149. THE INFLUENCE OF HALIDES ON THE OXIDATION OF ASCORBIC ACID

By L. W. MAPSON

*From the Nutritional Laboratory, University of Cambridge
and Medical Research Council*

(Received 5 November 1941)

PAST work on the influence of inorganic salts on the stability of ascorbic acid is somewhat conflicting. De Caro & Giani [1934] reported that the salts of Ringer's solution had a stabilizing effect on ascorbic acid; Kellie & Zilva [1935] noted that sodium, potassium and calcium chlorides decreased the oxidation of the vitamin at 37° and considered that they acted by inhibiting the catalytic action of copper. Barron *et al.* [1936], however, could not confirm the inhibiting effect of NaCl on the catalytic oxidation of ascorbic acid by copper at a pH of 6.00 in a phosphate buffer.

Klodt & Stieb [1938] found that the addition of NaCl to orange juice increased the stability. Høygaard & Rasmussen [1938] reported less destruction of the vitamin when potatoes, cabbage and liver were cooked in water containing NaCl than when they were cooked in distilled water; the increased retention of the vitamin due to the addition of the salt was, however, in no case greater than 19%, and with liver the effect of the salt was negligible.

The evidence from these studies is therefore confusing, and still less clear are the conditions under which such a salt as NaCl acts. Finally, it has not been shown conclusively which ion of NaCl is active, although Kellie and Zilva's work implied that it was the halide ion, since they obtained positive results with calcium, potassium and sodium chlorides.

The present paper deals with a more detailed investigation into the influence of NaCl and related salts. The results indicate clearly that the catalytic oxidation by Cu is either augmented or diminished, depending on conditions, by chloride, bromide or iodide ions.

EXPERIMENTAL

Determinations of ascorbic acid were carried out chemically by the visual method of Harris & Ray [1933] and Birch *et al.* [1933] using 2:6-dichlorophenol-indophenol. Before titration, all solutions were made up to volume with sufficient 20% metaphosphoric acid to produce a final concentration of 5%. When iodides were used ascorbic acid was determined by titration with iodine. pH was determined electrometrically with the quinhydrone electrode.

All solutions were made up with water freshly distilled from a pyrex glass still, and unless otherwise stated, the water was aerated thoroughly before each experiment to ensure that it was fully saturated with air. This procedure was essential, for if freshly boiled distilled water was used, the oxidation of ascorbic acid was much reduced.

Ascorbic acid was added initially to all solutions in a concentration of 10 mg./100 ml. of solution. All reagents used were of analytical purity.

PART I. EXPERIMENTS AT 100°

The destruction of ascorbic acid in acid solutions heated to 100° was due entirely to oxidation, since it was prevented if the O_2 was completely removed beforehand. Thus ascorbic acid in the presence of the weak organic acids, citric, malic, tartaric or acetic, was not oxidized if N_2 or CO_2 were bubbled through the solution for 15–20 min. before heating. The losses of ascorbic acid in the experiments recorded in this paper were therefore caused by oxidation, and not by any effect of heat *per se* on the acid. Ascorbic acid was relatively stable in solutions of citric, malic, tartaric or acetic acids at room temperature in the presence of O_2 , but the rate of destruction increased rapidly with rise of temperature.

The rate of oxidation of pure ascorbic acid in a solution of 0.1 *M* acetic acid heated at 100° is shown in Fig. 1. It proceeded rapidly for the first 30 min.,

Fig. 1. Oxidation of ascorbic acid in 0.1 *M* acetic acid at 100° and pH 2.87.
----- Solution cooled to room temperature and aerated.

and then virtually ceased. This was due to the elimination of O_2 from the heated solution, for if the latter were cooled to room temperature, re-aerated and then reheated, more ascorbic acid was oxidized, until all the O_2 had again been driven out of the solution (see Fig. 1). The stability of ascorbic acid at 100° has accordingly been determined by heating for 30 min. solutions of the acid in flasks fitted with steam condensers and immersed in a boiling water bath. This period of heating was selected as the longest that could be employed before lack of O_2 brought the reaction to an end. After heating, the flasks were cooled quickly to room temperature and their contents made up to a measured volume with metaphosphoric acid.

When NaCl was added to solutions of ascorbic acid in citric, tartaric, malic, acetic or sulphuric acids, the rate of oxidation of the acid was depressed in comparison with that of control solutions in which no salt was present (see Fig. 1). The protective influence of the NaCl increased with the amount of salt

added, provided that the pH of the solution was not more acid than 2.0 or less acid than 6.0. Typical results showing this influence of NaCl are given in Table 1. Control solutions, to which NaCl was added without ascorbic acid, were heated for 30 min. at 100° and did not reduce the indophenol dye. The addition of salt in the concentrations used did not significantly alter the pH (Table 1). In view

Table 1

In each instance the solution originally contained 10 mg. ascorbic acid/100 ml.

Solution	Molarity of added NaCl	pH	Ascorbic acid after heating 30 min. at 100° mg./100 ml.	% destruction of ascorbic acid	Ascorbic acid retained in solution with salt compared with control = 100
1% citric acid	—	2.35	6.50	35	100
	0.021	2.36	7.90	21	122
	0.042	2.34	8.50	15	131
	0.085	2.34	9.10	9	140
	0.170	2.33	9.60	4	148
	0.250	2.33	9.70	3	150
	0.50	2.32	9.80	2	151
1% tartaric acid	—	2.30	6.40	36	100
	0.042	2.29	7.60	24	119
	0.085	2.30	7.90	21	123
	0.170	2.28	8.50	15	133
	0.250	2.28	9.10	9	142
	0.50	2.27	9.20	8	144
1% malic acid	—	2.47	6.80	32	100
	0.042	2.46	8.20	18	142
	0.085	2.46	8.50	15	146
	0.170	2.45	9.10	9	134
0.1N acetic acid	—	2.87	6.50	35	100
	0.042	2.87	9.25	7.25	142
	0.085	2.87	9.50	5	146
	0.170	2.88	9.90	1	153
	0.250	2.87	10.00	Nil	154
	0.50	2.86	10.00	Nil	154
0.1N H ₂ SO ₄ (adjusted with NaOH to pH 2.30)	—	2.30	4.20	58	100
	0.042	2.30	8.80	12	210
	0.085	2.30	9.30	7	221
	0.170	2.29	9.40	6	225
	0.250	2.28	9.50	5	226
	0.50	2.27	9.70	3	231

of possible practical applications attention has been chiefly directed to the three fruit acids, citric, tartaric and malic, but the investigation has also included such acids as acetic, glycollic, succinic and sulphuric. The same general results have, however, been obtained with all the acids so far examined, differences between them being of a quantitative, not qualitative nature.

Many inorganic salts have now been tested in the same manner to determine their influence on the oxidation of ascorbic acid. In most cases the pH of the solutions was not significantly altered by the addition of the various salts. When any significant change in pH would have been introduced by the addition of the salt, a mixture of salt and acid of the salt was added. With sodium fluoride, the pH was maintained constant by adjustment with H₂SO₄.

Typical results are shown in Table 2. Of all the salts tested, only those containing chloride, bromide or iodide ions showed any inhibitory influence on the oxidation of ascorbic acid. Sulphates, nitrates, acetates and phosphates

Table 2

In each instance the solution originally contained 10 mg. ascorbic acid/100 ml.

Solution	Molarity of salt added	pH	Ascorbic acid after heating 30 min. at 100° mg./100 ml.	% destruction of ascorbic acid	Ascorbic acid retained in solution with salt compared with control = 100
1% malic acid	NaH ₂ PO ₄				
	—	2.47	5.90	41	100
	0.042	2.60	4.60	54	78
	0.085	2.73	4.25	57.5	72
	0.170	2.93	3.70	63	63
1% malic acid	NaH ₂ PO ₄ -H ₃ PO ₄				
	—	2.47	6.20	38	100
	0.04	2.47	6.08	40	97
	0.08	2.47	5.90	41	95
	0.17	2.47	5.80	42	93.5
	0.34	2.47	5.75	42.5	92.5
1% malic acid	Na ₂ SO ₄				
	—	2.46	6.30	37	100
	0.042	2.46	5.50	45	87
	0.085	2.50	5.40	46	86
	0.170	2.54	5.60	44	89
1% malic acid	CaSO ₄				
	—	2.46	5.90	41	100
	0.010	2.47	5.36	46	91
	0.020	2.48	4.95	50.5	84
1% malic acid	Acetic acid				
	—	2.47	6.10	39	100
	0.042	2.44	6.00	40	98.5
	0.085	2.41	6.05	39.5	99
	0.170	2.38	6.10	39	100
1% malic acid	(NH ₄)NO ₃				
	—	2.46	6.00	40	100
	0.042	2.46	6.00	40	100
	0.085	2.46	5.90	41	98
	0.170	2.46	5.80	42	97
	0.250	2.46	5.70	43	96
1% malic acid	(NH ₄) ₂ SO ₄				
	—	2.46	6.20	38	100
	0.042	2.48	6.00	40	97
	0.085	2.50	5.90	41	95
	0.170	2.57	6.00	40	97
	0.340	2.58	5.90	41	95
1% malic acid	KBr				
	—	2.47	6.40	36	100
	0.042	2.47	9.10	9	142
	0.085	2.48	9.60	4	150
	0.170	2.49	10.00	0	157
1% malic acid	KCl				
	—	2.47	5.90	41	100
	0.021	2.46	7.40	26.0	125
	0.042	2.47	7.85	21.5	133
	0.085	2.48	8.40	16	143
	0.170	2.45	8.70	13	148
	0.340	2.45	8.80	12	149
1% malic acid	MgCl ₂				
	—	2.46	5.90	41	100
	0.021	2.46	7.60	24	129
	0.042	2.45	8.70	13	147
	0.085	2.42	9.30	7	157

Table 2 (cont.)

Solution	Molarity of salt added	pH	Ascorbic acid after heating 30 min. at 100° mg./100 ml.	% destruction of ascorbic acid	Ascorbic acid retained in solution with salt compared with control =100
1% malic acid	NaF —	2.53	6.20	38	100
	0.021	2.54	6.10	39	98.5
	0.042	2.57	5.50	45	89
	0.085	2.52	5.20	48	84
	0.170	2.51	4.50	55	73
1% malic acid	BaCl ₂ —	2.47	6.10	39	100
	0.021	2.47	7.60	24	125
	0.042	2.47	8.60	14	141
	0.085	2.44	9.10	9	150
1% tartaric acid	CaCl ₂ —	2.30	6.40	36	100
	0.021	2.28	7.80	22	122
	0.042	2.26	8.40	16	132
	0.085	2.22	9.40	6	148
1% citric acid	KI —	2.35	6.50	35	100
	0.021	2.49	8.70	13	134
	0.042	2.63	9.00	10	139
	0.085	2.75	9.20	8	142
	0.170	2.95	9.40	6	145
	0.340	3.13	9.60	3	148

either had no effect or had a somewhat deleterious influence. Fluorides, it will be noted, in a concentration varying from 0.02 to 0.34 *M* afforded no protection in any of the acid solutions examined.

Fig. 2. Comparison of effects of various chlorides on oxidation of ascorbic acid. Solution: 1% citric acid, pH 2.35, heated 30 min. at 100°.

The results demonstrate that the effective ion is the halide ion, and this supposition is confirmed when quantitative comparisons are made of the effi-

ciency with which the chlorides of calcium, barium, magnesium, potassium and sodium protect ascorbic acid when added in concentrations equivalent to their content of halide. The effectiveness of salts of the type MCl and MCl_2 in inhibiting the oxidation of ascorbic acid in a solution of 1% citric acid, pH 2.35, is shown in Fig. 2. The degree of protection obtained depends on the concentration of the halide ion added, and is independent of the anion of the salt. In concentrations exceeding 0.025 N halide the protective influence of salts of the type MCl_2 is somewhat less than salts of the type MCl .

Influence of copper. In the results so far recorded reagents of the usual analytical purity were used. These, however, invariably contain traces of heavy metals such as Cu. Barron *et al.* [1936] have shown that ascorbic acid is not autoxidizable in pure solution, but reacts with O_2 only when Cu is present as a catalyst. They found that at 25° Cu at a concentration of 1 part in 20 million (46 $\mu g./l.$) was still sufficient to catalyse the oxidation at pH 3.17 in a citrate buffer.

The amounts of Cu present in the reagents used in the present experiments were determined with sodium diethyl-dithio-carbamate according to the method of Callan & Henderson [1929]. As modified by Eden & Green [1940], the reagent is capable of detecting Cu at a dilution of 1 part in 100 million.

The glacial acetic acid contained 1.7 parts of Cu in 10^6 , the citric, tartaric and malic acids 1–1.5 parts in 10^6 and the glass distilled water no detectable amount. The ascorbic acid contained 1 part per million, but owing to the very small amount added, 10 mg./100 ml., the amount of Cu thus introduced into the solution was insignificant (1 part in 10^{10}).

The main sources of Cu were thus the acids. In the final concentrations the amount of Cu present was 1 part in 100×10^6 for 0.1 M acetic acid; for tartaric, citric and malic acids 1 part in 70×10^6 . These concentrations, small though they are, were still sufficient to cause catalytic oxidation of ascorbic acid, as the following experiment shows.

Glacial acetic acid was distilled in an all-glass Pyrex apparatus and made up in a concentration of 0.1 M , ascorbic acid added and the stability of the latter tested by the routine procedure. An estimation of the Cu present in the distilled acid showed that it had been reduced to approximately 1 part in 4×10^6 , giving a final concentration in the solutions used for the test of 1 part in 650×10^6 . With this amount, the loss of ascorbic acid due to oxidation was negligible—in one experiment there was a loss of 3%, in another there was no loss.

By the addition of known small amounts of Cu to the redistilled acetic acid, the amount of Cu necessary to cause oxidation of ascorbic acid under the standard conditions could be ascertained. These points are illustrated in Table 2A.

Table 2A

In each instance the solution originally contained 10 mg. ascorbic acid/100 ml.

Solution	Cu present parts/million	Ascorbic acid after heating 30 min. at 100° mg./100 ml.	% destruction of ascorbic acid
0.1 M acetic acid (glass distilled)	0.0015	9.7	3
"	0.0015	10.0	0
"	0.0033	9.0	10
"	0.01	7.5	25
"	0.05	6.0	40
"	0.10	3.6	64
"	0.5	1.6	84
"	2.5	0.5	95

A concentration of more than 1 part of Cu in 650×10^6 must therefore be avoided if ascorbic acid is to be completely protected against oxidation at 100° .

Attempts were made to eliminate Cu from the other acids studied, but results have not been entirely successful. Citric acid, even after four or five recrystallizations, still contained 1 part in 2×10^6 , and when made up in a 1% solution gave a concentration of 1 part in 200×10^6 . Tests with this citric acid showed that, as the concentration of Cu was reduced, less ascorbic acid was lost (Table 2B).

Table 2B

In both instances the solution originally contained 10 mg. ascorbic acid/100 ml.

Solution	Cu present parts/million	Ascorbic acid after heating 30 min. at 100° mg./100 ml.	% destruction of ascorbic acid
1% citric acid	0.014	6.0	40
1% citric acid (4th recrystallization)	0.005	7.5	25

As mentioned in the introduction, Kellie & Zilva [1935] found that the oxidation of ascorbic acid in solutions to which Cu was added was much less if NaCl were present. Cu salts easily form complex salts with the halogens, and this offers the simplest explanation of the protective action of the halide ions.

To test this hypothesis various amounts of Cu as CuSO_4 were added to different acid solutions, and the stability of ascorbic acid at 100° was determined in the presence of added halide salts in the usual manner. Experiments in which acetic and citric acid solutions were used, which are typical of the general results obtained, are illustrated in Figs. 3 and 4, respectively. The acetic acid was freed from Cu by distillation, and allowance was made for the Cu present as impurity in the citric acid. It is clear that the halide ion, if added in sufficient concentration, inhibits the catalytic oxidation of ascorbic acid by Cu, and that the extent of this inhibition depends on the actual concentration of Cu present; the greater the concentration of Cu, the greater the concentration of the halide necessary to obtain the same protection. To suppress entirely the oxidation of ascorbic acid in 0.1M acetic acid at pH 2.87 with Cu present in the following concentrations, 0.12×10^{-7} , 5×10^{-7} and 25×10^{-7} , chloride must be present in concentrations of 0.2, 0.7 and 1.6 M, respectively. With corresponding amounts of Cu, more NaCl was needed to suppress the oxidation to an equivalent extent in the citric acid, than in the acetic acid solution.

When chloride was present in very low concentration it had a small but definite deleterious influence on the stability of ascorbic acid. The range of concentration of chloride throughout which this slight destructive influence was observed, increased as the content of Cu present in solution was increased. For example, under the conditions shown in Fig. 3, with Cu present in a concentration of 0.12×10^{-7} , NaCl had to be added in excess of 0.001M to suppress the oxidation below that in the control solution, and the range of salt throughout which the destructive influence was found was 0–0.001M. With Cu in a concentration of 25×10^{-7} , on the other hand, it was necessary to have the halide present in a concentration of more than 0.02 M before any protection was obtained, and the range of concentration in which the destructive effect was observed correspondingly increased.

Relative efficiencies of chloride, bromide and iodide ions at 100° . The efficiency with which the three halide ions protect ascorbic acid from oxidation at 100° has been determined in the range of concentrations of 0.0001–0.5 M. Neither

Fig. 3. Relation between concentration of Cu and chloride in oxidation of ascorbic acid. Solution: 0.1 *M* acetic acid, pH 2.87, heated 30 min. at 100°.

Fig. 4. Oxidation of ascorbic acid as influenced by Cu and halide, in solution of citric acid, 1%. pH 2.35, heated 30 min. at 100°.

Fig. 5. Comparison of effects of various halides on oxidation of ascorbic acid. Solution: 0.1 *M* acetic acid, pH 2.87, Cu = 5×10^{-7} , heated 30 min. at 100°.

Table 3

In each instance the solution originally contained 10 mg. ascorbic acid/100 ml.

Solution	Molarity of salt added	pH	Ascorbic acid after heating 30 min. at 100° mg./100 ml.	% destruction of ascorbic acid	Ascorbic acid retained in solution with salt compared with control = 100
1% malic acid	KCl				
	—	2.43	6.00	40	100
	0.02	2.43	7.40	26	123
	0.04	2.43	7.85	21.5	131
	0.08	2.43	8.40	16	140
	0.17	2.43	8.70	13	135
1% malic acid	KBr				
	—	2.43	6.20	38	100
	0.02	2.43	8.20	18	132
	0.04	2.43	9.10	9	147
	0.08	2.43	9.60	4	155
	0.17	2.43	10.00	0	162
1% malic acid	KI				
	—	2.43	6.20	38	100
	0.02	2.43	9.10	9	147
	0.04	2.43	9.60	4	155
1% tartaric acid	KCl				
	—	2.25	6.40	36	100
	0.02	2.25	6.85	31.5	107
	0.04	2.25	7.25	27.5	113
	0.08	2.25	7.90	21	124
	0.17	2.25	8.45	15.5	132
1% tartaric acid	KBr				
	—	2.25	6.40	36	100
	0.02	2.25	8.10	19	127
	0.04	2.25	8.60	14	135
	0.08	2.25	8.90	11	139
	0.17	2.25	9.10	9	143
1% tartaric acid	KI				
	—	2.25	6.40	36	100
	0.02	2.25	8.90	11	139
	0.04	2.25	9.40	6	147
1% citric acid	KCl				
	—	2.35	5.90	41	100
	0.02	2.35	7.80	22	132
	0.04	2.35	8.50	15	144
	0.08	2.35	9.00	10	152
	0.17	2.35	9.50	5	161
1% citric acid	KBr				
	—	2.35	5.90	41	100
	0.02	2.35	8.20	18	139
	0.04	2.35	9.00	10	152
	0.08	2.35	9.30	7	158
	0.17	2.35	9.60	4	163
1% citric acid	KI				
	—	2.35	5.90	41	100
	0.02	2.35	9.10	8	155
	0.04	2.35	9.30	6	158
	0.08	2.35	9.50	4	161
	0.17	2.35	9.70	2	164
	0.34	2.35	10.00	0	169

KCl nor KBr when added in these concentrations to the acid solutions examined caused any appreciable change in pH , but KI, in the highest concentrations used, altered it slightly; when this was so the pH was readjusted with dilute H_2SO_4 . The relative influence of these ions in amounts varying from 0.001 to 0.34 M on the oxidation of ascorbic acid in a solution of 0.1 M acetic acid, pH 2.87, with Cu present at a concentration of 2.5×10^{-7} , is illustrated in Fig. 5, and further examples with other acid solutions are shown in Table 3. The order of efficiency of the ions is chloride < bromide < iodide. Some typical results obtained with weaker solutions than 0.02 M of halide are shown in Table 4.

Table 4

In each instance the solution originally contained 10 mg. ascorbic acid/100 ml.

Solution	Molarity of salt added	Concentration of Cu present	Ascorbic acid after heating 30 min. at 100° mg./100 ml.	% destruction of ascorbic acid
0.1 M CH_3COOH , pH 2.87	KCl			
	—	5×10^{-7}	1.8	82
	0.0001	5×10^{-7}	1.3	87
	0.001	5×10^{-7}	0.7	93
	0.005	5×10^{-7}	0.9	91
0.1 M CH_3COOH , pH 2.87	KBr			
	0.001	5×10^{-7}	1.6	84
	0.0001	5×10^{-7}	1.2	88
	0.001	5×10^{-7}	0.6	94
	0.005	5×10^{-7}	1.5	85
0.1 M CH_3COOH , pH 2.87	KI			
	0.01	5×10^{-7}	2.4	76
	0.0001	5×10^{-7}	1.1	89
	0.001	5×10^{-7}	0.7	93
	0.005	5×10^{-7}	2.3	77
	0.01	5×10^{-7}	3.3	67

Both the bromide and iodide, like the chloride, when present in very small amounts, accelerated the oxidation of ascorbic acid compared with solutions without halide. Table 4 shows also that the minimum concentration of halide necessary to obtain protection is least with iodide and greatest with chloride.

Influence of pH . Kellie & Zilva [1935] observed the beneficial effect of NaCl in preventing the oxidation of ascorbic acid in water. They did not state the pH of their solution, but it must have been of the order of pH 4.6, for the concentration of ascorbic acid they used was 35 mg./100 ml. Barron *et al.* [1936], who could not confirm Kellie and Zilva's results, used a buffered citrate solution of pH 6.0. In view of the findings reported in this paper, these divergent results seem to the writer to be explicable by differences in the pH of their solutions.

The influence of pH on the protective action of the halide ions has, therefore, been studied in solutions of three different acids, citric, acetic and sulphuric, over a pH range varying with the acids from 1.20 to 6.5. No results of such a study are valid unless other factors are also controlled. It is obvious that Cu must be one of these, and, as is pointed out later in the paper, the control of the concentration of hydroxy compounds, especially hydroxy-acids, is important.

Solutions of pH varying from 2.30 to 6.50 were prepared, using a mixture of citric acid and sodium citrate, and the oxidation of ascorbic acid, with and without added halide salts, was studied. The concentration of the total citrate, present either as acid or as sodium citrate, was controlled by adding citric acid and sodium citrate in equivalent proportions. Two series of such solutions were

prepared with a total amount of citrate of 0.1% and 1%. Cu in all the solutions was maintained constant, in the 1% citrate solution at 0.25×10^{-7} and in the 0.1% solution at approximately 0.025×10^{-7} .

Figs. 6 and 7 illustrate the results obtained; it is clear that the maximum protection given by the chloride is obtained between pH values of 2.30–4.00 in both the weaker and the stronger citrate solutions. As the pH becomes progressively more alkaline than 4.0 there is an abrupt fall in the protection afforded, to a value approximating to zero in the neighbourhood of pH 6.0. The stability

Fig. 6.

Fig. 7.

Fig. 6. Influence of pH on protective action of chloride. Citric buffer (0.1% total citrate), $\text{Cu} = 0.025 \times 10^{-7}$, solution heated 30 min. at 100° .

Fig. 7. Influence of pH on protective action of chloride. Citric buffer (1.0% total citrate), $\text{Cu} = 0.25 \times 10^{-7}$, solution heated 30 min. at 100° .

of ascorbic acid in the control solutions was similarly affected. Results obtained with mixtures of molar acetic acid and sodium acetate solutions, with Cu present in a concentration of 5×10^{-7} , showed the same influence of pH on the protective action of the halide ions. Further data, obtained with solutions of 0.1N H_2SO_4 , neutralized with NaOH or NaHCO_3 to pH values ranging from 1.20 to 6.0, support the same conclusion; there was, however, the difference that in solutions more acid than pH 2.0, the oxidation of ascorbic acid was considerably reduced, with the result that the protection due to the halide ion was correspondingly decreased. At a pH of 1.20, there was no advantage in adding a halide salt; indeed, with the higher concentrations of salt, the action of the halide was deleterious (see Table 5). The explanation of this finding is not clear.

The results indicate that the influence of pH on the protective action of the halide ions is significantly affected neither by the nature of the acid radical present, nor by its concentration, nor by the amount of Cu present. It is possible

Table 5

In each instance the solution originally contained 10 mg. ascorbic acid/100 ml.

Solution	Molarity of NaCl added	pH	Ascorbic acid after heating 30 min. at 100° mg./100 ml.	% destruction of ascorbic acid
H ₂ SO ₄ , 0.1 N	—	1.20	9.0	10
	0.04	1.20	9.3	7
	0.08	1.20	9.4	6
	0.17	1.20	9.3	7
	0.25	1.20	8.8	12
	0.50	1.20	7.8	22
H ₂ SO ₄ , 0.1 N (partly neutralized)	—	1.90	5.4	46
	0.04	1.90	8.1	19
	0.08	1.90	9.0	10
	0.17	1.90	9.2	8
	0.34	1.90	9.4	6
H ₂ SO ₄ , 0.1 N (partly neutralized)	—	2.30	4.2	58
	0.04	2.30	8.8	12
	0.08	2.30	9.3	7
	0.17	2.30	9.5	5
	0.25	2.30	9.6	4
	0.50	2.30	9.9	1

that other substances not present in these solutions, but present in natural products, may alter the influence of pH. This effect of pH partly explains why Kellie & Zilva [1935] obtained a positive result with NaCl, and Barron *et al.* [1936] a negative one.

Influence of hydroxy compounds. A further factor of importance is the nature and concentration of other acid anions. If reference be made to Table 1 it is evident that the efficiency with which the added salt prevents the oxidation of ascorbic acid in the different acid solutions, varies considerably. In these preliminary experiments the acids were not used in equivalent concentrations, and—even of more importance—the amount of Cu in different solutions varied. Direct comparison of the efficiency of the halide ion in protecting ascorbic acid from oxidation in these solutions could not therefore be made.

If the concentration of Cu and the pH are controlled, and the acids are used in equivalent concentration, direct comparison is possible. A series of experiments has been carried out in which the protective influence of the halide ion has been determined in solutions of the acids at concentrations of 1, 0.1 and 0.01 *M*. Cu was added in all cases to give a concentration of 1 part in 2×10^6 , allowance being made for the Cu present as impurity in the acids and other reagents used.

When the natural pH of the acid solution together with the ascorbic acid added was lower than pH 2.30, it was adjusted with NaOH to 2.30. In the case of the weaker acid solutions, when the pH was higher than 2.30 no adjustment was made, since it has already been shown that the protective effect due to halide was constant and at a maximum within a pH range of 2.3–4.0.

Experiments have been carried out with citric, tartaric, glycollic, acetic, malic and succinic acids. All the results obtained are in general agreement and indicate that the protective influence of the halide ion varies with the nature and concentration of the acid anion used. With increasing concentration of the hydroxy-acids the protective influence of the halide ion is decreased, whereas acids containing no hydroxyl group have no such influence. For example, NaCl, when added in a concentration of 0.34 *M*, had no protective

Fig. 8. Relation between concentrations of glycolate and chloride in oxidation of ascorbic acid. $\text{Cu} = 5 \times 10^{-7}$; 30 min. heating at 100° .

Fig. 9.

Fig. 9. Relation between concentrations of acetate and chloride. $\text{Cu} = 5 \times 10^{-7}$; 30 min. heating at 100° .

Fig. 10.

Fig. 10. Relation between concentrations of citrate and chloride. $\text{Cu} = 5 \times 10^{-7}$; 30 min. heating at 100° .

Table 6

In each instance the solution originally contained 10 mg. ascorbic acid/100 ml.

Solution	Molarity of NaCl added	pH	Cu present parts/million	Ascorbic acid after heating 30 min. at 100° mg./100 ml.	% destruction of ascorbic acid
Tartaric acid, 0.01 <i>M</i>	—	2.30	0.5	3.80	62
	0.02	2.30	0.5	4.00	60
	0.04	2.30	0.5	4.50	55
	0.08	2.30	0.5	5.00	50
	0.17	2.30	0.5	6.80	32
	0.34	2.30	0.5	8.40	16
Tartaric acid, 0.1 <i>M</i>	—	2.35	0.5	5.30	47
	0.02	2.35	0.5	4.80	52
	0.04	2.35	0.5	4.90	51
	0.08	2.35	0.5	5.20	48
	0.17	2.35	0.5	6.00	40
	0.34	2.35	0.5	6.30	37
Tartaric acid, 1 <i>M</i>	—	2.30	0.5	6.30	37
	0.02	2.30	0.5	6.30	37
	0.04	2.30	0.5	6.10	39
	0.08	2.30	0.5	6.20	38
	0.17	2.30	0.5	5.80	42
	0.34	2.30	0.5	5.60	44
Succinic acid, 0.01 <i>M</i>	—	3.07	0.5	2.50	75
	0.04	3.07	0.5	5.40	46
	0.08	3.07	0.5	6.60	36
	0.17	3.07	0.5	7.70	23
Succinic acid, 0.6 <i>M</i>	—	2.83	0.5	2.40	76
	0.04	2.83	0.5	4.50	55
	0.08	2.83	0.5	5.60	44
	0.17	2.83	0.5	6.90	31

Fig. 11. Influence of glucose on protective action of chloride. Solution: 0.1 *M* acetic acid, pH 2.87; Cu = 5×10^{-7} ; 30 min. heating at 100°.

influence on the oxidation of ascorbic acid in molar solutions of citric, tartaric, malic or glycollic-acids, but in molar solutions of acetic and succinic acids the degree of protection obtained was considerable. Indeed, with the non-hydroxy acids the protective influence of the salt was not markedly different in solutions varying in concentration from 0.01 to 1 *M*. In the weaker solutions (0.01–0.1 *M*) of the hydroxy-acids, the action of the salt was well marked. The results with glycollic, acetic and citric acids are illustrated in Figs. 8, 9 and 10, and with the other acids in Table 6.

In the absence of halide ions the extent of the oxidation of ascorbic acid depends on the nature and concentration of the acid anion. In equivalent concentration the hydroxy-acids depress the oxidation to a greater extent than do acids like acetic, and this effect is most marked in the more concentrated solutions. Indeed, with the non-hydroxy-acids examined, if *pH* is controlled, the oxidation is independent of the concentration of the acid anion. In an experiment with succinic acid at a concentration of 0.01 *M* (*pH* 3.07) and 0.6 *M* (*pH* 2.90) the losses at 100° were 75 and 76 %, respectively.

Other hydroxy compounds behaved like the hydroxy-acids. Fig. 11 shows the results of an experiment in which the effect of increasing amounts of glucose on the protective action of the halide ion was determined. Here, as with the hydroxy-acids, as the concentration of the sugar was increased, so the degree of protection due to the halide ions was reduced, though not to the same marked extent as with the hydroxy-acids. The same effect was seen with ethyl alcohol.

PART II. INFLUENCE OF HALIDE IONS ON THE OXIDATION AT 20°

The results outlined in Part I of this paper have shown that the stability of ascorbic acid in solution at 100° depends on the relative amounts of Cu and halide ions present, and that the action of these ions is modified by change in *pH*, and by the presence of hydroxy compounds. To determine the influence of temperature on this system, the oxidation of ascorbic acid has also been studied at a lower temperature, viz. 20°. The catalytic influence of Cu on the oxidation is increased by a rise of temperature, Barron *et al.* [1936] reporting a temperature coefficient of 1.65 for the reaction. The experiments to be described below fully support these conclusions, and in addition show how the action of the halide ion is influenced by temperature.

At 100° the stability of ascorbic acid was tested by determining its loss over a given period of time (30 min.). At 20° the solubility of O₂ in the solutions used was sufficient to allow the reaction ascorbic acid → dehydroascorbic acid to proceed to completion. This enabled the rate of oxidation to be readily followed. The reaction was studied at 20° in solutions of 0.1 *M* acetic acid at *pH* 2.87, or in 1 % citric acid at *pH* 2.35, in the presence of varying amounts of Cu. The influence of chloride, added as NaCl, in concentrations varying from 0.02 to 1.6 *M* was examined. Two experiments in this series, typical of others, are illustrated in Figs. 12 and 13. The general results show that the influence of the halide ion at 20°, as at the higher temperatures, depends on its concentration. With the lower concentrations there is a marked increase in the rate at which the ascorbic acid is oxidized, compared with control solutions without halide, and not until a considerable amount of halide is present is the oxidation reduced below the rate for the control. Qualitatively, the results are similar to those obtained at 100°. At the lower temperature, however, chloride must be present in an amount considerably in excess of that necessary under corre-

sponding conditions at 100° in order to suppress the oxidation to the same extent. These results are the more remarkable when it is remembered that the catalytic activity of Cu is much greater at the higher temperature.

Fig. 12.

Fig. 13.

Fig. 12. Rate of oxidation of ascorbic acid at 20° as influenced by varying concentrations of NaCl. Solution: $0.1M$ acetic acid, pH 2.87, $Cu = 25 \times 10^{-7}$. Numbers on curve refer to order of concentration of NaCl used.

Fig. 13. Rate of oxidation of ascorbic acid at 20° as influenced by NaCl in presence of a hydroxy-acid. Solution: 1% citric acid, pH 2.35, $Cu = 25 \times 10^{-7}$. Numbers on curve refer to order of concentration of NaCl used.

The graphs in Figs. 14 and 15 show more clearly the relationship between the concentrations of Cu and chloride, and the stability of the ascorbic acid. The ordinates represent the percentage of ascorbic acid retained during a period of 30 min. at 20° . These values were obtained either by direct experimental observation or by intrapolation from curves such as those shown in Figs. 12 and 13. The results are thus directly comparable with those in the similar experiments carried out at 100° and illustrated in Figs. 3 and 4. For ease of comparison the former graphs have been replotted on the same scale and are shown as dotted lines in Figs. 14 and 15.

At the lower temperature the deleterious action of the chloride, in the weaker concentrations, is more pronounced, both in the magnitude of the destruction brought about and also in the range of concentration throughout which its influence extends. It should, however, be noted that the amount of NaCl necessary to suppress completely the oxidation in the presence of a given concentration of Cu is not appreciably modified by change of temperature. As at the higher temperature, the extent to which the chloride adversely affects the stability of ascorbic acid depends on the amount of Cu present. For example, in $0.1M$ acetic acid at 20° , with a concentration of Cu of 0.12×10^{-7} , the maximum extra loss over that in the control solution is 3.5% , and no protection is obtained until the chloride is present in a concentration of $0.1M$,

whereas in the same solution with Cu at 25×10^{-7} , the maximum extra loss is 60 %, and protection is only obtained when the halide ion is present in a concentration greater than 0.55 *M*.

Fig. 14. Comparison of effects of varying concentrations of chloride and Cu, at 20° and 100°. Solution: 0.1 *M* acetic acid, pH 2.87. Oxidation period = 30 min. — Solution at 20°, solution at 100°.

Fig. 15. Influence of varying concentrations of chloride and Cu, at 20° and 100°, in solution of a hydroxy-acid. Solution: 1.0% citric acid, pH 2.35. Oxidation period = 30 min. — Solution at 20°, solution at 100°.

Efficiencies of the chloride, bromide and iodide at 20°. Quantitatively the same results have been obtained with the two other halide salts, bromide and iodide. Fig. 16 shows the relative influence of the three halides on the oxidation during a period of 2 hr. at 20° in a solution of acetic acid (0.1 *M*). The efficiencies of the ions in suppressing the oxidation of ascorbic acid are chloride < bromide < iodide.

If we compare the results illustrated in Fig. 16 and the corresponding results at 100° (Fig. 5), it is evident that in contrast with chloride and bromide the protective influence of the iodide is not significantly altered by temperature.

A summary of these data is given in Table 7; this shows that an increase of temperature from 20 to 100° decreases by 1/20 and 1/15 respectively, the amount of chloride and bromide that it is necessary to add to depress the oxidation of ascorbic acid below that in a control solution without halide. The concentration of the iodide remains unaltered.

Fig. 16. Relative effects of different halides on oxidation of ascorbic acid at 20°. Solution: 0.1 *M* acetic acid, pH 2.87, Cu = 5×10^{-7} , oxidation period = 2 hr.

The highest concentrations shown in the last column represent the smallest amount of each halide salt which it was necessary to add to suppress entirely the oxidation of ascorbic acid in the acetic acid solution. For each salt these concentrations, it will be noted, are not significantly affected by change in temperature.

Table 7

Solution	Temperature	Range of concentration of the halide ion throughout which the stability of ascorbic acid is decreased	Concentrations of halide ion throughout which the stability is increased
Acetic acid, 0.1 <i>M</i> , with Cu present in a concentration of 5×10^{-7}	20°	KCl 0-0.23 <i>M</i>	0.23-0.52 <i>M</i>
	20°	KBr 0-0.12 <i>M</i>	0.12-0.38 <i>M</i>
	20°	KI 0-0.005 <i>M</i>	0.005-0.11 <i>M</i>
	100°	KCl 0-0.011 <i>M</i>	0.011-0.50 <i>M</i>
	100°	KBr 0-0.008 <i>M</i>	0.008-0.32 <i>M</i>
	100°	KI 0-0.005 <i>M</i>	0.005-0.12 <i>M</i>

Influence of O₂. The influence of the concentration of O₂ upon the stability of ascorbic acid in the presence of chloride has been determined at 20°. The rate of oxidation of the acid was measured in a solution of 0.1 *M* acetic acid, with Cu present in a concentration of 5×10^{-7} . Separate solutions had previously been brought into equilibrium with either O₂, air or a gas mixture containing 5% O₂ and 95% N₂ (Fig. 17), and this equilibrium was maintained throughout the experiment by the continuous bubbling of the gases through their respective solutions. The results are summarized in Fig. 18 and show that an increase in the concentration of O₂ enhances the destructive influence of the chloride. It is worth noting that the oxidation of the acid in the control solu-

Fig. 17. Rate of oxidation of ascorbic acid at 20° as influenced by O₂ and concentration of chloride. Solution: 0.1 M acetic acid, pH 2.87, Cu = 5×10^{-7} . Top panel = 5% O₂ + 95% N₂. Middle panel = air. Bottom panel = 100% O₂. Numbers on curves refer to order of concentration of NaCl used.

tions was not increased when air was replaced by pure O_2 . A reduction in the rate of oxidation occurred, however, when air was replaced by the gas mixture of 5% O_2 and 95% N_2 .

Fig. 18. Action of chloride at 20° as influenced by amount of available O_2 . Solution: $0.1 M$ acetic acid, $pH\ 2.87$, $Cu = 5 \times 10^{-2}$. Oxidation period = 2 hr.

DISCUSSION

The evidence presented in this paper shows that the oxidation of ascorbic acid catalysed by Cu is influenced to a large extent by the addition of the halide salts, chloride, bromide and iodide. At a temperature of 100° chloride and bromide were relatively more effective in suppressing the oxidation than at lower temperatures, chiefly because the range of concentrations throughout which their deleterious action was observed was decreased. In practice this means that a greater concentration of the halide salt is necessary to suppress the oxidation of the acid at ordinary temperatures. In contrast, the action of iodide is not significantly altered by change in temperature. The action of the halides at all temperatures is correlated with the amount of Cu present, for the extent both of their destructive and protective influences varies with the concentration of the Cu.

The complete removal of Cu from a solution renders ascorbic acid stable to O_2 and unaffected by the presence or absence of halide ions. Very small amounts of Cu are sufficient at a temperature of 100° to catalyse the oxidation of the acid; in the experiments reported here, Cu had to be reduced to approximately 0.015×10^{-7} before the oxidation of the acid was virtually suppressed.

It seems probable that the inhibitory action of the halide ions is due to the formation of a complex ion between them and Cu. An explanation of the destructive influence of the halide ion is far from clear. The fact that the magnitude of this effect is connected with the amount of Cu present indicates that Cu participates in these reactions. A plausible explanation is that the halide ion forms a hydroxy-acid complex, and the O_2 so activated is more readily available with Cu for the catalytic oxidation of ascorbic acid. When sufficient halide has been added, the copper-halide complex is formed and the oxidation

thereby suppressed. Admittedly no evidence in support of this explanation has been obtained, apart from the fact that the destructive influence is more marked as the O_2 in the solution is increased or as the temperature is lowered—both conditions in which the formation of such a hypothetical oxy-halide ion should be favoured.

The precise mechanism of the action of the hydroxy-acids and other hydroxy compounds in decreasing the effectiveness of the halide salts is also not clear. It seems probable that they act in the same manner as these salts, by forming complexes with Cu. If this is true, they must compete with the halide ions for Cu, for the higher the concentration of the hydroxy compound, the greater the concentration of salt needed to obtain the halide effect. Moreover, in solutions like molar citric acid, in which there is no protective influence of added salt up to concentrations of 0.34 *M*, the percentage loss of ascorbic acid is of the order of 36, but in solutions of 0.01 *M* citric acid with the same concentration of salt, the percentage destruction of ascorbic acid has been reduced to 6. Any hypothetical complex formed between the citrate ion and Cu must, therefore, prevent the formation of the complex between halide and Cu. This, however, presupposes 100 % complex formation between Cu and citrate, and yet oxidation of ascorbic acid is not under these conditions reduced to zero. The implications of this argument suggest that the complex formed between Cu and hydroxy compound still retains some catalytic activity.

Whatever the real explanation, from the practical point of view it is clear that a greater retention of the vitamin can be obtained in a solution of a weak hydroxy acid plus salt than in a solution of a more concentrated hydroxy acid without salt at 100°. In a continuation of this work it is hoped to throw more light on these points.

The degree of protection of ascorbic acid which can be secured by adding halide salts to any foodstuff or processed extract will depend on the concentration in which they are added, on the temperature, on the pH, on the content of Cu and on the nature and concentration of other substances such as hydroxy-acids and sugars. It is probably true that in a complex system like a foodstuff, other factors not recognized here may influence the action of these salts and speculation is therefore unprofitable. It is certainly true, however, that these halide effects would appear to be more generally applicable to fresh fruits and processed fruit products, than to vegetables which have a natural pH of 6.0–7.0 and are not generally processed in acid media.

The degree to which salt can be used with advantage in foods to preserve vitamin C will depend not only on the factors enumerated above but on the amount of salt it is possible to add without detracting from taste and flavour. This limit will vary with different foods, but concentrations of salt of from 1 to 2 % can be used in cooking vegetables, and concentrations up to 0.5 % can be added to most fruits, without affecting palatability.

SUMMARY

1. The oxidation of ascorbic acid was studied under standardized conditions in various acid solutions heated to 100° for 30 min.
2. Ascorbic acid was not oxidized at 100° if Cu was reduced to 1 part in 650×10^6 of solution.
3. The oxidation was greatly reduced if chloride, bromide or iodide salts were added in sufficient amount, the extent of the reduction being proportional to the concentration of the halide present. In weaker concentrations, however,

the halides had a slight destructive effect. Both the destructive and protective influences of the salts were correlated with the amount of Cu present.

4. The action of the halide salts was independent of the nature of the cation, though slightly influenced by the valency of the salt.

5. The relative efficiencies of the three halides in stabilizing ascorbic acid were of the order, iodide > bromide > chloride. Fluoride had no protective action.

6. The protective influence of the halides was operative over a pH range of 2.0–6.0, with a maximum effect at pH 2.3–4.0, but diminished rapidly between 4.0 and 6.0, or below 2.0. At pH 1.20 a high concentration of halide was deleterious.

7. Hydroxy-acids antagonized the protective action of the halide salts.

8. The influence of the halides on the rate of oxidation of ascorbic acid at 20° corresponded qualitatively with their influence at 100°. Quantitatively, however, the destructive effects of chloride and bromide at lower concentrations were markedly increased, but that of the iodide not significantly affected by the alteration of temperature.

9. An increase in the concentration of O₂ enhanced the destructive influence of the halides at 20°.

I wish to thank Drs C. S. Hanes and F. H. Isherwood for valuable suggestions, and Dr L. J. Harris for his helpful criticism and advice; to Mr L. D. Ward I wish to express my thanks for his technical assistance.

The work described above was carried out as part of the programme of the Food Investigation Board, and is published by permission of the Department of Scientific and Industrial Research and the Medical Research Council.

REFERENCES

- Barron, De Meio & Klemperer (1936). *J. biol. Chem.* **112**, 625.
Birch, Harris & Ray (1933). *Biochem. J.* **27**, 590.
Callan & Henderson (1929). *Analyst*, **54**, 650.
De Caro & Giani (1934). *Z. phys. Chem.* **228**, 13.
Eden & Green (1940). *Biochem. J.* **34**, 1202.
Harris & Ray (1933). *Biochem. J.* **27**, 303.
Høygaard & Rasmussen (1938). *Nature, Lond.*, **142**, 293.
Kellie & Zilva (1935). *Biochem. J.* **29**, 1028.
Klodt & Stieb (1938). *Arch. exp. Path. Pharmac.* **188**, 21.