C-6 OXIDATION OF ASCORBIC ACID: A MAJOR METABOLIC PROCESS IN ANIMALS

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Summary. Metabolic activity of the C-6 carbon of ascorbic acid has been examined using two experimental methods. 43% of the label from ascorbic-6-^{3}H acid is converted to water in the monkey after i.v. injection. The rate of formation of $^{3}H_2O$ corresponds to the rate of excretion of organic ascorbate metabolites in the urine as measured using ascorbic-6-^{3}H or ascorbic-6-^{14}C acid. Periodate degradations of whole urine from rats or monkeys given ascorbic-6-^{14}C acid by i.v. injection show that 45% of the labeled metabolites do not give formaldehyde from the C-6 carbon, indicating the C-6 carbon is no longer a primary alcohol or that the C-5 and/or C-6 hydroxyl groups are derivitized. There is no significant excretion of $^{14}CO_2$ in these experiments indicating the metabolites do not enter general carbohydrate catabolism. The combination of these results show that about 45% of the C-6 carbon of ascorbic acid is oxidized *in vivo*. Because the monkeys had an adequate dietary intake of ascorbic acid and the rats synthesize ascorbic acid as needed, the results also indicate that the precursor of the oxidation is a slowly exchanging form of ascorbic acid in which the C-6 carbon is in the primary alcohol oxidation state.

Metabolic activity of the side chain of ascorbic acid, carbons 5 and 6, has not normally been considered in ascorbic acid biochemistry in higher animals. Several studies indicate such a process should be further explored. Hornig (1) suggests that the 6-carboxy derivative of ascorbate-2-sulfate might be a metabolite of ascorbic acid. Loewus (2) has suggested oxidation-reduction of the C-5 carbon of ascorbate precursors in some plants. We find the number and diversity of urinary metabolites of ascorbic acid in higher animals is difficult to explain without suggesting C-6 oxidized metabolites (3), (4).

In this paper we describe results from two experimental approaches which provide clear evidence that the side chain of ascorbic acid is metabolically active and subject to C-6 oxidation. Biological implications of these observations are discussed.

MATERIALS AND METHODS

Labeled Compounds. Ascorbic-6-^{14}C acid was made as described (5). Ascorbic-6-^{3}H acid was a gift from Drs. U. Gloor and F. Weber, F. Hoffman-LaRoche & Co., Basle, Switzerland.

Animal Procedures. Macaque monkeys weighing about 3 kg were injected i.v. with labeled ascorbic acid after 12 hours on no food. No food was given for the next 24 hours. They were then fed a normal monkey ration ad lib which provided about 300 mg ascorbic acid per day. 24 hour urines were collected at intervals. Where appropriate, breath $^{14}CO_2$ measurements were made at intervals. Rats were injected subcutaneously with labeled ascorbic acid and 24 hour urines collected. Injected amounts were: monkeys, 253 µCi in 50 mg ascorbic-6-^{3}H; monkeys, 62.3 µCi in 25 mg ascorbic-6-^{14}C; rats, 33.2 µCi in 10 mg ascorbic-6-^{14}C.

Assay of ^{3}H Labeled Urine. Urines were assayed for total radioactivity and for specific activity of water. From these values and urine volumes, 24 hour excretions of tritium in organic forms were calculated.

Assay of ^{14}C Labeled Urines. Total radioactivity was measured. Five to eight ml aliquots were filtered using a UM-2 filter, diluted to 25 ml, and 2.5 mM ascorbic acid added as carrier. Periodate degredation was done as described (6). Formaldehyde was isolated as the dimedone derivative and counted.

RESULTS AND DISCUSSION

Figure 1 shows the urine $^{3}H_2O$ specific activity and organic radioactivity for a monkey given ascorbic-6-^{3}H acid. The curves are parallel and follow a first order process with $T_{1/2}$ of 20 days. A short burst of organic-^{3}H in the initial days of the experiment is believed due to labeled impurities in the sample. In a duplicate experiment with another monkey a $T_{1/2}$ of 17 days was observed. The half time for excretion of ascorbic acid and matabolites has previously been reported as 20 d in both monkey (7) and man (8). On the average 27% of the daily ^{3}H excretion in urine is present as H_2O. In addition the monkey excreted $^{3}H_2O$ as insensible loss from breath and skin. Experiments using

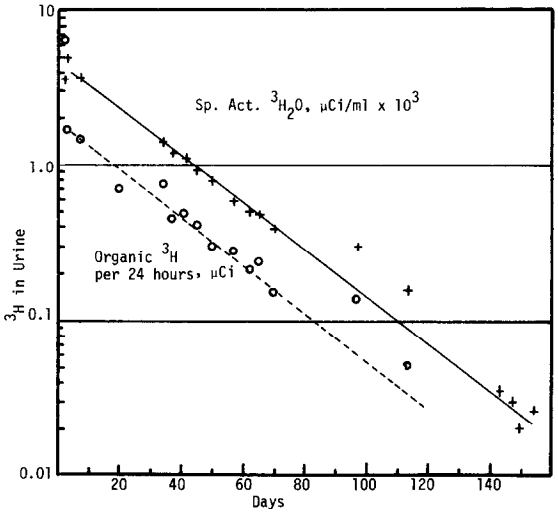


Figure 1. Urinary excretion of ^3H after i.v. injection of ascorbic-6-^3H acid in a macaque monkey: solid line with crosses, specific activity of urine water, µCi/ml x 10^3; dashed line with circles, total µCi organic ^3H excreted per 24 hours. The organic tritium values after 120 days were too low to measure reliably. $T_{1/2}$ for these excretion processes is 20 days.

alcohol-Dry Ice-cooled traps to collect such water show that these losses are about equal to urine ^3H_2O. Thus the amount of ^3H excreted as water is 43% of the total. These data clearly show that the C-6 carbon of ascorbic acid is metabolically active in a process that causes a significant release of the C-6 hydrogens.

Results of periodate degradation of urine from a rat and the monkey given ascorbic-6-^{14}C acid are shown in Table I. In both animals the late urines show that 45% of the radioactivity cannot be recovered as the formaldehyde derivative. Processes that could account for non-production of HCHO-^{14}C include a) oxidation of the C-6 carbon, b) derivitization of the 5 or 6 hydroxyl groups or c) reduction of the C-5 or C-6 carbons. Loss of ^3H

TABLE I

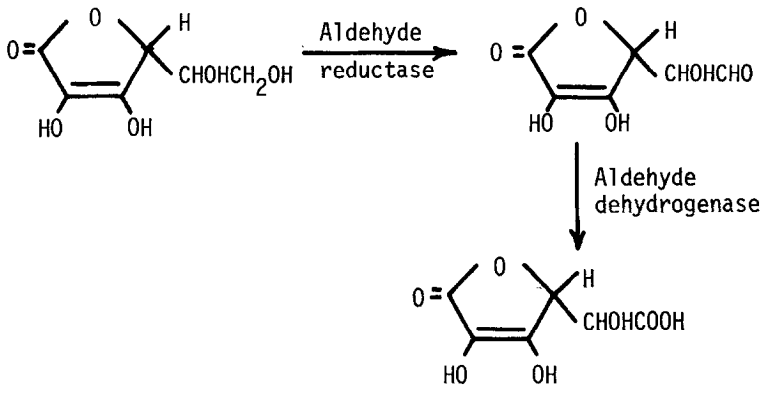
Periodate degradation of aliquots of 24 hour urine samples from a rat and a monkey given ascorbate-6-^{14}C. Percent of total ^{14}C that was recovered as the dimedone derivative of HCHO-^{14}C.

Monkey		Rat	
Day	HCHO-^{14}C	Day	HCHO-^{14}C
1	55%	2	75%
6	49	9	62
29	58	18	55
39	57	23	56
41	51		
43	55		
57	48		

to H_2O in the tritium experiments clearly show that the C-6 carbon is being oxidized.

The slow increase in percent C-6 carbon oxidized in the rat data indicates that the precursor of the C-6 oxidation process is a compound with a long turnover time. Such an inference also can be made from the monkey data. When the "ascorbate pool" of monkeys is labeled the half-time for excretion of the label is about 20 days, whether or not dietary ascorbic acid is given. Thus the labeled ascorbate pool does not exchange rapidly with dietary ascorbic acid. The parallel curves for ^3H_2O and organic ^3H in figure 1 indicate that the ^3H_2O must be released as the "ascorbate pool" is turned over. If the body water turnover time was similar to the ascorbate pool turnover time, this argument would fail, but other experiments by the authors show the body water turnover has a $T_{1/2}$ of about seven days in monkeys, distinctly different from the 20 d $T_{1/2}$ for ascorbate.

It seems reasonable to suggest the following metabolic process based on the above observations.



It is not known if the substrate for such a reaction is ascorbic acid itself or a derivative. The 2-methyl and 2-sulfate derivative are both known metabolites of ascorbate (9, 10).

This process is of biological interest for at least two reasons. First, C-6 oxidized derivatives of ascorbate have functional groups that allow easy covalent bonding of these metabolites to proteins and polysaccharides. Such covalently bonded forms of ascorbic acid would retain the unique enediol lactone ring in an unsubstituted form and potential catalytic functions of ascorbate itself.

Secondly, ascorbic acid and its postulated side chain oxidation products are structurally very similar to those observed in catecholamine catabolism. When norepinephrine is degraded by monoamine oxidase, the aldehyde formed is oxidized and reduced to an acid and alcohol. The alcohol, aldehyde and acid have the same side chain as our postulated ascorbate metabolites. In addition, the catechol ring and ascorbate ring have many properties in common, being highly conjugated, nearly planar, similar in size, and good reducing agents. Catechol-O-methyl transferase methylates both norepinephrine and ascorbate (9) and sulfated derivatives of both are observed. These structural

similarities lead to many questions, especially whether the catechols and ascorbate are degraded by similar enzymes and whether any intermediates are competitive or interactive.

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