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COLORADO UNIV BOULDER DEPT OF CHEMISTRY  
ASCORBIC ACID, BIOLOGICAL FUNCTION AND CHEMISTRY.(U)  
AUG 78 B M TOLBERT

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6 ASCORBIC ACID, BIOLOGICAL FUNCTION AND CHEMISTRY.

9 ANNUAL PROGRESS REPORT.

10 Project Director:  
Bert M. Tolbert / Ph.D.

11 Aug 1978  
(For the Period 1 May 1975 - 3 Jun 1976)

12 5 p.

Supported by

U.S. ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND  
Fort Detrick  
Frederick, Maryland 21701

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Contract No. DA-49-193-MD-2611

16 3A1611P2B71R

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM																		
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER																		
4. TITLE (and Subtitle) Ascorbic Acid, Biological Function and Chemistry		5. TYPE OF REPORT & PERIOD COVERED Annual Report 1 May 1975 - 3 June 1976																		
7. AUTHOR(s) Bert M. Tolbert, Ph. D.		6. PERFORMING ORG. REPORT NUMBER																		
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Chemistry University of Colorado Boulder, Colorado 80309		8. CONTRACT OR GRANT NUMBER(s) DA-49-193-MD-2611																		
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick, Frederick, Maryland 21701		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 61102A 3A161102B71R.02.006																		
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		12. REPORT DATE August 1978																		
		13. NUMBER OF PAGES 5																		
		15. SECURITY CLASS. (of this report) Unclassified																		
16. DISTRIBUTION STATEMENT (of this Report)  Approved for public release; distribution unlimited		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE																		
17. DISTRIBUTION STATEMENT (of the abstract entered in block 20, if different from Report)																				
18. SUPPLEMENTARY NOTES																				
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Ascorbic acid                      ascorbate sulfatase isoascorbic acid                    ascorbic acid synthesis erythorbic acid                     C-6 oxidation ascorbic acid metabolism		<table border="1"> <tr> <td>ACCESSION NO.</td> <td></td> </tr> <tr> <td>RTD</td> <td>WITH SIGNATURE</td> </tr> <tr> <td>NOT</td> <td>NOT SIGNED</td> </tr> <tr> <td>RECORDED</td> <td></td> </tr> <tr> <td>JUSTIFICATION</td> <td></td> </tr> <tr> <td>BY</td> <td></td> </tr> <tr> <td>DISTRIBUTION/AVAILABILITY CODE</td> <td></td> </tr> <tr> <td>Dist.</td> <td>AVAIL. AND/OR SPECIAL</td> </tr> <tr> <td>A</td> <td></td> </tr> </table>	ACCESSION NO.		RTD	WITH SIGNATURE	NOT	NOT SIGNED	RECORDED		JUSTIFICATION		BY		DISTRIBUTION/AVAILABILITY CODE		Dist.	AVAIL. AND/OR SPECIAL	A	
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## INTRODUCTION

↘ This is a report on the concluding year of a study in depth on the chemistry and metabolism of ascorbic acid and isoascorbic acid or erythorbic acid. The broad objectives of the research were to study in detail the biochemistry of these compounds to provide the fundamental background for further studies on the nutritive and stress requirement for the soldier for vitamin C. A further aspect of the study was to determine the metabolic interaction of ascorbic acid and erythorbic acid, since the latter is an optical isomer of vitamin C and also a common food additive, especially in military emergency rations. ↙

During the period of this report, three major projects were studied - the first was the nature and enzymic properties of ascorbate sulfatase; the second was whether C-6 oxidation of ascorbic acid was a significant process in ascorbic acid metabolisms; and the third was an effort to prepare a C-6 oxidized ascorbic acid by synthetic methods, so that its presence or absence in biological tissue could be tested.

## REPORT ON ASCORBATE SULFATE SULFOHYDROLASE

Ascorbate is a ubiquitous metabolite of ascorbic acid in higher animals. It is a vitamin in several species of fish. But most important of all, it is stable to air oxidation and does not hydrolyze at neutral pH's. Thus it has special nutritive value for fish. Man surely ingests a considerable amount in his diet.

If ascorbate sulfate is to serve as a source of ascorbic acid there should be an enzyme that can hydrolyze this compound back to ascorbic acid. We have discovered such a compound in a number of animals, and examined in detail the properties of the enzyme from cow liver. This report is attached.

Two interesting questions remain: ascorbate sulfatase is very similar to an important animal enzyme called aryl sulfatase A. The absence of aryl sulfatase A in humans results in the genetic disease metachromatic leukodystrophy. What is the relationship between these enzymes and what is their metabolic role? The other problem is whether ascorbate sulfatase serves to hydrolyze any significant amount of ascorbate sulfate, and whether the ascorbate sulfate has a biological role. At present, it seems ascorbate is both an excretion form of ascorbic acid, and has biological function, perhaps as an hypolipodemic agent.

## REPORT ON C-6 OXIDATION OF ASCORBIC ACID

Whether there is significant C-6 oxidation of ascorbic acid in higher animals was tested in two ways. First, a periodate degradation for ascorbic acid was developed, see attached reprint (1976) and this method was applied to urine of monkeys and rats given [6-<sup>14</sup>C] - ascorbic acid. The experiments, see attached reprint, showed that about 45% of all ascorbic acid metabolites were no longer in the -CH<sub>2</sub>OH oxidation state, characteristic of ascorbic acid.

To confirm this result [6-<sup>3</sup>H] - ascorbic acid was injected into monkeys and their excretion of <sup>3</sup>H in urine measured. The excretion was determined for <sup>3</sup>H-water in the urine and for organic bound <sup>3</sup>H in urine. This study, see attached reprint, showed that again about 45% of the tritium had been released from the ascorbic acid metabolites and appeared in the urine.

The biological significance of this side chain metabolism of ascorbic acid could be related to some special function of ascorbic acid - vitamin C.

### PREPARATION OF SACCHAROASCORBIC ACID

If ascorbic acid is subject to C-6 oxidation, a likely product is ascorbic acid with a side chain terminal carbon oxidized to a carboxyl group, or saccharoascorbic acid. Preparation of this compound was attempted in various ways. Success was finally achieved using the method shown in Figure 1. Details of the procedure were published in 1978.

Harkrader, R.J., Plunkett, L.M., and Tolbert, B.M., "Periodate Degradation of Labeled Ascorbic Acid," *Analytical Biochemistry* 72, 310-314 (1976).

About a month after the new contract year began, October 15, 1975, support for this work was terminated effective January 15th. This date was later extended to June 30th with no additional funds to allow a more orderly termination of the graduate students who were doing the work described in this report.

Their work has been of outstanding quality and their results have been the basis of significant further studies on the metabolism of ascorbic acid. The problem remains to obtain solid scientific results from which optimum intakes of ascorbic acid can be determined for the soldier under various conditions to maximize his ability as a military man.

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