THE INHIBITORS OF ENZYMATIC AND CUPRIC ION OXIDATION OF VITAMIN C

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The oxidation of ascorbic acid and its retardation by various substances have been studied by several workers. Noteworthy examples of substances which are found to inhibit the oxidation of ascorbic acid are glutathione and cysteine (De Caro and Giani, 1934; Bersin *et al.*, 1935; Barron *et al.*, 1936; Ghosh and Rakshit, 1936; Hopkins and Morgan, 1936); metaphosphoric acid (Fujita and Iwatake, 1935; Levy, 1936; Hinsberg, 1937; Musulin and King, 1936), Pyrophosphate (Giri, 1937; Giri and Doctor, 1938; Krishnamurthy and Giri, 1941 *a*; Lugg, 1942); tannin from Indian Gooseberry (*Phyllanthus emblica*) (Damodaran and Nair, 1936); and oxalic acid (Krishnamurthy and Giri, 1941 *c*; Seshagiri Rao and Giri, 1942; Ponting, 1943). The importance of the discovery of such inhibitors of vitamin C oxidation lies in their application to the determination of vitamin C content of foodstuffs in order to prevent the oxidation of the vitamin during extraction, the preparation of stable aqueous solutions of the vitamin and in the study of the nature of catalytic systems present in plant and animal tissues which oxidise the vitamin.

Among the inhibitors of vitamin C oxidation metaphosphoric acid has been widely used by various workers for extraction of the vitamin from plant and animal materials. Pyrophosphate which was found to stabilise the vitamin (Giri, 1937) has also been used by several workers for extraction and stabilisation of the vitamin (Mitra, *et al.*, 1940; Lugg, 1942; Klodt and Steib, 1938; Steinman and Dawson, 1942).

Some of the inhibitors of vitamin C oxidation which exert their effect on cupric ion oxidation without exerting any influence on the enzymic oxidation may be useful in eliminating the catalytic effect of copper while studying the enzymic oxidation of the vitamin. Thus Krishnamurthy and Giri (1941 *a*) showed that pyrophosphate has no influence on the enzymic oxidation of vitamin C while it retards considerably the cupric ion oxidation of the vitamin. This property of pyrophosphate was made use of in investigations on the mechanism of the ascorbic acid-ascorbic acid oxidase

reaction by Steinman and Dawson (1942). Furthermore, Giri and Seshagiri Rao (1942) in a preliminary note have reported that some of the purine derivatives such as xanthine, uric acid, guanine, theophylline have the specific property of inhibiting only the cupric ion oxidation without exerting any influence on the enzymic oxidation of the vitamin. Recently Snow and Zilva (1942) have utilised the property of these inhibitors in their studies on the nature of the catalytic system in tea infusions which catalyse the aerobic oxidation of ascorbic acid.

The foregoing findings together with the observation that the enzyme ascorbic acid oxidase is a copper protein compound (Stotz *et al.*, 1937) suggested that a comparative study of the influence of inhibitors on copper oxidation and enzymic oxidation of the vitamin might be advisable. In a previous note (Giri and Krishnamurthy, 1941; Krishnamurthy and Giri, 1941 *c*) it was shown that certain purine derivatives inhibit the oxidation of ascorbic acid by Cu and these results on the stabilising action of purines have been confirmed recently by other workers (*cf.*, Bergel, 1944). The present paper deals with a more detailed study of these and other inhibitors of vitamin C oxidation.

Experimental

The oxidation of vitamin C was followed both by manometric and titrimetric methods. The manometric method consisted in measuring the oxygen uptake from solution of vitamin C shaken in air in Warburg manometers. The buffer and the catalyst Cu together with the substance whose influence on the oxidation is to be examined were placed in the main chamber of the vessel, vitamin C solution being kept in the side arm and dropped into the main vessel when temperature equilibrium was reached. The readings were taken at definite intervals of time.

The water used for the preparation of buffers and other solutions was twice distilled in a pyrex distillation apparatus.

The vitamin used in the present investigation was B. D. H. ascorbic acid. All the substances used were of the highest grade of purity, either Merck's or Kahlbaum's pure products.

The pH of all solutions used in testing their influence on the oxidation of the vitamin was always adjusted to the pH of the experimental solution (pH 7·2). Some of the purine derivatives which are difficultly soluble in water are dissolved first in minimum amount of alkali and diluted to the required strength. These solutions when added in such low concentrations as were used in the experiments, were found to have no significant influence

on the pH of the solution. All the solutions were always freshly prepared for each experiment.

In Table I are presented the results obtained manometrically on the influence of the compounds under investigation on the oxidation of vitamin C by Cu.

TABLE I

The influence of purines and other substances on the oxidation of vitamin C

(By manometric method)

The experimental cup contained 0·8 c.c. M/15 phosphate buffer (pH 7·2); 0·2 c.c. copper sulphate solution containing 0·71 γ Cu and 1·5 c.c. of buffer containing the substance whose influence on the oxidation is to be determined. The side arm contained 2 mg. ascorbic acid dissolved in 0·5 c.c. of water and the central chamber contained 0·2 c.c. of 20 per cent. KOH and filter paper. The vessels were placed in the manometers with the stopcocks open and introduced into the bath, which was accurately controlled (± 0·01 C.) at the desired temperature 30° C., and the flasks equilibrated for five minutes. The stopcocks were then closed the vitamin C solution dropped into the main vessel and the readings were taken at definite intervals of time.

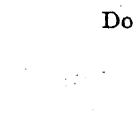
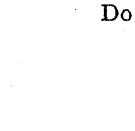
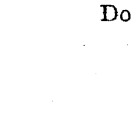
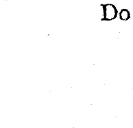
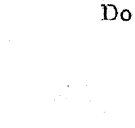
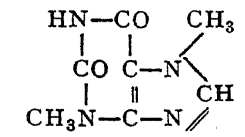
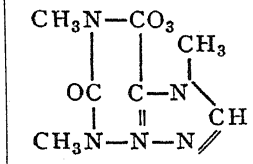
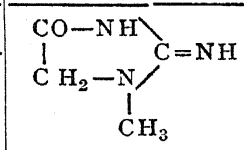
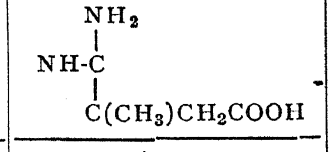
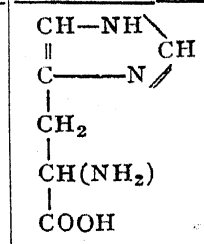
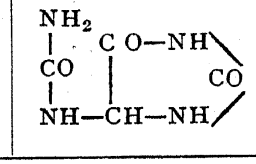
	Substance added	Formula	Concentration 10^{-4}(M)	μl O_2 uptake time in minutes					
				5	10	15	20	25	30
Ascorbic acid +Cu	..	..	..	17	43	69	95	114	132
Do	xanthine		1·7 8·5	0 0	0 0	0 0	0 0	0 0	0 0
Do	uric acid		1·5 7·5	0 0	0 0	0 0	0 0	0 0	0 0
Do	adenine		1·6 8·0	0 0	0 0	0 0	0 0	0 0	0 0
Do	guanine		1·7 8·5	0 0	0 0	0 0	0 0	0 0	0 0
Do	theophylline		1·4 7·0	6 0	9 0	13 0	.. 0	.. 0	17 0

TABLE I—*Contd.*

	Substance added	Formula	Concentration 10^{-4}(M)	μl O_2 uptake time in minutes					
				5	10	15	20	25	30
Do	theobromine		7·0	10	40	65	85	103	120
Do	caffeine		6·4	10	35	62	80	101	126
Do	yeast nucleic acid	$C_{20}H_{42}N_{13}P_3O_{23}$	0·0025% 0·0125%	0 0	0 0	0 0	0 0	0 0	0 0
Do	creatinine (2,3-dihydro-2-imino-1-methyl-4(5) imidazolone]		2·2 11·0	8 ..	22 2	35 5	50 7	62 9	77 10
Ascorbic acid +Cu	creatine		9·5	15	40	62	88	106	130
Do	histidine (α-amino-β-imidazolyl-propionic acid or β-imidazolyl-alanine		1·8	..	10	25	..	..	58
Do	allantoin		1·76 7·8	.. ..	21 4	36 8	52 12	67 15	84 19

The results indicate that the purine derivatives xanthine, adenine, guanine, uric acid, theophylline and yeast nucleic acid completely inhibit the oxidation of the vitamin, while theobromone and caffeine have no significant influence on the oxidation at pH 7·2 and in the concentrations used in the experiments. Creatinine is found to inhibit the oxidation, while creatine is without effect on the reaction. Histidine and allantoin also inhibit the oxidation

With a view to confirming the results obtained manometrically the rate of oxidation of the vitamin in presence and absence of the substances was followed by estimating the vitamin by the usual titration method. For purposes of comparison the well-known inhibitors sodium diethyldithiocarbamate and 8-hydroxyquinoline were also included.

The results are presented in Table II.

The results confirm the observations made by manometric method.

TABLE II

The influence of purines and other substances on the oxidation of Vitamin C

(*By titration method*)

The reaction mixtures contained 10 c.c. of phosphate buffer M/15 (pH 7·2), 2 c.c. of ascorbic acid solution containing 5 mg. of the vitamin, 3 c.c. of $CUSO_4$ $5H_2O$ solution containing 10·7 γ Cu^{++} and 5 c.c. water or the solution containing the substance. The systems were let in open conical flasks of 100 c.c. capacity at a temperature of 35° C. in a thermostat. At the beginning of the experiments and thereafter at short intervals, 5 c.c. aliquots of the reaction mixture were taken and after acidification with glacical acetic acid, the vitamin content was determined by titration.

	Substance added	Concentration M	Mg. vitamin C after		
			0	30	60 min.
Ascorbic acid +Cu	..	..	5·0	2·2	0·73
Do	xanthine	$3·3 \quad 10^{-4}$	5·0	5·0	5·0
Do	uric acid	$3·0 \quad 10^{-4}$	5·0	5·0	5·0
Do	adenine	$1·85 \quad 10^{-3}$	5·0	5·0	5·0
Do	guanine	$3·3 \quad 10^{-4}$	5·0	5·0	5·0
Do	theophylline	$2·8 \quad 10^{-4}$	5·0	5·0	5·0
Do	yeast nucleic acid	0·005%	5·0	5·0	5·0
Do	creatinine	$1·8 \quad 10^{-4}$	5·0	4·5	4·0
Do	histidine	$1·2 \quad 10^{-3}$	5·0	4·7	4·0
Do	allantoin	$3·1 \quad 10^{-3}$	5·0	4·7	3·7
Do	Sodium diethyl-dithio carbamate	$1·79 \quad 10^{-3}$	5·0	5·0	5·0
Do	8-hydroxyquino-line	$1·72 \quad 10^{-3}$	5·0	4·0	3·0

The results show that all the purine derivatives inhibit the oxidation of the vitamin in the absence of added Cu at pH 7·2. It is interesting to note from Table III that oxalic acid and 8-hydroxyquinoline which protect

TABLE III

The influence of purines and other substances on the oxidation of vitamin C at pH 7·2 in the absence of added Cu

The reaction mixture contained 10 c c. M/15 phosphate buffer (pH 7·2), 5 c.c. ascorbic acid solution containing 5 mg. of the vitamin and 5 c.c. of water or the solution containing the substance under investigation. The total volume of the reaction mixture was made up to 20 c.c. The results are presented in Table III.

	Substance added	Concentration M	Mg. of vitamin C after incubation for		
			0 hr.	24 hrs.	48 hrs.
Vitamin C	..	..	5·0	0	0
Do	xanthine	$1·64\ 10^{-3}$	5·0	3·6	3·1
Do	uric acid	$1·49\ 10^{-3}$	5·0	3·6	3·1
Do	adenine	$1·85\ 10^{-3}$	5·0	3·6	3·1
Do	guanine	$1·65\ 10^{-3}$	5·0	3·6	3·1
Do	theophiline	$1·39\ 10^{-3}$	5·0	2·9	2·2
Do	8-hydroxy quinoline	$1·72\ 10^{-3}$	5·0	0	0
Do	sodium diethyl dithio carbamate	$1·79\ 10^{-3}$	5·0	3·6	..
Do	Oxalic acid	$1·98\ 10^{-3}$	5·0	0	0

the vitamin at acid pH (Table IV) do not exert any protection against oxidation of the vitamin at the alkaline pH 7·2.

The influence of the inhibitors of cupric ion oxidation of vitamin C on the enzymic oxidation of the vitamin.—The observation that the enzyme ascorbic acid oxidase is a copper-protein compound (Stotz *et al.*, 1937) suggested that a comparative study of the influence of the inhibitors on Cu oxidation and enzymic oxidation of the vitamin might be useful in throwing light on the nature of the Cu-protein linkage.

For the enzymic oxidation the enzyme ascorbic acid oxidase was prepared from pumpkin (*Cucurbita maxima*) and snake gourd (*Tricosanthus anguina*) (Krishnamurthy and Giri, 1941 *b*). The enzyme was prepared by extracting the finely minced vegetable with 30 per cent. alcohol and dialysing the extract for about 16 hours in collodion bags.

The amount of the enzyme solution used for the experiments was so adjusted that the rates of oxidation of the vitamin by the enzyme and Cu were practically the same. The results are presented in Table IV.

TABLE IV

The influence of the inhibitors of Cu-oxidation of vitamin C on the enzymic oxidation of the vitamin

The reaction mixture consisted of 10 c.c. M/5 acetate buffer (pH 5·6), 5 c.c. vitamin C solution containing 5 mg. V. C.), $CuSO_4$ 5 H_2O solution containing 8·02 γ cu^{++} + (for Cu-oxidation) or enzyme solution (for enzymic oxidation) and the solution of the inhibitor, the total volume being adjusted to 20 c.c. Incubation temperature, 35° ± 01° C.

Substance	Concentration of the substance	mg. of Vitamin C found in reaction mixtures containing								
		Vitamin C + Cu^{++}			Vitamin C + ascorbic acid oxidase (from pumpkin)			Vitamin C + ascorbic acid oxidase (from snake gourd)		
		0 min.	30 min.	60 min.	0 min.	30 min.	60 min.	0 min.	30 min.	60 min.
Nil ..	..	5	2·8	1·6	5	3·0	2·0	5	2·8	1·7
Sod. diethyl. dithiocarbamate	$1·79 \times 10^{-3}$M	5	5	5	5	5	5	5	5	5
8-Hydroxyquinoline	$1·72 \times 10^{-3}$M	5	5	5	5	5	4·9	5	4·4	4·4
Adenine ..	$1·85 \times 10^{-3}$M	5	4·9	4·6	5	3·0	2·0	5	2·8	1·6
Uric acid ..	$1·49 \times 10^{-3}$M	5	4·9	4·6	5	3·0	1·8	5	2·9	1·7
Guanine ..	$1·65 \times 10^{-3}$M	5	4·9	4·9	5	3·0	1·8	5	2·9	1·6
Xanthine ..	$1·64 \times 10^{-3}$M	5	4·9	4·9	5	3·0	1·9	5	2·8	1·7
Theophylline ..	$1·39 \times 10^{-3}$M	5	4·7	4·6	5	3·0	1·8	5	2·8	1·7
Creatinine ..	$2·21 \times 10^{-3}$M	5	4·6	4·2	5	3·0	1·9	5	2·9	1·7
Oxalic acid ..	$1·98 \times 10^{-3}$M	5	4·7	4·2	5	3·0	2·0	5	2·0	1·8

The results show that sodium-diethyl-dithiocarbamate and 8-hydroxyquinoline inhibit both the Cu and enzymic oxidation of vitamin C, while the purine compounds, creatinine and oxalic acid, inhibit only the Cu oxidation of the vitamin without any inhibiting action on the enzymic oxidation of the vitamin.

The specific inhibiting action of purines and other substances on the Cu oxidation of vitamin C in presence of the enzyme ascorbic acid oxidase.—The specific property of some of the inhibitors in inhibiting the Cu oxidation of the vitamin without exerting any influence on the enzymic oxidation of the vitamin, may be useful in studying the nature of the catalytic systems present in plants which oxidise the vitamin. The inhibitors can be used for preventing the action of Cu on the vitamin when investigating the nature and action of the enzyme ascorbic acid oxidase on vitamin C. In view of the importance of such inhibitors in their application to the study of the nature of catalytic systems for the oxidation of the vitamin, experiments were carried out on the influence of the inhibitors on the Cu oxidation of the vitamin in presence of the enzyme ascorbic acid oxidase.

The results of these experiments are presented in Table V.

TABLE V

The inhibiting action of purines and other compounds on the Cu oxidation of Vitamin C in presence of the enzyme ascorbic acid oxidase

The reaction mixture consisted of 10 c.c. M/5 acctate buffer (pH 5·6), 5 c.c. vitamin C solution, containing 5 mg., 1 c.c. of the enzyme (from snake gourd), 0·75 c.c. of $CuSO_4$ $5H_2O$ containing 8·2 γ Cu and 3·25 c.c. water or the solution of the substance whose influence on the oxidation of the vitamin is to be investigated. Temperature, 35 ± 0·1 C.

Reaction mixture	Inhibitor	Concentration of inhibitor M	Mg. of vitamin C		
			0 min	30 min.	60 min.
Vitamin C + enzyme ..	..	..	5·0	2·9	1·7
Vitamin C + enzyme + Cu ..	..	..	5·0	2·3	1·1
Vitamin C + enzyme + Cu ..	oxalic acid ..	$1·98\ 10^{-3}$	5·0	3·1	1·0
Vitamin C + enzyme + Cu ..	adenine ..	$1·85\ 10^{-3}$	5·0	2·85	1·7
Vitamin C + enzyme + Cu ..	uric acid ..	$1·49\ 10^{-3}$	5·0	2·9	1·9
Vitamin C + enzyme + Cu ..	creatinine ..	$1·21\ 10^{-3}$	5·0	2·9	1·3

The above results clearly indicate that the substances investigated annul the inhibition of vitamin C oxidation by Cu in presence of the enzyme as ascorbic acid oxidase, the enzymic oxidation being unaffected as before.

DISCUSSION

From the observations reported it is evident that the oxidation of vitamin C by Cu is completely inhibited by xanthine, adenine, guanine, uric acid, theophylline and yeast nucleic acid, while caffeine and theobromine have no influence on the oxidation under the experimental conditions described. In order to determine which portion of the purine derivatives is responsible for the observed effect, the influence of other compounds having the iminazole group have been tested and the results of these tests can be summarised as follows:

1. The iminazole component is esssential for the inhibiting action of the purine derivatives and other iminazole compounds investigated. This is supported by the following observations:

(*a*) In addition to the purine derivatives, creatinine (2, 3-dihydro-2-imino-1-methyl-4-(5)-imidazolone), histidine (β-imodazolylalanine) and allantoin, which contain the iminazole component, exert inhibiting action on the oxidation, although the extent of inhibition is not so great as that with the purine derivatives, under similar experimental conditions.

(*b*) The inhibiting action is destroyed by the breakdown of the iminazole ring structure. Thus creatinine, which contains the iminazole ring, exerts inhibition, while creatine which is formed from creatinine by breaking the ring structure, does not exert similar action on the oxidation of the vitamin.

2. The imino group of the purine derivatives appear to be directly concerned with the inhibitory action of these compounds as the replacement of the hydrogen atoms of the imino groups causes loss of the inhibitory property. Thus the purine derivatives, xanthine, adenine, guanine and uric acid whose imino groups are free, exert considerable inhibition, while caffeine whose imino groups are completely methylated has no such inhibiting action. The inhibiting property of the purine compounds tested depends, therefore, upon the presence of free imino groups in the molecule.

3. Among the imino groups of the purine derivatives, the imino group 7 appears to exert a decisive influence on the oxidation of the vitamin, since the replacement of the hydrogen atom of the imino group by methyl group causes a complete loss of the inhibitory property, although the other imino groups are free. Thus while theophylline which contains free 7 imino group acts as inhibitor, theobromine with its 7 imino group methylated does not affect the oxidation. The evidence on the whole appears in favour of the view that the inhibiting action of the purine derivatives is due to the free 7-imino group of the purines.

The mechanism of inhibition.—As to the mechanism of inhibition of vitamin C oxidation, it is conceivable that the inhibitor forms a complex with copper, thereby preventing the action between the metal and the substrate as in the case of glutathione (Hopkins and Morgan, 1936). The complex thus formed is probably of such a type that transformation of Cu^{I} to Cu^{II}, or the reverse is not possible. This transformation is necessary for the Cu to exert its catalytic effect on the vitamin. Copper combined with the substance, which functions as inhibitor, may not retain its catalytic properties, as in the ionic state.

The results are of interest in indicating the existence of substances in tissues other than glutathione, which exert powerful protection against the oxidation of vitamin C. The fact that purine derivatives, nucleic acids and creatinine are widely distributed in the biological kingdom lends biological significance to these results and points to the possibility that the deleterious effects of Cu which is widely distributed in all living cells together with the vitamin, are diminished or completely eliminated by such substances. An

attempt is being made to study further the reactions involved, with a view to the elucidation of the mechanisms concerned in the retardation of the oxidation by the substances investigated.

Classification of the inhibitors of vitamin C oxidation.—We have also observed (Table IV) that some of the substances when used in concentrations at which they inhibited completely the oxidation of the vitamin by copper, are ineffective on the enzymic oxidation of the vitamin, a point of interest indicating the difference between the enzymic and Cu-oxidation. Sodium diethyldithio carbamate and 8-hydroxiquinolin, however, inhibit both the enzymic and cupric ion oxidation of the vitamin. The various inhibitors of vitamin C oxidation so far known from literature are listed and classified in Table VI according to the effects they produce on the enzymic, cupric ion and other types of oxidation of the vitamin.

The inhibitors may be classified into two main categories: (1) Inhibitors like oxalic acid and purine derivatives which inhibit the oxidation of the vitamin by Cu without exerting any influence on the enzymic oxidation and (2) Inhibitors like sodium diethyl dithio carbamate and 8-hydroxyquinolin which inhibit both the enzymic and cupric ion oxidations.

One of the special advantages of the specific property of the inhibitors belonging to the first category in preferentially retarding the Cu oxidation of the vitamin, is that in their presence the effect of Cu can be eliminated while studying the action of other catalytic systems such as the enzyme ascorbic acid oxidase on the vitamin. Thus pyrophosphate which was shown by Krishnamurthy and Giri (1941 *a*) to inhibit the Cu oxidation of ascorbic acid without exerting any influence on the enzymic oxidation has been used by Steinman and Dawson (1942) in their studies on ascorbic acid-ascorbic acid oxidase reaction in order to prevent the action of Cu in the reaction mixture. Similarly Seshagiri Rao and Giri (1942) have used the inhibitors (oxalic acid) for eliminating the influence of Cu in the reaction mixture on ascorbic acid in their studies on the influences of ascorbic acid on amylase. The rapid oxidation of ascorbic acid in certain plant press juices and vegetables when exposed to air may be due to the catalytic effect of Cu or enzymes. In such cases these inhibitors which preferentially retard the Cu oxidation of the vitamin may prove to be very useful tools in the study of the nature of the catalytic systems in plants which oxidize vitamin C; for any oxidising system containing free ionised copper can be detected by the inhibition produced on adding any one of the above inhibitors to the system.

TABLE VI

Inhibitors of Vitamin C oxidation

I Inhibition ; O No effect on the oxidation

Substance	Auto-oxidation	Oxidation by Cu	Enzymic oxidation	Oxidation by other systems	Reference
			I. Organic compounds		
1. Thiol-and disulphide compounds—					
Glutathione ..	I	I	I	..	Hopkins and Morgan (1936), Bersin *et al.* (1935), Mawson (1935)
do ..	..	I	0	..	Barron *et al.* (1936*b*)
do ..	I	..	..	..	Ghosh and Rakshit (1936)
do ..	..	..	..	Photo-oxidation I	Hopkins (1938), Arcus and Zilva (1940)
Cysteine ..	I	I	..	..	Barron *et al.* (1936*b*)
do ..	..	..	..	Oxidation by tea infusion I	Snow and Zilva (1942)
Cystine ..	I	..	..	..	Rudolph (1938) ; Mawson (1935)
Sodium and hydrogen sulphide	..	I	I	..	Stotz *et al.* (1937)
	..	I	..	..	Seshagiri Rao and Giri (1942), Mawson (1935)
Potassium thiocyanate	..	I	I	..	Stotz *et al.* (1937)
thiourea ..	..	I	I (reversible)	..	McCarthy *et al.* (1939) ; Kawerean and Fearon (1940)
2. Proteins and amino acids—					
Proteins and amino acids	..	I	0	..	Barron *et al* (1936)
Casein and edestin ..	I	..	..	..	Bergel (1944)
Egg albumin ..	..	I	..	..	Krishnamurthy and Giri (1941*c*)
do	..	..	..	Oxidation by tea infusion I	Snow and Zilva (1942)
Dried ovalbumia ..	I	..	..	..	Rudolph (1938)
Peptone ..	..	I	..	..	Krishnamurthy and Giri (1941*c*)
Glycine ..	..	I	0	..	Stotz (1940)
do ..	..	0 (PH6·6)	..	..	Mcfarlane (1936)
do ..	..	..	..	Oxidation by tea infusion 0	Snow and Zilva (1942)
do ..	I	..	..	..	Mystkowski and Losocka (1939)
Leucine and aspartic acid	I	—	..	..	Mystkowski and Lasocka (1939)
Phenylalanine ..	I	..	..	..	Rudolph (1938)
Histidine ..	..	I	..	..	Seshagiri Rao and Giri (this paper)
3. Purine compounds					
Adenine ..	I (PH 7·2)	I	0	..	Giri and Krishnamurthy (1941)
					Seshagiri Rao and Giri (this paper)
Xanthine ..	I (do)	I	0	..	*Idem* ; Bergel (1944)
Uric acid ..	I (do)	I	0	..	*Idem* ; Bergel (1944)
Guanine ..	I (do)	I	0	..	*Idem*
Theophylline ..	I (do)	I	0	..	*Idem*
Yeast nucleic acid ..	..	I	..	..	*Idem*
Sodium urate ..	..	I	..	Oxidation by tea infusion	Snow and Zilva (1942)

TABLE VI (*Continued*)

Substance	Auto-oxidation	Oxidation by Cu	Enzymic oxidation	Oxidation by other systems	Reference
4. Other compounds—					
Sodium diethyl dithiocarbamate	I	I	I	..	Stotz *et al.* (1937) Seshaglri Rao and Giri
do ..	..	I	I (reversible)	..	McCarthy *et al.* (1939)
do ..	..	..	..	Oxidation by tea infusion 0	Snow and Zilva (1942)
8-Hydroxy-quinolin..	I (PH 7·2)	I	I	..	Seshagiri Rao and Giri (this paper)
do ..	..	I	I	..	Stotz *et al.* (1937)
do ..	..	..	0	..	Barron *et al.* (1936*b*)
Potassium ethyl xanthate	..	I	I	..	Stotz *et al.* (1937) McCarthy *et al.* (1939)
Creatinine ..	..	I	..	..	Giri and Krishnamurthy (1941)
Salicyldioxime ..	..	I	I	..	McCarthy *et al.* (1939)
l-adrenalin ..	I (pH 7·4)	..	..	..	Yamamoto (1936)
Allantoin ..	..	I	..	..	Seshagiri Rao and Giri (this paper)
Pyridine ..	..	I	I	..	Stotz *et al.* (1937)
Protoporphyrin ..	0	I	..	..	Schreus and Schummer (1940)
Oxalic acid ..	I (pH 5·0) 0 (pH 7·2)	I	0	..	Krishnamurthy and Giri (1941*c*), Seshagiri Rao and Giri (this paper) Ponting (1943)
Citric and tartaric acids	..	I	..	..	Krishnamurthy and Giri (1941*c*)
Chlorophyll ..	I	..	..	..	Rakshit (1938)
Lecithin (egg) ..	..	I	..	..	Krishnamurthy and Giri 1941*c*)
Acqueous extracts of animal tissues liver, kidney, muscle, spleen, intestines, and erythrocytes	I	I	..	..	Kellie and Zilva (1935), Mawson (1935) De Caro and Giani (1934*c*) Giri and Shourie (1939), Schreus and Schummer (1940)
Leucocytes ..	0	0	..	..	Kellie and Zilva (1935)
II. Inorganic compounds					
Metaphosphoric acid	I	I	..	..	Fujita and Iwatake (1935) Musulin and King (1936) Hinsberg (1937)
Pyrophosphate ..	I	I			Giri (1937), Giri and Doctor (1938); Krishnmurthy and Giri (1941*a*), Steinman and Dawson (1942)
Sodium chloride ..	I	I		..	Decaro and Giani (1934), Kellie and Zilva (1935) Mystkowaski and Lasocka(1939), Mapson (1941)
do ..	..	..	0	..	Mystkowaski (1942)
Potassium ferrocyanide	..	I	I	..	Stotz *et al.* (1937)
Sodium azide ..	..	I	I	..	McCarthy *et al.* (1939)
Boric acid ..	..	..	..	Oxidation by tea infusion I	Snow and Zilva (1942)
Hydrogen-cyanide ..	..	I	I	..	Hopkins and Morgan (1936), Stotz *et al.* (1937) McCarthy *et al.* (1939)

The inhibitors of the enzymic oxidation of ascorbic acid.—Stotz *et al.* (1937) examined the influence of a number of compounds which inhibit the catalytic oxidation of ascorbic acid by Cu on the enzyme ascorbic acid oxidase. They found that sodium diethyldithiocarbamate, 8-hydroxyquinolin, pyridine, potassium thiocyanate, sodium cyanide, potassium ethyl xanthate, potassium ferrocyanide and sodium sulphide which acted as copper inhibitors produced nearly complete poisoning of the enzyme as well as inorganic copper and copper-protein mixture. On the basis of these results the authors suggest that ascorbic acid oxidase is a copper-protein compound and that the activity of ascorbic acid oxidase is related to the presence of copper in combination with proteins. On the other hand, Barron *et al.* (1936 *b*) found that glutathione, proteins and aminoacids protected ascorbic acid from oxidation through the agency of catalytic metals such as Cu, but not from oxidation by enzymes such as the ascorbic acid oxidase of squash. Later Krishnamurthy and Giri (1941 *a*) found that pyrophosphate which inhibits the Cu oxidation of ascorbic acid does not exert any significant influence on the enzymic oxidation of the vitamin. Mystkowski (1942) has shown that the oxidation of ascorbic acid by Cu is inhibited by NaCl, while the activity of ascorbic acid oxidase from cucumber is not influenced by it. The results reported in the present investigation also show that except 8-hydroxyquinolin and sodium diethyldithiocarbamate all the compounds investigated, namely adenine, uric acid, guanine, xanthine, theophylline, creatinine and oxalic acid do not exert any inhibition on the enzymic oxidation of ascorbic acid, while the Cu oxidation of the vitamin is considerably inhibited in their presence. It is clear therefore that all substances which inhibit the Cu oxidation of the vitamin need not necessarily inhibit the enzymic oxidation. Our present knowledge of the chemical nature of the enzyme ascorbic acid oxidase is too limited to allow a fundamental approach to the interpretation of the nature of the difference observed on the effect of the inhibitors on the enzymic and Cu oxidation of the vitamin, but nevertheless it offers interesting field for further exploration.

Summary

1. The influence of xanthine, adenine, uric acid, theophylline, guanine, creatinine, oxalic acid, sodium diethyldithiocarbamate and 8-Hydroxyquinolin on the oxidation of vitamin C by Cu and ascorbic acid oxidase has been studied.

2. Sodium diethyldithiocarbamate and 8-hydroxyquinolin inhibit both the enzymic and Cu oxidation of vitamin C. The other compounds investigated inhibit only the Cu oxidation without exerting any influence on the enzymic oxidation of the vitamin.

3. The bearing of these results on the nature of ascorbic acid oxidase and their application to the study of the nature of catalytic systems in plants which oxidise the vitamin have been discussed. Various types of inhibitors of vitamin C oxidation have been listed and properly classified.

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REFERENCES

Arcus, C. L., and Zilva, S. S. .. *Biochem. J.*, 1940, **34**, 61.
Barron, E. S. G., DeMelo and Klemperer, F. .. *J. Biol. Chem.*, 1936 *a*, **112**, 625.
———, Barron, A. G., and Klemperer, F. .. *Ibid.*, 1936 *b*, **116**, 563.
Bergel, F. .. *Chemistry and Industry*, 1944, **14**, 127.
Bersin, Th., Koster, H., and Jusatz, H. J. .. *Zeit. Physiol. Chem.*, 1935, **235**, 12.
Damodaran, M., and Nair, K. R. .. *Biochem, J.*, 1936, **30**, 1014.
De Caro and Giani, M. .. *Zeit. Physiol. Chem.*, 1934, **228**, 13.
Fujita, A., and Iwatake, D. .. *Biochem. Zeit.*, 1935, 277, 291.
Ghosh, J. C., and Rakshit, P. C. .. *Ibid.*, 1936, **289**, 15.
Giri, K. V. .. *Ind. J. of Med. Res.*, 1937, **25**, 443.
——— and Doctor, N. S. .. *Ibid.*, 1938, **26**, 165.
——— and Shourie, K. L. .. *Ibid.*, 1939, **27**, 650.
——— and Krishnamurthy, P. V. .. *Nature* (Lond.), 1941, **147**, 59.
——— and Seshagiri Rao, P. .. *Proc. 20th Ind. Science Congress*, Baroda., 1942, 96.
Hinsberg, K. .. *Biochem. Zeit.*, 1937, **290**, 125.
Hopkins, F. G. .. *Compt. rend. trav. lab. Carlsberg*, 1938, **22**, 226.
——— and Morgan, E. J. .. *Biochem. J.*, 1936, **30**, 1446.
Kawerean, E., and Fearon, W. R. .. *Soc. Proc. Roy.*, Dublin Soc.
Kellie, A. E., and Zilva, S. S. .. *Biochem JJ.*, 1935, **29**, 1028.
Klodt and Stieb .. *Arch. exp. path. pharmak.*, 1938, **190**, 341.
Krishnamurthy, P. V., and Giri, K. V. .. *Ind. J. of Med. Res.*, 1941 *a*, **29**, 71.
——————— .. *J. Ind. Chem. Soc.*, 1941 *b*, **18**, 7.
——————— .. *Ibid.*, 1941 *c*, **18**, 191.
Levy, L. F. .. *Nature*, 1936, **138**, 933.
Lugg, J. W. H. .. *Australian J. of Exptl. Biol. and Medical Sciences*, 1942, **20**, 273.
Mapson, L. W. .. *Biochem. J.*, 1941, **35**, 1332.
Mawson, C. A. .. *Ibid.*, 1935, **29**, 569.
McCarthy, J. F., Green, L. F., and King, C. G... *J. Biol. Chem.*, 1939, **128**, 455.
Mcfarlane, W. D. .. *Biochem. J.*, 1936, 30, 147, 2.
Mitra, Mitra and Roy .. *J. Ind. Chem. Soc.*, 1940, **17**, 247.

Musulin, R. R. and King, C. G.	.. *J. Biol. Chem.*, 1936, **116**, 409.
Mystkowski, E. M.	.. *Biochem. J.*, 1942, **36**, 494.
——— and Lasocka, D.	.. *Ibid.*, 1939, **33**, 1460.
Ponting, J. D.	.. *Ind. and Eng. Chem.*, Anal Edn., 1943, **15**, 389.
Rakshit, P. C.	.. *Biochem. Z.* 1938, **297**, 153.
Rudolph, W.	.. *Naturwiss.*, 1938, **26**, 155.
Sampson, W.	.. *J. Amer. Chem. Soc.*, 1939, **61**, 389.
Schreus, H. Th. and Schummer, H.	.. *Biochem. Z.*, 1940, **304**, 18.
Schutz, A. F. and Umschweif, B.	.. *Ibid.*, 1934, **268**, 326.
Seshagiri Rao, P. and Giri, K. V.	.. *Proc. Ind. Acad. of Sci.*, 1942, **16B**, 190.
Snow, G. A. and Zilva, S. S.	.. *Biochem. J.* 1942, **36**, 641.
Steinman, H. G. and Dawson, C. R.	.. *J. Amer. Chem. Soc.*, 1942, **64**, 1212.
Stotz, E., Harrer, C. J. and King, C. G.	.. *Science*, 1937, **86**, 35; *J. Biol. Chem.*, 1937, **119**, 511.
Stotz, E.	.. *J. Biol. Chem.*, 1940, **133** C.
Yamamoto, M.	.. *Zeit. fur Physiol. Chem.*, 1936, **243**, 266.

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