table>

Results are compatible with the observation that ascorbic acid L-[^13]C is metabolized to $^13$CO$_2$ in man (3) as well as in other species (4, 8, 24).

About 40 and 20% of the urinary oxalate were derived from ascorbic acid and glycine, respectively, in the present study, whereas glycine was the precursor of about 40% of the urinary oxalate excreted by another normal subject in a previous similar study (10). It appears that glycine and a C$_3$ fragment derived from carbon atoms 1 and 2 of ascorbic acid are about equally important as metabolic precursors of the urinary oxalate, but that other metabolic pathways may make small but significant contributions. The C$_3$ fragment which is transferred in the transketolase reaction at the oxidation level of glycolic aldehyde (25) may be of importance in this connection, as may the reactions leading from 6-hydroxyglutaric acid to glyoxylate (26). A much smaller fraction of the urinary oxalate was derived from ascorbic acid in a patient with primary hyperoxaluria whose total urinary oxalate excretion was about 5 times the normal amount (27).

Since the isotope enrichment in the oxalate isolated on the first day after ascorbic acid-L-[^13]C was given lies within the 95% confidence limits of the line describing the subsequent experimental values (Fig. 1), the oxalate pool is very small relative to the ascorbic acid pool. This is compatible with the values found by Elder and Wyngaarden (7), of 4.9 and 8.6 mg for the size of the oxalate metabolic pool, and 34 and 63 mg per day for the turnover rate.

Our values for the turnover rate of the ascorbic acid metabolic pool (77.0 and 68.0 mg per day) are larger than the known intake of ascorbic acid (50 mg per day). We consider it very improbable that sufficient ascorbic acid to account for this discrepancy was present in our low ascorbic acid diet, although the presence of some 2,3-diketogulonic acid in the food cannot be excluded.

TABLE II

Comparison of values for ascorbic acid metabolic pool size, turnover rate, and half-life with those obtained by other investigators

<table>
<thead>
<tr>
<th>Source</th>
<th>Pool size</th>
<th>Turnover rate</th>
<th>Half-life</th>
<th>Turnover to respiratory CO$_2$</th>
<th>Ascorbic acid intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>mg/kg/day</td>
<td>days</td>
<td>%</td>
<td>mg/day</td>
</tr>
<tr>
<td>This paper</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td>47.1</td>
<td>1.3</td>
<td>24.6</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Experiment 2</td>
<td>60.7</td>
<td>1.2</td>
<td>30.1</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Hellman and Burns (2)</td>
<td>26</td>
<td>1.4</td>
<td>13</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>1.0</td>
<td>15</td>
<td></td>
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<tr>
<td></td>
<td>19</td>
<td>0.66</td>
<td>20</td>
<td></td>
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</tr>
<tr>
<td>Baker et al. (6)</td>
<td>29.4</td>
<td>0.19</td>
<td></td>
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<tr>
<td></td>
<td>29.5</td>
<td>0.19</td>
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<td></td>
<td>29.7</td>
<td>0.19</td>
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<td>22.5</td>
<td>0.19</td>
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<td>27.6</td>
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<td></td>
<td>20.5</td>
<td>0.19</td>
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<td></td>
</tr>
<tr>
<td>Abt et al. (3)</td>
<td>15</td>
<td>0.35</td>
<td>26.5</td>
<td>53</td>
<td>25</td>
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<td>12.8</td>
<td>0.46</td>
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</table>
The mobilization of stored ascorbic acid is also unlikely because the subject took the 50-mg ascorbic acid supplement daily throughout the study. The observation that D-glucuronolactone is converted to ascorbic acid in man (6, 28) suggests that the lack of L-gulono- 

xidase, which makes L-ascorbic acid an essential nutrient for man and some other species (29), may not exclude some synthesis of the vitamin from suitable precursors in the intact human subject.

SUMMARY

Urinary ascorbic acid, including dehydroascorbic acid and 2,3-diketogulonic acid, has been used to sample the ascorbic acid metabolic pool after the oral administration of a single physiological dose of ascorbic acid-1-13C. The pool size and turnover rate, the fractional rates of turnover to urinary ascorbic acid and oxalate, and the proportion of the urinary oxalate which arises from ascorbic acid catabolism have been determined in two studies on a normal adult male subject. The fraction of the urinary oxalate formed from glycine was also determined in the same subject.

Oxalate formation and urinary excretion of ascorbic acid accounted for about half of the total ascorbic acid turnover. This observation agrees with the findings of other investigators, who showed that the other half of a tracer dose of ascorbic acid gave rise to respiratory CO2 from carbon atom 1. Most of the urinary oxalate arises from ascorbic acid or glycine metabolism; the source of the remainder (about 40% of the total) is not known.

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