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RESEARCH ON ASCORBIC ACID (REPORT NO 4):

THE ACTION OF GLUTATHIONE ON ASCORBIC ACID (PART II)

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Received

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A. FOREWORD

In Report No 1, this author demonstrated chemically ~~analytic~~ that the protective action of glutathione on ascorbic acid is proportional to the amount of glutathione used and that the presence of any active enzyme is not required to effect this protective action. At the same time, in regard to this reaction in a living organism, this writer stated that the presence ~~influence~~ of an active enzyme is not necessarily required for the protective action of glutathione on ascorbic acid because it is considered that the quantitative relationship between glutathione and ascorbic acid and other various biochemical conditions ~~influences~~ are better accomplished in the living organism than ~~in vitro~~ in vitro.

This report, extending the above experiment, discusses a test made to determine whether glutathione can still protect ascorbic acid when the acid is about to undergo sudden oxidation by specific outside influences. For this purpose, strong ascorbic acid oxidase was employed as a catalyst on ascorbic acid and the protective action of glutathione was tested during the oxidation

*[Note: 藤村吉之助, which can be also read Kichinosuke FUJIMURA.]

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process.

The above test was made also to determine the minimum concentration strength necessary to elicit clear-cut protective action from glutathione on a given quantity of ascorbic acid and to test the effect of this minimum concentration on reaction system concentration of hydrogen ions. The following is the report on above-described tests.

B. TEST RESULTS AND THE EXAMINATIONS

1. The Effect of Glutathione When Ascorbic Acid Oxidase is used as a catalyst on Ascorbic Acid

(a) Preparation of Ascorbic Acid Oxidase:

320 cc suspension solution of ascorbic acid oxidase was made according to the methods of S. W. Johnson and S. S. Zilva.

(b) Determination of Activity Degree of Ascorbic Acid Oxidase Suspension Solution:

The activity degree of ascorbic acid oxidase suspension solution made by the above-cited methods was determined by testing with solutions of compositions given in Table 1.

TABLE 1

COMPOSITION OF TEST SOLUTIONS USED IN DETERMINING THE ACTIVITY DEGREE OF ASCORBIC ACID OXIDASE SUSPENSION SOLUTION

	(cc)
Ascorbic Acid solution (100 mg%)*	25
Ascorbic Acid Oxidase suspension solution	10
Sörensen phosphate buffer solution pH=5.28	65
Total quantity	100

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* In preparation of ascorbic acid solution, used sörensen phosphate buffer solution pH 5.26 as a solvent. Made at Bubeck and Dolder Basel, Switzerland.

The above-described test solution was carefully placed in a 24 degree thermostat and the ascorbic acid in the thermostat was measured at definite intervals. The method of measuring was the same as that described in Report No 1 (3). However, the test solution was tested with 5 cc of 10% dilute solution. All the ascorbic acid solutions and glutathione solutions used in all the following tests were made on the day of use. The test results are shown in Table 2.

TABLE 2

QUANTITY CHANGE OF ASCORBIC ACID RESULTING FROM ASCORBIC ACID OXIDASE

	Test Solution		A	B
at 0 time	Titration value (cc)	I	0.99	1.003
		II	0.99	1.003
		M	0.99	1.003
	Residue (mg)		17.41	17.64
after 1 hour	Titration value (cc)	I	-	0.825
		II	-	0.825
		M	-	0.825
	Residue (mg)		-	14.51

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			A	B
after 2 hours	Titration value (cc)	I	-	0.70
		II	-	0.72
		M	-	0.71
	Residue (mg)		-	12.46
after 4 hours	Titration value (cc)	I	0.98	0.438
		II	0.98	0.438
		M	0.98	0.438
	Residue (mg)		17.24	7.70

As is clear from Table 2, when the ascorbic acid oxidase is applied to ascorbic acid, the ascorbic acid rapidly reduces with the passage of time, and in four hours it becomes only 1/2.5 of the original quantity. On the other hand, comparative test A does not show a marked change. By this fact, it can be clearly recognized that the above-described suspension solution of ascorbic acid oxidase can sufficiently accelerate the oxidation of ascorbic acid. Utilizing this fact, the following test was conducted:

(c) The Effect of Glutathione on Ascorbic Acid When Ascorbic Acid Oxidase is Applied to Ascorbic Acid

To determine whether or not glutathione can protect ascorbic acid without being affected by an enzyme, even when the suspension solution of ascorbic acid oxidase acts upon ascorbic acid to accelerate its oxidation, a given quantity of the above-mentioned ascorbic acid oxidase suspension solution was added to a given quantity of ascorbic acid solution. To this was added varying

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quantities of glutathione solution. The effect was then tested.

The composition of the test solution was that listed in Table 3, and the measurement results of ascorbic acid and glutathione solution are listed in Tables 4 and 5 and in Graph I.

Table 3

COMPOSITION OF SOLUTION USED TO DETERMINE PROTECTIVE ACTION OF GLUTATHIONE WHEN ASCORBIC ACID WAS TREATED WITH ASCORBIC ACID OXIDASE

(Temperature 24° ± 1°, Titrated with 1cc of 10x dilute test solution)

Items	Test Solutions	A	B	C
Ascorbic Acid solution ⊕	(cc)	2	2	2
(500 mg %)	(mg)	10	10	10
Ascorbic Acid Oxidase suspension solution	(cc)	10	10	10
Glutathione solution ⊕	(cc)	0	12.5	30
(200 mg %)	(mg)	0	25	60
Sörensen phosphate buffer solution pH=5.28	(cc)	38	25.5	8
Total quantity	(cc)	50	50	50

⊕ sign indicates that the solvent used in preparing ascorbic acid and glutathione solutions was Sörensen phosphate buffer solution pH 5.28. Glutathione was made by Oberschöneweide-Berlin.

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Table 4

QUANTITY CHANGE OF ASCORBIC ACID WHEN TREATED SIMULTANEOUSLY WITH ASCORBIC ACID OXIDASE AND GLUTATHIONE

(Testing Temperature, 24° ± 1°. The pH values of test solution were the same both before and after test; the same colorimetric method was used in each of the following tests)

Items		Test Solution	A	B	C
After 10 minutes	Titration value (cc)	I	0.365	0.764	0.865
		II	0.365	0.762	0.865
		M	0.365	0.763	0.865
	Residue (mg)		6.23	8.39	9.51
After 1.5 hours	Titration value (cc)	I	0.225	0.750	0.850
		II	0.225	0.750	0.860
		M	0.225	0.750	0.855
	Residue (mg)		3.87	8.25	9.40
After 4 hours	Titration value (cc)	I	0.036	0.745	0.850
		II	0.037	0.745	0.850
		M	0.0365	0.745	0.850
	Residue (mg)		0.40	8.19	9.34
After 5.5 hours	Titration value (cc)	I	0.0025	-	-
		II	0.0025	-	-
		M	0.0025	-	-
	Residue (mg)		0.027	-	-
After 24 hours	Titration value (cc)	I	-	0.715	0.835
		II	-	0.715	0.835
		M	-	0.715	0.835
	Residue (mg)		-	7.86	9.18

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Items		Test Solution	A	B	C
After 48 hours	Titration value (cc)	I	-	0.644	0.780
		II	-	0.646	0.780
		M	-	0.645	0.780
	Residue (mg)		-	7.09	8.58
After 72 hours	Titration value (cc)	I	-	0.620	0.735
		II	-	0.620	0.735
		M	-	0.620	0.735
	Residue (mg)		-	6.82	8.08

Table 5

QUANTITY CHANGE OF GLUTATHIONE WHEN ASCORBIC ACID WAS TREATED SIMULTANEOUSLY WITH BOTH ASCORBIC ACID OXIDASE AND GLUTATHIONE

(Testing temperature was 24° ± 1°. The pH values of test solution were the same both before and after the test)

Items		Test Solution	A	B	C
After 10 minutes	Titration value (cc)	I	-	0.722	1.53
		II	-	0.722	1.53
		M	-	0.722	1.53
	Residue (mg)		-	27.68	58.67
After 1.5 hours	Titration value (cc)	I	-	0.72	1.496
		II	-	0.72	1.494
		M	-	0.72	1.495
	Residue (mg)		-	27.61	57.32

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Items		Test Solution	A	B	C
After	Titration	I	-	0.706	1.51
4	value	II	-	0.704	1.51
hours	(cc)	M	-	0.705	1.51
	Residue (mg)		-	27.03	58.00
After	Titration	I	-	0.710	1.506
24	value	II	-	0.710	1.504
hours	(cc)	M	-	0.710	1.505
	Residue (mg)		-	27.22	57.71
After	Titration	I	-	0.696	1.500
48	value	II	-	0.694	1.500
hours	(cc)	M	-	0.695	1.500
	Residue (mg)		-	26.65	57.52
After	Titration	I	-	0.670	1.495
72	value	II	-	0.670	1.495
hours	(cc)	M	-	0.670	1.495
	Residue (mg)		-	25.69	57.32

From Table 4 comparative test A shows that ascorbic acid rapidly reduces with passage of time, and is almost completely oxidized after four hours. Despite such rapid disappearance of ascorbic acid in test A, when glutathione is added in other comparative tests, ascorbic acid is very well preserved; there were residues of 6.82 mg in test B and 8.08 mg in test C even after 72 hours of testing period. This fact can be more clearly understood from Graph I.

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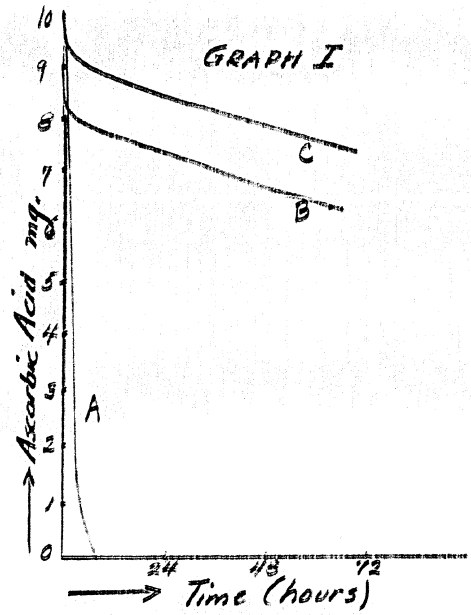

It is clear, therefore, that glutathione still protects ascorbic acid even when ascorbic acid is treated with ascorbic acid oxidase; furthermore, it is clear that in this case the presence of an active enzyme is not necessary to elicit this protective action. Moreover, the quantitative change of glutathione in this case shows the same tendency as that set forth in Table 3.

2. Tests at Varying Temperatures with Varying Concentrations of Hydrogen Ions Influencing the Protective Action of Glutathione on Ascorbic Acid

It has already been noted in Report No 3(1) that the protective action of glutathione on ascorbic acid varies with the amount of glutathione applied to a given quantity of ascorbic acid. Few tests, however, have been made along this line. Consequently, this writer, using the following method, determined the effects

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yielded upon the protective action by varying the compositions of hydrogen ion concentration and the temperatures of the reaction solution.

First, each of the test solutions described in Table 6 were prepared, and the quantity of ascorbic acid in them was measured after oxidation in thermostats of various temperatures.

Table 6

COMPOSITION OF TEST SOLUTIONS USED FOR TESTING AT VARIED TEMPERATURES AND VARIED CONCENTRATIONS OF HYDROGEN IONS AFFECTING THE PROTECTIVE ACTION OF GLUTATHIONE ON ASCORBIC ACID

Item \ Test Solutions		A	B	C	D	E	F
Ascorbic Acid solution	(cc)	5	5	5	5	5	5
(100 mg %)	(mg)	5	5	5	5	5	5
Glutathione solution	(cc)	0	2	4	6	7 [illegible]	10
(0.1 mg %)	(mg)	0.000	0.002	0.004	0.006	0.008	0.01
Buffer solution*	(cc)	45	43	41	39	37	35
Total quantity	(cc)	50	50	50	50	50	50

* Type of buffer solution and pH values were changed at each test, and each buffer solution was used as a solvent for the ascorbic acid and glutathione solutions.

(a) Effects of Temperature Change With Hydrogen Ions Concentration of pH=2.00 (N/1000 HCl)

Test solutions of Table 6 were placed in thermostats with

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temperatures of 12° ± 1°, 24° ± 1° and 36° ± 1° and the ascorbic acid remaining in each solution was measured one hour later. Results are shown in Tables 7 to 9 (4cc of 10x dilute was used to measure the test solution).

Table 7

QUANTITATIVE CHANGE OF ASCORBIC ACID WHEN pH OF THE TEST SOLUTION =2.00, AND TEST TEMPERATURE = 12° ± 1°

(pH values were the same both before and after the test)

Item	Test Solution		A	B	C	D	E	F
0 minute	Titration value (cc)	I	0.28	0.28	0.28	0.285	0.28	0.28
		II	0.28	0.28	0.28	0.280	0.285	0.285
		M	0.28	0.28	0.28	0.2825	0.2825	0.2825
	Residue (mg)		3.08	3.08	3.08	3.11	3.11	3.11
30 minutes	Titration value (cc)	I	0.265	0.265	0.265	0.27	0.27	0.28
		II	0.265	0.265	0.265	0.27	0.27	0.275
		M	0.265	0.265	0.265	0.27	0.27	0.275
	Residue (mg)		2.91	2.91	2.91	2.97	2.97	3.05
90 minutes	Titration value (cc)	I	0.25	0.25	0.25	0.25	0.26	0.26
		II	0.25	0.25	0.25	0.26	0.26	0.26
		M	0.25	0.25	0.25	0.255	0.26	0.26
	Residue (mg)		2.75	2.75	2.75	2.80	2.86	2.86
24 hours	Titration value (cc)	I	0.12	0.12	0.12	-	0.125	0.125
		II	0.12	0.12	0.12	-	0.120	0.120
		III	0.12	0.12	0.12	-	0.1225	0.1225
	Residue (mg)		1.32	1.32	1.32	-	1.35	1.35

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Table 8

QUANTITATIVE CHANGE OF ASCORBIC ACID WHEN pH OF THE TEST SOLUTION =2.00 AND TEST TEMPERATURE = 24° ± 1°

(pH values were the same both before and after the test)

Item	Test Solution		A	B	C	D	E	F
0 minute	Titration value (cc)	I	0.260	0.265	0.275	0.280	0.280	0.28
		II	0.265	0.265	0.275	0.275	0.275	0.28
		M	0.2625	0.265	0.275	0.2775	0.2775	0.18
	Residue (mg)		2.91	2.91	3.02	3.05	3.05	3.05
30 minutes	Titration value (cc)	I	0.235	0.235	0.24	0.24	0.24	0.240
		II	0.235	0.235	0.24	0.24	0.24	0.245
		M	0.235	0.235	0.24	0.24	0.24	0.2425
	Residue (mg)		2.56	2.56	2.64	2.64	2.64	2.67
90 minutes	Titration value (cc)	I	0.215	0.215	0.215	0.22	0.22	0.22
		II	0.215	0.215	0.220	0.22	0.22	0.22
		M	0.215	0.215	0.2175	0.22	0.22	0.22
	Residue (mg)		2.36	2.36	2.39	2.42	2.42	2.45
24 hours	Titration value (cc)	I	0.02	0.015	0.020	0.020	0.020	0.020
		II	0.015	0.015	0.015	0.015	0.015	0.015
		M	0.0175	0.015	0.0175	0.0175	0.0175	0.0175
	Residue (mg)		0.19	0.16	0.19	0.19	0.19	0.19

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Table 9

QUANTITATIVE CHANGE OF ASCORBIC ACID WHEN pH OF THE TEST SOLUTION =2.00 AND TEST TEMPERATURE = 36° ± 1°

(pH values were the same both before and after the test)

Item		Test Solution	A	B	C	D	E	F
0 minute	Titration value (cc)	I	0.17	0.17	0.17	0.175	0.18	0.18
		II	0.17	0.17	0.17	0.175	0.18	0.18
		M	0.17	0.17	0.17	0.175	0.18	0.18
	Residue (mg)		1.87	1.87	1.87	1.92	1.98	1.98
30 minutes	Titration value (cc)	I	0.16	0.16	0.165	0.17	0.18	0.18
		II	0.16	0.16	0.165	0.17	0.18	0.18
		M	0.16	0.16	0.165	0.17	0.18	0.18
	Residue (mg)		1.76	1.76	1.81	1.87	1.98	1.98
90 minutes	Titration value (cc)	I	0.13	0.13	0.130	0.14	0.14	0.14
		II	0.13	0.13	0.135	0.14	0.15	0.15
		M	0.13	0.13	0.1325	0.14	0.145	0.145
	Residue (mg)		1.43	1.43	1.46	1.54	1.59	1.59
24 hours	Titration value (cc)	I	0.025	0.025	0.03	0.03	0.030	0.030
		II	0.030	0.030	0.03	0.03	0.035	0.035
		M	0.0275	0.0275	0.03	0.03	0.0325	0.0325
	Residue (mg)		0.30	0.30	0.33	0.33	0.36	0.36

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(b) Results of Changing Temperatures at Hydrogen Ions of Concentration pH = 5.28 (Sörensen Phosphate Buffer Solution)

Test solutions tabulated in Table 6 were placed in thermostats with temperatures of 12° ± 1°, 24° ± 1°, and 36° ± 1° and after a given period the quantity of ascorbic acid was measured by the same method used in (a). The results are listed in Tables 10 to 12. 4cc of 10% dilute of the test solution were used for measurement.

Table 10

QUANTITATIVE CHANGE OF ASCORBIC ACID WHEN pH OF THE TEST SOLUTION =5.28 AND TEST TEMPERATURE = 12° ± 1°

(pH values were the same both before and after the test)

Item	Test Solution		A	B	C	D	E	F
0 minute	Titration value (cc)	I	0.165	0.165	0.17	0.18	0.18	0.185
		II	0.165	0.165	0.17	0.18	0.18	0.185
		M	0.165	0.165	0.17	0.18	0.18	0.185
	Residue (mg)		1.81	1.81	1.87	1.98	1.98	2.03
30 minutes	Titration value (cc)	I	0.095	0.095	0.095	0.100	0.100	0.100
		II	0.095	0.095	0.095	0.100	0.100	0.100
		M	0.095	0.095	0.095	0.100	0.100	0.100
	Residue (mg)		1.04	1.04	1.04	1.10	1.10	1.10

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Table 11

QUANTITATIVE CHANGE OF ASCORBIC ACID WITH pH OF THE TEST SOLUTION

=5.28 AND TEST TEMPERATURE = 24° ± 1°

(pH values were the same both before and after the test)

Item	Test Solution		A	B	C	D	E	F
0	Titration	I	0.16	0.16	0.16	0.16	0.17	0.17
minute	value	II	0.16	0.16	0.16	0.16	0.16	0.17
	(cc)	M	0.16	0.16	0.16	0.16	0.165	0.17
	Residue (mg)		1.76	1.76	1.76	1.76	1.62	1.67
30	Titration	I	0.05	0.05	0.05	0.06	0.06	0.09
minutes	value	II	0.05	0.05	0.05	0.06	0.06	0.09
	(cc)	M	0.05	0.05	0.05	0.06	0.06	0.09
	Residue (mg)		0.55	0.55	0.55	0.66	0.66	0.99
90	Titration	I	0.01	0.01	0.01	0.01	0.01	0.01
minutes	value	II	0.01	0.01	0.01	0.01	0.01	0.01
	(cc)	M	0.01	0.01	0.01	0.01	0.01	0.01
	Residue (mg)		0.11	0.11	0.11	0.11	0.11	0.11

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Table 12

QUANTITATIVE CHANGE OF ASCORBIC ACID WITH pH OF THE TEST SOLUTION =5.26 AND TEST TEMPERATURE = 36° ± 1°

(pH values were the same both before and after the test)

Item	Test Solution		A	B	C	D	E	F
0 minute	Titration value (cc)	I	0.12	0.12	0.12	0.125	0.125	0.13
		II	0.12	0.12	0.12	0.120	0.125	0.13
		M	0.12	0.12	0.12	0.1225	0.125	0.13
	Residue (mg)		1.32	1.32	1.32	1.35	1.37	1.43
30 minutes	Titration value (cc)	I	0.04	0.04	0.04	0.05	0.045	0.045
		II	0.04	0.04	0.05	0.04	0.045	0.045
		M	0.04	0.04	0.045	0.045	0.045	0.045
	Residue (mg)		0.44	0.44	0.49	0.49	0.49	0.49
90 minutes	Titration value (cc)	I	0.02	0.02	0.02	0.025	0.025	0.025
		II	0.02	0.02	0.02	0.020	0.020	0.020
		M	0.02	0.02	0.02	0.0225	0.0225	0.0225
	Residue (mg)		0.22	0.22	0.22	0.25	0.25	0.25

(c) Results of Varying Temperatures With Hydrogen Ion Concentration of pH = 7.00 (Sörensen Phosphate Buffer Solution)

The test solutions described in Table 6 underwent reactions in thermostats of 12° ± 1°, 24° ± 1° and 36° ± 1° temperatures, and the quantity of ascorbic acid was determined after a given period of time by utilizing the same method of tests (a) and (b). The

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results are shown in Tables 13 to 15. The analysis was made with use of 10x dilute of the test solution.

Table 13

QUANTITATIVE CHANGE OF ASCORBIC ACID WITH pH OF THE TEST SOLUTION

pH 7.00 AND TEST TEMPERATURE = 12° ± 1°

(pH values were the same both before and after the test)

Item	Test Solution		A	B	C	D	E	F
0 minute	Titration value (cc)	I	0.12	0.12	0.12	0.12	0.125	0.125
		II	0.12	0.12	0.12	0.13	0.125	0.125
		M	0.12	0.12	0.12	0.125	0.125	0.125
	Residue (mg)		1.32	1.32	1.32	1.37	1.37	1.37
30 minutes	Titration value (cc)	I	0.04	0.04	0.04	0.04	0.04	0.04
		II	0.04	0.04	0.04	0.04	0.04	0.04
		M	0.04	0.04	0.04	0.04	0.04	0.04
	Residue (mg)		0.44	0.44	0.44	0.44	0.44	0.44
90 minutes	Titration value (cc)	I	0.01	0.01	0.01	0.01	0.01	0.01
		II	0.01	0.01	0.01	0.01	0.01	0.01
		M	0.01	0.01	0.01	0.01	0.01	0.01
	Residue (mg)		0.11	0.11	0.11	0.11	0.11	0.11

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Table 14

QUANTITATIVE CHANGE OF ASCORBIC ACID WITH pH OF THE TEST SOLUTION

=7.00 AND TEST TEMPERATURE = 24° ± 1°

(pH values were the same both before and after the test)

Item	Test Solution		A	B	C	D	E	F
0	Titration	I	0.11	0.11	0.115	0.12	0.12	0.12
minute	value	II	0.11	0.11	0.115	0.12	0.12	0.12
	(cc)	M	0.11	0.11	0.115	0.12	0.12	0.12
	Residue (mg)		1.21	1.21	1.26	1.32	1.32	1.32
30	Titration	I	0.035	0.045	0.045	0.045	0.045	0.045
minutes	value	II	0.035	0.045	0.045	0.045	0.045	0.045
	(cc)	M	0.035	0.045	0.045	0.045	0.045	0.045
	Residue (mg)		0.38	0.49	0.49	0.49	0.49	0.49
90	Titration	I	0.01	0.01	0.01	0.01	0.01	0.01
minutes	value	II	0.01	0.01	0.01	0.01	0.01	0.01
	(cc)	M	0.01	0.01	0.01	0.01	0.01	0.01
	Residue (mg)		0.11	0.11	0.11	0.11	0.11	0.11

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Table 15

QUANTITATIVE CHANGE OF ASCORBIC ACID WITH pH OF THE TEST SOLUTION

=7.00 AND TEST TEMPERATURE = 36° ± 1°

(pH values were the same both before and after the test)

Item	Test Solution		A	B	C	D	E	F
0	Titration	I	0.08	0.08	0.08	0.085	0.085	0.09
minute	value	II	0.08	0.08	0.08	0.085	0.090	0.09
	(cc)	M	0.08	0.08	0.08	0.085	0.0875	0.09
	Residue (mg)		0.88	0.88	0.88	0.93	0.96	0.99
30	Titration	I	0.02	0.02	0.02	0.025	0.03	0.03
minutes	value	II	0.02	0.02	0.02	0.025	0.03	0.03
	(cc)	M	0.02	0.02	0.02	0.025	0.03	0.03
	Residue (mg)		0.22	0.22	0.22	0.275	0.33	0.33
90	Titration	I	0.01	0.01	0.01	0.01	0.01	0.01
minutes	value	II	0.01	0.01	0.01	0.01	0.01	0.01
	(cc)	M	0.01	0.01	0.01	0.01	0.01	0.01
	Residue (mg)		0.11	0.11	0.11	0.11	0.11	0.11

In assembling the results of the tests listed in Tables 7 to 9, 10 to 12, and 13 to 15, the minimum concentration effecting protective action of glutathione on ascorbic acid is approximately constant for each test solution. In other words, in comparison with test A, the result of the test B is always the same under each circumstances and does not show any protective action, but protective

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action begins to appear partly in test C, and the action becomes pronounced in test D. The protective action becomes very pronounced with tests beyond D. The protective action of glutathione on ascorbic acid has no relation to the kind of buffer solution or the temperature of its reaction system and hydrogen ion concentration. Only two kinds of buffer solution were used; phosphate buffer solution and N/1000 HCl, but this statement is true at least within the limits of the experiment. The protective action appears when the glutathione and ascorbic acid are mixed in the proportions used in test solutions C and D. Consequently, it may be concluded that the minimum concentration necessary to elicit the protective action of glutathione on ascorbic acid [redacted] within the conditions of the above test, that is, with temperature from 12° to 36° and pH from 2.00 to 7.00, is that of 0.004 to 0.006 mg of glutathione added to 5 mg ascorbic acid.

In an animal organism, this kind of protective action will not only be exerted by glutathione, but also by other various unknown factors. Consequently, it is still too early to apply this result immediately in vivo, but in general, it would probably be safe to accept the above minimum concentration for application in vivo.

Borsook and his co-researchers (4) tested the reducing function of glutathione on dehydro-ascorbic acid and reported that the reduction potential of ascorbic acid is not related to the oxidizing agent or to the composition of the buffer solution of its reaction system. Furthermore, in regard to the hydrogen ion concentration, the reduction potential increases as the pH increases.

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They also stated that this reduction potential is stronger than the formation velocity of irreversable materials in dehydro-ascorbic acid.

However, according to the above tests, the protective action of glutathione on ascorbic acid has no relation to the pH value within the range pH 2.00 to 7.00. If ascorbic acid once becomes dehydro-ascorbic acid by aerobic condition in the test solution and is then again reduced to ascorbic acid by glutathione, then the protective action of glutathione on ascorbic acid with pH value of 7.00 (since the composition of the buffer solution which was used as the test solution has no bearing according to the theory of Lersook) should be more pronounced than with pH values of 2.00 or 5.20. This theory does not conform with results of the tests made by this writer, and in the mechanism of protective action of glutathione on ascorbic acid, if glutathione reduces it at the moment of oxide formation in each oxidation process of ascorbic acid, the reduction effect of glutathione may differ in its method of reduction between the case of oxidizing products and the case of dehydro-ascorbic acid.

Many tests have been made on the degree of stability of ascorbic acid and it is a generally well known fact that ascorbic acid is comparatively stable at a low temperature or in an acid area, but unstable in a high temperature or in an alkali area. However, these tests were mostly made in living tissue. Crystalline ascorbic acid solutions were used in the tests of Table 7 to 9, 10 to 12, and 13 to 15 and proved the above fact by compositions of various temperatures and pH. In other words, when hydrogen ion concentration is held constant, ascorbic acid is far more stable

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under low temperature than high temperature, and under constant temperature, it is far more stable when the hydrogen ion concentration is low than when the hydrogen ion concentration is large. In the relationship of temperature and hydrogen ion concentration, pH 2.00 and 36° ± 1° (Table 9) compared with pH 7.00 and temperature 12° ± 1° (Table 13), 90 minutes after the test started, the residual weight of ascorbic acid in the test solutions from A to F in the former (Table 9) was 1.43 to 1.59 mg, but on the other hand, the latter (Table 13) shows only 0.11 mg of residue in each test solution. From this result, it is understood that pH is a more important factor than temperature in regard to the degree of stability of ascorbic acid.

C. SUMMARY

In regard to the protective action of crystalline glutathione on ascorbic acid solution:

1. When ascorbic acid is subjected to oxidation decomposition by strong ascorbic acid oxidase in the presence of glutathione, the ascorbic acid is well protected by the glutathione and the extent of protective action is in direct ~~ratio~~ proportion to the quantity of glutathione present. Furthermore, the presence of an enzyme is not required to elicit this protective action.

2. The minimum concentration necessary for glutathione to exert protective action on ascorbic acid was determined by varying the reaction temperatures and the hydrogen ion concentrations. In other words, at temperatures between 12° to 36° and pH values between

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2.00 to 7.00, 0.004 to 0.006 mg of glutathione is the minimum concentration necessary to exert protective action on 5 mg of ascorbic acid, and this minimum concentration has no relation to the test temperature or to the pH value.

3. Crystalline ascorbic acid, as within living tissues, is comparatively stable in an acid area or at low temperatures and unstable in an alkali area or at high temperatures. Furthermore, it was made clear that in regard to the stability of ascorbic acid the hydrogen ion concentration is a more important factor than temperature so far as the limits of the above test is concerned.

In conclusion, I want to express my deep appreciation to my teacher, Doctor Kinsuke Kondo, Doctor of Agriculture Science at Kyoto Imperial University, for his guidance and encouragement and also for his efforts in reviewing this Report.

E N D

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