Note added in proof 30 *July* 1945. Since this paper was written, Light, R. F., Alscher, R. P. & Frey, C. N. (1944, *Science*, **100**, 225) have reported that hypoprothrombinaemia, which often causes cerebral haemorrhage, may be induced in rats by massive doses of vitamin A, and may be prevented by the simultaneous addition of vitamin K.

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Influence of Halides on the Oxidation of Ascorbic Acid

2. ACTION OF Cl^- ON THE CUPRIC-CUPROUS SYSTEM

By L. W. MAPSON, *The Nutritional Laboratory, University of Cambridge, and the Medical Research Council*

(*Received* 23 *February* 1945)

Several workers have shown that Cl^- retards the aerobic oxidation of ascorbic acid by Cu (De Caro & Giani, 1934; Kellie & Zilva, 1935; Mystkowski & Lasocka, 1939). Mapson (1941) found that Br^- and I^- inhibited this reaction even more than does Cl^-, but that F^- had no inhibitory effect. Moreover, at low concentrations, the ions Cl^-, Br^- and I^- increased the catalytic activity of the Cu in the reaction, the degree of acceleration being influenced *inter alia* by pH, concentration of Cu and oxygen tension. An attempt has been made in the present work to determine the mechanism by which the halide ions act on this system.

EXPERIMENTAL

Methods

Reagents. The chemical salts used were of A.R. purity. Glass-distilled water was used.

Peroxidase. Szent-Györgyi (1928), Tauber (1936) and Huzak (1937) found that only crude preparations of peroxidase were able to promote the oxidation of ascorbic acid by H_2O_2, whereas purified preparations were unable to do so. This we were able to confirm. Dr T. Mann kindly set at our disposal a series of peroxidase preparations ranging from crude extracts to material of high purity; the purest preparations had no catalytic effect on the oxidation of ascorbic acid by H_2O_2, though they did after the addition

of carriers such as flavone. The peroxidase preparation used in the experiments in this paper had a purpuro-gallin number of 430 and contained a sufficient concentration of natural carrier to render it effective in catalyzing the oxidation of ascorbic acid by H_2O_2.

Catalase. A preparation was made according to the method of Sumner & Dounce (1937).

Ascorbic acid. Estimations of ascorbic acid were carried out by titration against standard 2:6-dichlorophenolindophenol. Solutions were mixed with an equal volume of 10% HPO_3 before titration against the dye; under such conditions H_2O_2 does not interfere in the estimation.

Anaerobic oxidation of ascorbic acid by H_2O_2. Solutions under test were first freed of O_2 by the passage of O_2-free N_2. Ascorbic acid and H_2O_2 were added and the reaction followed by determining the ascorbic acid content of the solution at different time intervals. Throughout the experiment O_2 was excluded by passing a continuous stream of N_2 through the solutions.

Aerobic oxidation of ascorbic acid. Air was bubbled through the solutions throughout the experiment, the ascorbic acid content being determined at different time intervals.

Reduction of cupric salts by ascorbic acid. The rate of reduction of cupric salts was measured by determining the rate of decolorization of blue cupric salt photometrically. In those cases in which the reaction was very rapid, its progress was followed by the use of the continuous flow apparatus originally devised by Hartridge & Roughton (1923), and recently used in conjunction with a photoelectric colorimeter by Harris & Mapson (1944) for the determination of the rate of reaction of certain reductants with the indophenol dye. Solutions were freed of O_2 before use to prevent reoxidation of the cuprous salts formed in the reaction.

Oxidation of cuprous salts by air or O_2. The progress of the reaction was followed by estimating the cuprous content of solutions at different time intervals. Cuprous salts were determined by oxidation with a solution of indophenol dye previously mixed with 10% HPO_3, the excess dye being determined by back titration against ascorbic acid.

Oxidation of cuprous salts by H_2O_2. Owing to the rapidity of this reaction its progress was followed by measuring in the flow apparatus the rate of production of the blue colour of the cupric salt formed on oxidation. Solutions were freed of O_2 before use.

RESULTS

The formation of H_2O_2 *during the aerobic oxidation of ascorbic acid catalyzed by* Cu

Barron, De Meio & Klemperer (1936) first postulated that H_2O_2 was formed during the aerobic oxidation of ascorbic acid catalyzed by Cu. Lyman, Schultze & King (1937) and Dekker & Dickinson (1940) showed that a peroxide, assumed then to be H_2O_2, was produced during the reaction; the former workers showed this by observing that the O_2 absorption during the reaction was greater than the theoretical amount needed, and could not be accounted for by the further oxidation of the dehydroascorbic acid; the latter workers reached the same conclusion because they obtained a positive test with titanium sulphate. This view was subsequently supported by Steinman & Dawson (1942), who showed that the O_2 absorption was always greater than that theoretically expected, unless catalase was present. We have confirmed that an O_2 uptake of one atom of O_2 per molecule of ascorbic acid is obtained only when catalase is present. Without the enzyme, the O_2 uptake is greater than this.

Exp. 1. Solutions of ascorbic acid containing Cu in small amounts were aerated until some 50% of the ascorbic acid had been oxidized; the unoxidized ascorbic acid was then oxidized by titration with indophenol dye. The resulting solution was then divided into several portions, to each of which the following tests were applied.

Enzyme added	Result
(1) Peroxidase	Immediate development of colour due to oxidation of leuco-indophenol dye
(2) Catalase	No development of colour
(3) Catalase, followed in 30 sec.–1 min. by peroxidase	No development of colour

Control solutions of ascorbic acid which had been completely oxidized by indophenol dye did not give a positive reaction unless peroxidase and H_2O_2 were added. The oxidation of the leuco-indophenol dye by the experimental solutions to which peroxidase was added, and the prevention of this oxidation by the prior addition of catalase, indicates the formation of H_2O_2, since no other peroxide is known to be decomposed by catalase.

Exp. 2. On addition of H_2O_2 to a strong solution of peroxidase the brown colour of the solution turns red and a peroxidase-H_2O_2 complex is formed, which gives a spectrum showing two absorption bands at 561 and 530·5 mμ. (Keilin & Mann, 1937). Such a spectrum was readily given by a solution of ascorbic acid to which both cupric ions and a peroxidase preparation had previously been added. Control tests showed that such a spectrum was not obtained when the peroxidase preparation was added to solutions of ascorbic acid, or to cupric ions, or to both, if oxidation of the vitamin was prevented.

Exp. 3. That the H_2O_2 formed in the Cu-catalyzed aerobic oxidation of ascorbic acid may, in the presence of an appropriate catalyst, be used to oxidize additional ascorbic acid was shown by the increase in the rate of reaction when a suitable peroxidase preparation was added (Fig. 4B). In the absence of Cu, the enzyme preparation caused no oxidation of the ascorbic acid.

The catalytic effect of Cl^- *on the* Cu*-catalyzed anaerobic oxidation of ascorbic acid by* H_2O_2

Dekker & Dickinson (1940) showed that the rate of oxidation of ascorbic acid when catalyzed by Cu was increased by small amounts of H_2O_2. In the absence of oxidizable substrates, Cu activates the decomposition of H_2O_2, an effect intensified by the presence of NaCl (Mystkowski, 1942). Mystkowski (1942) also found that NaCl inhibited the oxidation of ascorbic acid by H_2O_2 in the presence of Cu. From the experimental data given it appears that

these reactions with H_2O_2 were studied in the presence of O_2, and the effect of Cl^- on the reaction of ascorbic acid with H_2O_2 may have been obscured by an effect on the reaction with O_2, since the halide ion may retard the aerobic oxidation but accelerates the corresponding reaction of ascorbic acid with H_2O_2 (see below). That this was so seems probable from Mystkowski's data.

dependent on the presence of Cu. In the complete absence of Cu, Cl^- had no influence on the reaction. Thus ascorbic acid was not oxidized by H_2O_2 in solutions free of Cu, nor was there any oxidation when halide ions were added.

The conclusion that the effect of the Cl^- was due to its activating influence on the Cu is supported by the observed relationship between the accelerating effect of the Cl^- on the oxidation and the Cu content of the solution. The magnitude of the effect of the Cl^- was proportional to the

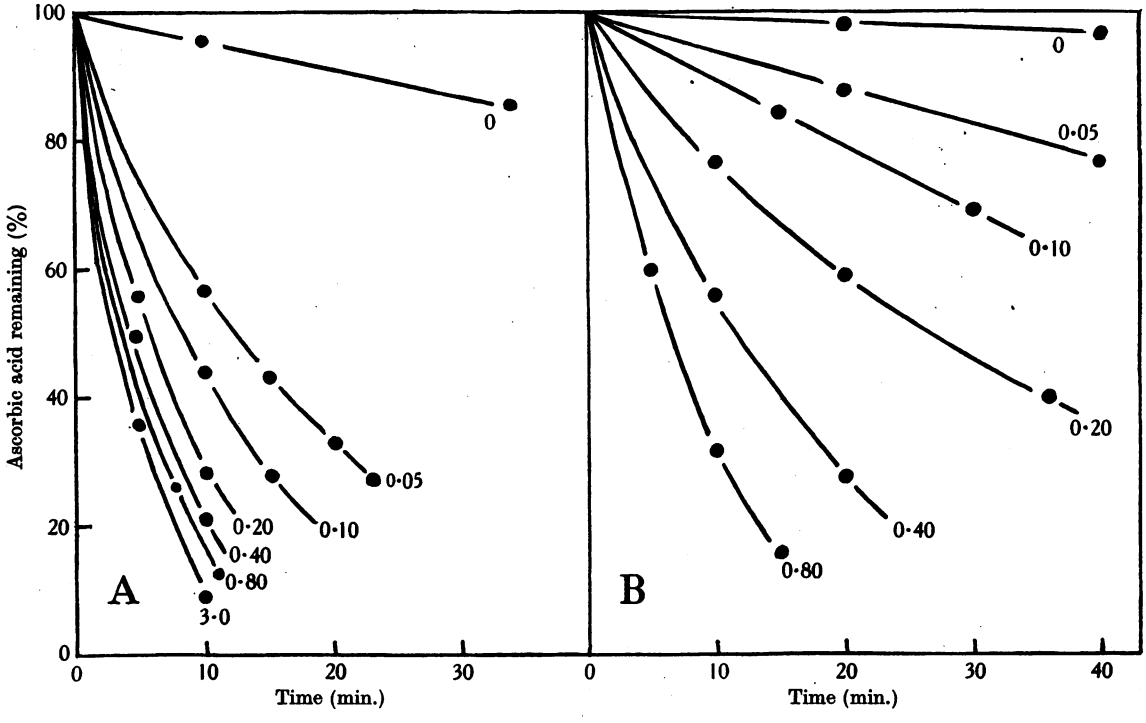


Fig. 1. Influence of Cl^- on the rate of anaerobic oxidation of ascorbic acid by H_2O_2. In both Exps. A and B, 0·57 mM-ascorbic acid was present in a solution containing 2·5 μg./ml. Cu, 0·8 mM-H_2O_2 together with the different molar concentrations of KCl indicated by the figures on the curves. In Exp. A, 0·1 M-acetic acid was present, while in Exp. B the solution contained 0·05 M-citric acid.

When Cu was present Cl^- markedly increased the rate of oxidation of ascorbic acid by H_2O_2. The effect of the Cl^- was *inter alia* dependent on three factors: (1) concentration of halide, (2) concentration of Cu, and (3) pH.

Exp. 4. *Concentration of halide.* The influence, on the reaction, of KCl in concentrations varying from 0·05 to 3·0 M in solutions of 0·1 M-acetic and 0·05 M-citric acids, is illustrated in Fig. 1. The catalytic effect of the Cl^- was proportional to its concentration, and, unlike the corresponding effect of the halide ion on the reaction of ascorbic acid with O_2, there was no reversal of the effect on increasing the concentration of the halide salt to 3 M. Br^-, like Cl^-, similarly caused an acceleration in the rate of the reaction in the concentrations used (0·05–0·4 M).

Exp. 5. *Concentration of* Cu. The effect of the Cl^- in activating the ascorbic acid-H_2O_2 reaction was found to be concentration of Cu present (Fig. 2). A similar relationship was shown to exist for the effect of the Cl^- in the reaction of ascorbic acid with O_2 (Mapson, 1941).

Exp. 6. pH. The influence of pH on the catalytic effect of the Cl^- in the reaction of ascorbic acid with H_2O_2 is shown in Fig. 3; the optimum pH was within the range 2·70–3·3. These results were obtained with buffer solutions containing various proportions of 0·05 M-citric acid and citrate; essentially similar results were obtained with mixtures of acetic acid and acetate.

Comparative effect of a peroxidase preparation and Cl^- *on the aerobic oxidation of ascorbic acid*

That Cl^- accelerates the Cu-catalyzed reaction between H_2O_2 and ascorbic acid was at first considered to be the main cause for the accelerative effect of the Cl^- on the Cu-catalyzed reaction of

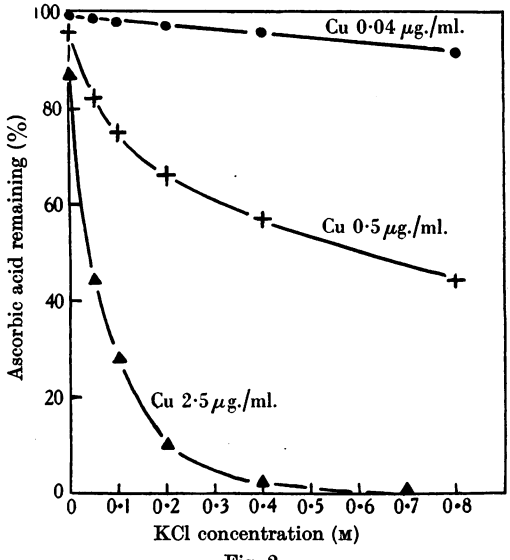


Fig. 2.

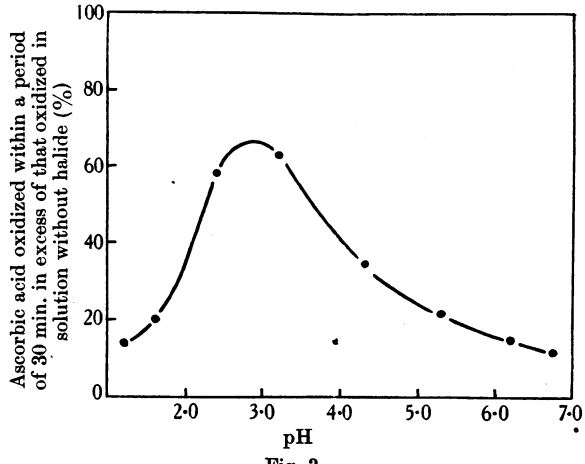


Fig. 3.

Fig. 2. Relation between concentration of Cu and catalytic activity of Cl^- on the reaction of ascorbic acid with H_2O_2. Solutions contained 0·57 mM-ascorbic acid and 0·8 mM-H_2O_2, together with different concentrations of Cu. Temp. 20°, and oxidation period 15 min.

Fig. 3. Influence of pH on the activity of Cl^- on the reaction of ascorbic acid with H_2O_2. 0·57 mM-ascorbic acid and 2·5 μg./ml. of Cu were both present in solution in 0·5 M-citric acid-citrate mixtures. The Cl^- was used in a concentration of 0·2 M. Temp. 20°.

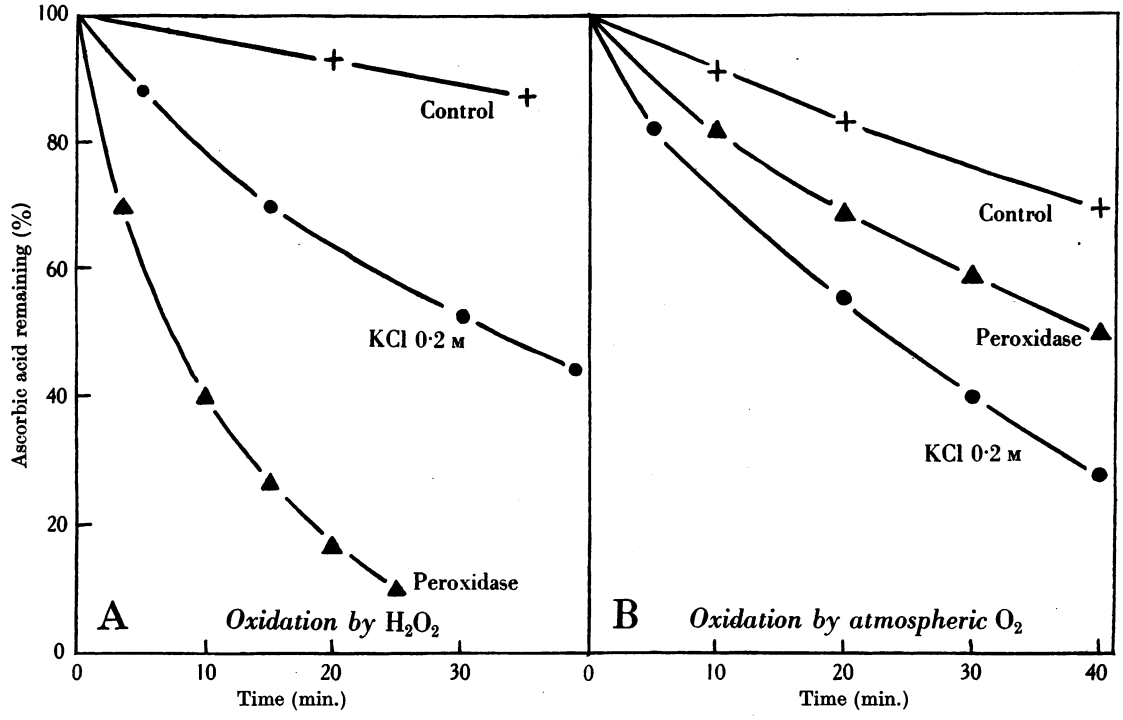
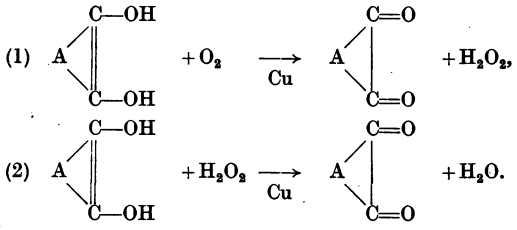


Fig. 4. The comparative effect of Cl^- (0·2 M) and of a peroxidase preparation on the Cu-catalyzed oxidation of ascorbic acid by H_2O_2 and atmospheric O_2. Solutions contained 0·57 mM-ascorbic acid, 0·8 mM-H_2O_2 and Cu 2·5 μg./ml. in solution in McIlvaine's phosphate-citric acid buffer pH 4·5 in a concentration of 15 ml. buffer to 30 ml. of final solution. Temp. 20°.

ascorbic acid with atmospheric O_2 (Mapson, 1941). On this view the influence of the Cl^- would be due to its catalytic effect on reaction (2) in the scheme shown below:



That this theory does not completely explain the mechanism of the action of Cl^- was shown in the following experiment.

Exp. 7. The relative effect of Cl^- and peroxidase on the rate of oxidation of ascorbic acid by H_2O_2 and atmospheric O_2, in the presence of Cu, is shown in Fig. 4. The concentration of halide salt used was 0·2M, which had previously been found to give the optimal accelerating effect of Cl^- on the Cu-catalyzed aerobic oxidation of ascorbic acid (Mapson, 1941). Fig. 4A shows that the acceleration in the oxidation of ascorbic acid by H_2O_2, due to the added peroxidase, was approximately twice as great as that due to the added Cl^-. In the aerobic oxidation (Fig. 4B) the Cl^- had a catalytic effect nearly twice as great as that due to the peroxidase. If the accelerating effect of the Cl^- in the Cu-catalyzed aerobic oxidation of ascorbic acid is due simply to an acceleration in the reaction of ascorbic acid with the H_2O_2 formed during the reaction, the peroxidase should have accelerated the aerobic oxidation at least as much as the Cl^-.

Moreover, in other experiments it was found that the O_2 absorbed per molecule of ascorbic acid oxidized, when the accelerating effect of Cl^- in the aerobic oxidation was most marked, was not invariably smaller in the presence of Cl^- than in its absence, as would be expected if the halide ion accelerated reaction (2) but not reaction (1).

H_2O_2 *reaction catalyzed by* Fe *salts*

Exp. 8. The catalytic effect of iron salts on oxidative reactions with H_2O_2 is well known. Unlike the effect of Cl^- on the Cu-catalyzed reaction, the rate of oxidation of ascorbic acid by H_2O_2 catalyzed by Fe was not affected by the addition of Cl^-. The solutions tested contained Fe as ferrous sulphate in a concentration of 2·5 μg./ml., 0·57 mM-ascorbic acid, 0·8 mM-H_2O_2, and 0–1·0M-KCl, in a phosphate-citric acid buffer pH 4·5. 50% of the ascorbic acid was oxidized within 50 min.

Influence of Cl^- *on the cupric-cuprous system*

Rate of reduction of cupric salts by ascorbic acid

Exp. 9. When ascorbic acid was added to a solution of cupric acetate in 0·1M-acetic acid the blue cupric salt was reduced to the yellowish cuprous salt which, in the absence of Cl^-, decomposed depositing Cu_2O. For this reason it was difficult to follow photometrically the rate of reaction of the cupric salt with ascorbic acid. If, however, a certain minimum concentration of Cl^- was present the cupric salt was reduced to colourless CuCl, which, in the presence of sufficient Cl^-, remained in solution. Such experiments showed that the rate of the reaction was progressively accelerated by increasing the concentration of Cl^- (Fig. 5A). The lowest Cl^- concentration in the reacting solutions which it was found possible to use was 0·15M, and even in this concentration CuCl precipitated on standing. Owing to the rapidity of the reaction the rate of the reduction of the cupric ion could be measured before the precipitate of the cuprous salt appeared. With the higher concentrations of chloride there was no difficulty.

Even in the absence of other anions, Cl^- accelerated the reduction of the Cu^{++} by ascorbic acid. Thus if ascorbic acid was added to a solution of $CuCl_2$ in water the cupric salt was reduced, the rate of this reaction being progressively accelerated by increasing concentrations of Cl^-.

Exp. 10. In solutions in which substances like glycine or citrate were present in concentrations of 0·05 and 0·2M, respectively, there was no significant reduction of the cupric salt by twice the equivalent amount of ascorbic acid, within a period of 2–3 min. at 20°. The reduction of the cupric salt was again accelerated by the addition of Cl^-, the rate of the reaction being increased with increasing concentrations of the halides (Fig. 5B).

Br^- also catalyzed these reactions to a degree which was somewhat greater than equivalent concentrations of Cl^-. Anions such as NO_3^-, $SO_4^=$, or $PO_4^{\equiv}$ had no influence on the reaction.

In his experiments on the Cu-catalyzed aerobic oxidation of ascorbic acid, Mystkowski (1942) noticed the deposition of Cu_2O, more being formed in the absence of Cl^- than in its presence. From this he concluded that the inhibitory action of the Cl^- was due to an inhibition of the $Cu^{++} \rightarrow Cu^+$ reaction. This interpretation of the results may be criticized on the grounds that the amount of Cu_2O deposited does not necessarily represent the true cuprous ion content, for Cu^+ is more soluble if Cl^- is present in sufficient quantity.

Rate of aerobic oxidation of the cuprous ion

Exp. 11. The influence of the ions, F^-, Cl^- and Br^-, on the aerobic oxidation of the Cu^+, was studied. 400 mg. CuCl were dissolved in the absence of O_2 in 50 ml. of 2M-KCl containing either 0·1M-acetic acid or 0·1M-citric acid. One ml. of this stock solution was then added to 49 ml. of the solution in which the reaction was to be tested, and which had previously been freed from O_2. Air or oxygen was then bubbled through the solution at a rate of 1 l./min. The results (Fig. 6) show that the rate of oxidation was progressively retarded as the concentration of the Cl^- was increased.

Br^- in equivalent concentration retarded the oxidation of the cuprous ions to a greater extent than Cl^-, but F^- in concentrations up to 1M had no influence. I^- could not be tested since it reacts with the cupric salts formed on oxidation. Other anions such as NO_3^-, $SO_4^=$ and $PO_4^{\equiv}$ had no significant influence.

The rate of the reaction depended also on the O_2 tension for, when air was replaced by O_2, the inhibitory influence of the halide was reduced (Fig. 7).

Since the retarding effect of halide salts on the oxidation of Cu^+ might have been due simply to a reduction of O_2 tension, we studied the reaction under conditions in which the O_2 tension was approximately the same, but in which the concentration of Cl^- was different. This was achieved by studying the rate of oxidation of the Cu^+ (0·0016M) in

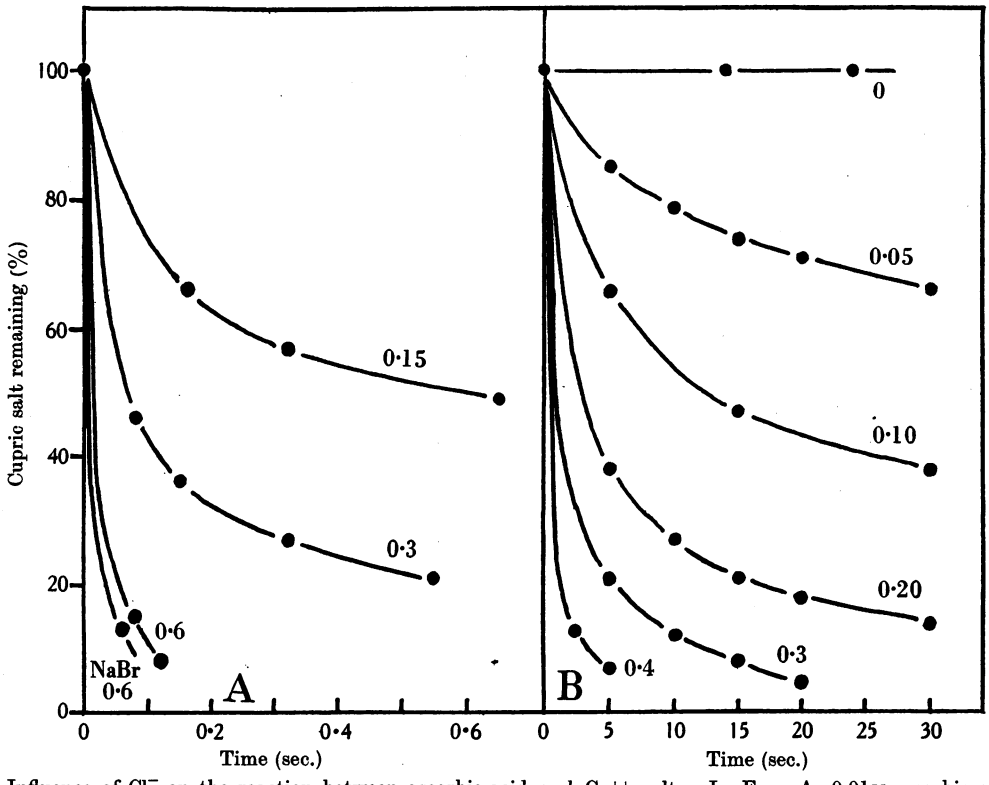


Fig. 5. Influence of Cl^- on the reaction between ascorbic acid and Cu^{++} salts. In Exp. A, 0·01M-ascorbic acid was allowed to react with an equal volume of a solution of 0·02M-cupric acetate in the presence of different concentrations of NaCl. In Exp. B, 0·01M-ascorbic acid was allowed to react with 0·01M-cupric acetate in solution in 0·1M-acetic acid containing 0·05M-glycine in the presence of different concentrations of NaCl. In both experiments, the molar concentrations of NaCl present are indicated by figures on the curves. Temp. 20°.

solution in 0·1M-acetic acid and 0·05M-NaCl in equilibrium with air (solution A), and in a similar solution, in equilibrium with O_2, in which the concentration of NaCl was 4·75M (solution B). The data show that the inhibitory action of the Cl^- was not due solely to a reduction in the O_2 tension.

	Time (sec.)	Cuprous salt oxidized (%)
Solution A (aerated)	30	74
	60	92
	Time (min.)	Cuprous salt oxidized (%)
Solution B (O_2 passed through)	1	3
	7	13
	15	22
	30	36

Formation of H_2O_2 during the oxidation of cuprous salts

Traube (1882) detected the formation of H_2O_2 during the oxidation of cuprous salts by air. We have been able to confirm this fact by the following tests. When sparingly soluble cuprous chloride was shaken with water, the solution gave a positive reaction for H_2O_2 with the peroxidase-indophenol test described earlier, but a negative reaction if catalase was added prior to the addition of peroxidase. In addition, a faint spectrum of the peroxidase-H_2O_2 complex could be detected on the addition of peroxidase. It was, however, more difficult to get positive reactions in solutions in which there was an excess of soluble cuprous ions, for the H_2O_2 formed during the oxidation rapidly reacted with excess of the former.

Rate of oxidation of cuprous salts by H_2O_2

Exp. 12. The rate of oxidation of cuprous salts was studied with H_2O_2 as the oxidizing agent. A solution of CuCl (0·081M), in M-KCl containing either 0·1M-citric acid or 0·2M-acetic acid, was allowed to react with an equa volume of an equivalent solution of H_2O_2. In the fina reaction mixture this gave a concentration of 0·5M-KCl, which was the minimum concentration that could be used to keep the cuprous salt in solution. For the higher concentrations of Cl^- used, additional KCl was added to the H_2O_2 solution.

The rate of oxidation of the cuprous salt in such solutions is shown in Fig. 8. In contrast to the results of the corresponding experiments on the oxidation of the cuprous salt by atmospheric O_2, the rate of the reaction was unaffected by alteration of the concentration of Cl^- from 0·5 to 2M.

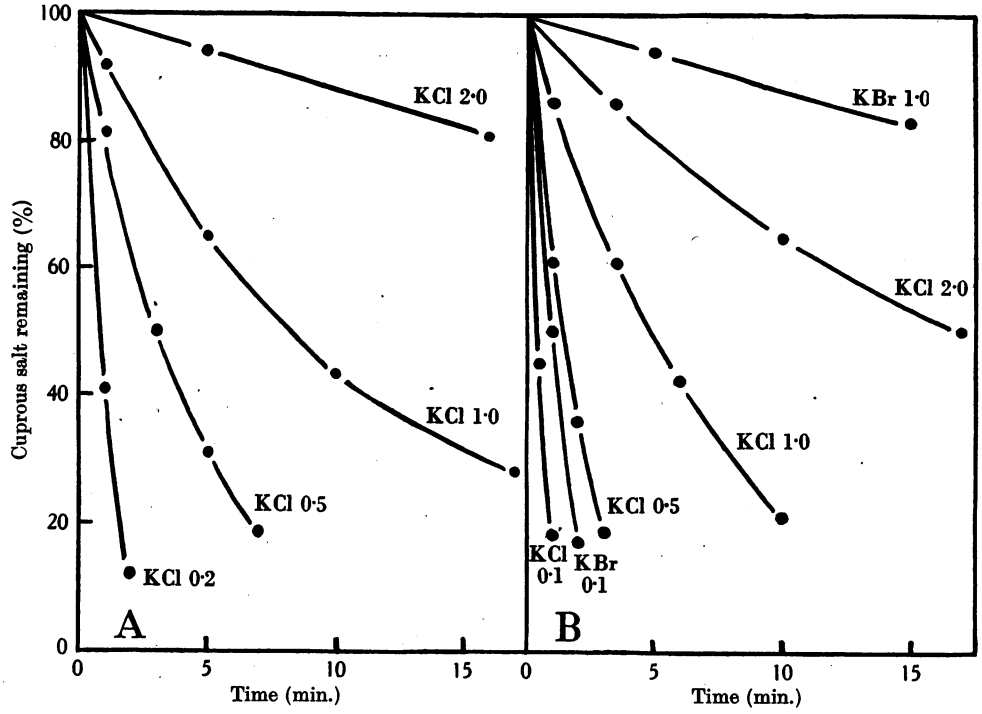


Fig. 6. Influence of Cl^- and Br^- on the oxidation of cuprous salts by air. In both Exps. A and B, the solution contained 0·0016 M-CuCl with different molar concentrations of either KCl or KBr. The molarity of the halide salts present is indicated by figures on the curves. In Exp. A the solution contained 0·1 M-acetic acid, in Exp. B the solution contained 0·1 M-citric acid. Temp. 20°.

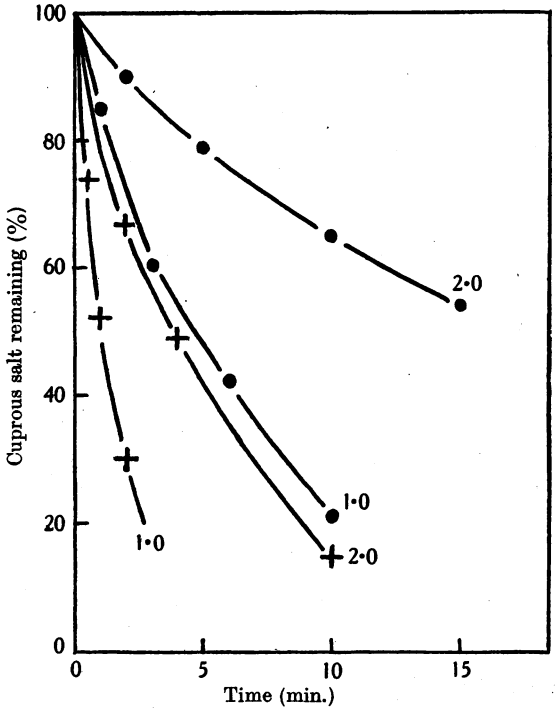


Fig. 7. Effect of O_2 tension on the inhibitory action of Cl^- on the oxidation of cuprous salts. The solution contained 0·0016 M-CuCl in 0·1 M-citric acid together with different molar concentrations of KCl indicated by the figures on curves. Air was passed through the solutions indicated by curves marked •——•, and O_2 through solutions indicated by curves marked +——+. Temp. 20°.

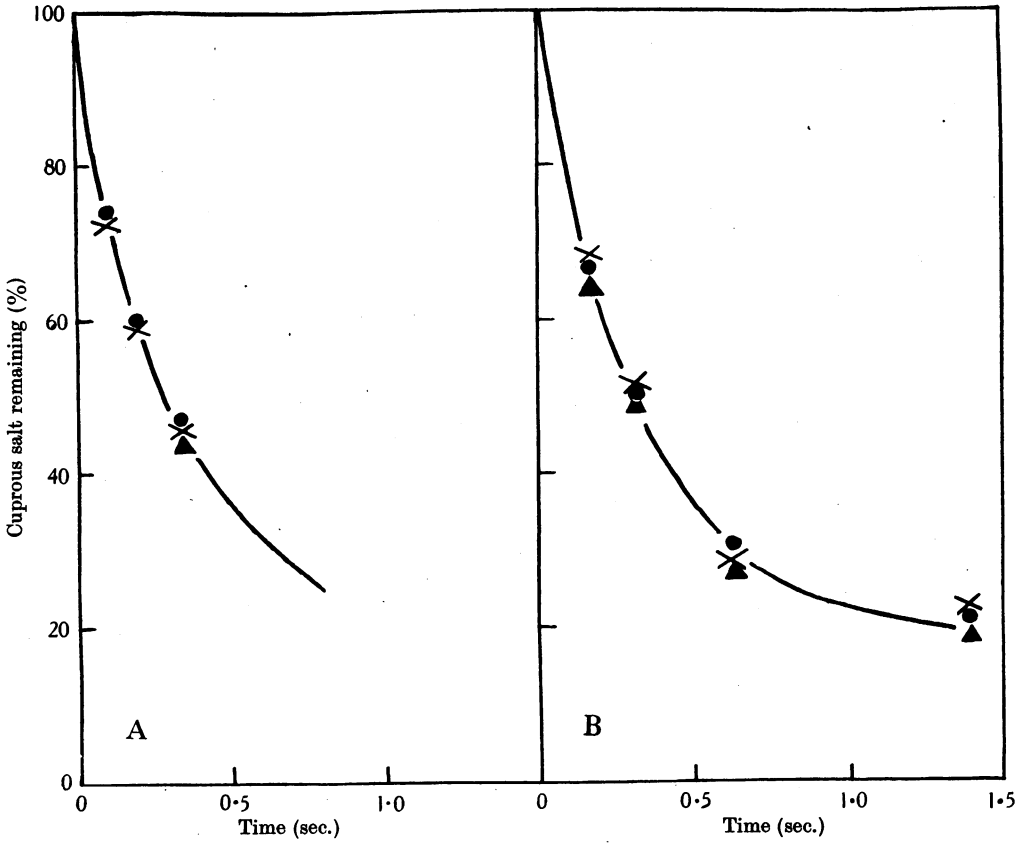
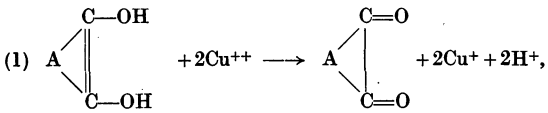


Fig. 8. Influence of Cl^- on the oxidation of cuprous salts by H_2O_2. In Exp. A a solution of 0·08M-CuCl in M-KCl and 0·1M-citric acid was allowed to react with an equal volume of a solution of 0·04M-H_2O_2, to which for the higher concentrations of KCl used further KCl was added. The molarity of KCl in the final reaction mixture is indicated as follows: ●——● 0·5M-KCl, ▲——▲ M-KCl, ×—— × 2·0M-KCl. In Exp. B, 0·2M-acetic acid replaced 0·1M-citric acid in the CuCl solution. Temp. 20°.

DISCUSSION

Many of the observed effects of Cl^- on the Cu-catalyzed oxidation of ascorbic acid by O_2 and H_2O_2 may possibly be explained by the influence of the halide on the following reactions:

(1) 

(2) $2Cu^+ + 2H^+ + O_2 \longrightarrow 2Cu^{++} + H_2O_2$,

(3) $2Cu^+ + 2H^+ + H_2O_2 \longrightarrow 2Cu^{++} + 2H_2O$.

Kodicek & Wenig (1938) proposed a similar scheme for reaction (1) based on the fact that when ascorbic acid was added to a solution of cupric ions, in the absence of O_2, a polarographic wave appears on the current-voltage curve, which is due to the deposition of free hydrions. A scheme representing the mechanism of the aerobic oxidation of ascorbic acid, similar to that above, was postulated by Barron *et al.* (1936), the chief difference being that they considered the H_2O_2 formed in reaction (2) to be immediately decomposed into H_2O and O_2. That the latter explanation is not entirely true is shown by the fact that at the end of the reaction the absorption of O_2 is always greater than the ratio of one atom of O_2 per molecule of ascorbic acid oxidized, unless catalase is present. The value of this ratio is never 2 (which would be expected if the H_2O_2 formed was not used in a secondary reaction or not decomposed) but usually about 1·2–1·8 (see Steinman & Dawson, 1942). This is more likely to be due to a reaction of H_2O_2 with the cuprous salt formed in reaction (1), than to the partial decomposition of H_2O_2 into H_2O and O_2. At 20° with H_2O_2 (0·008M) and Cu (5μg./ml.) there was little or no decomposition of the peroxide, which was not increased by adding KCl in concentrations up to 2M.

The experimental results described in this paper show that reaction (1) is accelerated by the Cl^-, reaction (2) is retarded, whilst reaction (3) is un-

affected. The influence of the Cl^- on these reactions may explain the observed effect of Cl^- on the aerobic oxidation of ascorbic acid, and the corresponding reaction with H_2O_2. Under aerobic conditions, in which all three reactions may occur, the accelerative influence of small concentrations of Cl^- on the oxidation of ascorbic acid might be interpreted as indicating that the effect of the Cl^- in inhibiting reaction (2) is more than compensated by its accelerative effect on reaction (1). It has been shown (Mapson, 1941) that the magnitude of the accelerative effect of the Cl^- and the range of concentration of the Cl^-, over which this effect on the Cu-catalyzed aerobic oxidation of ascorbic acid was observed, was progressively increased by raising the O_2 tension. This agrees with the fact that an increase in O_2 tension reduces the inhibiting effect of the Cl^- in reaction (2). Similarly, the smaller effect of low concentrations of Br^- in accelerating the aerobic oxidation of ascorbic acid is consistent with the greater activity of this ion in inhibiting reaction (2) than an equivalent concentration of Cl^-.

The effect of the larger amounts of halide ions, in reducing the rate of the Cu-catalyzed aerobic oxidation of ascorbic acid, agrees with their marked effect in inhibiting reaction (2). The relatively greater efficiency of Br^- than Cl^-, and the negative effect of the F^-, in reducing the rate of oxidation of ascorbic acid, is in accordance with the experimental observations of their effect on reaction (2).

The scheme also explains the accelerative influence of Cl^- on the Cu-catalyzed oxidation of ascorbic acid by H_2O_2. In this case there is no reversal of the effect of the halide ion when its concentration is increased, for reaction (2) is not involved and the corresponding reaction (3) is not retarded. The end result is that the rate of oxidation of the vitamin is progressively increased by raising the Cl^- concentration.

That the effective concentration of Cl^- should be related, in all these reactions, to the concentration of Cu present, is to be expected on the basis of an increase in the concentration of the active catalyst. The influence of the Cl^- on reaction (2) is most likely due to the complex salt formed between the Cu^+ and Cl^-, and to a lowering of O_2 tension in solution. The reason for the accelerative effect of the Cl^- on reaction (1) is not at present clear and requires further investigation.

SUMMARY

1. Hydrogen peroxide is formed during the Cu-catalyzed aerobic oxidation of ascorbic acid; it is also formed during the oxidation of cuprous salts.

2. Chloride ions, in concentrations up to 3M, accelerate the Cu-catalyzed anaerobic oxidation of ascorbic acid by H_2O_2; Cl^- has no effect on the reaction in the absence of Cu.

3. The influence of Cl^- on the ascorbic acid-H_2O_2 reaction varies with (1) the concentration of Cl^-; (2) the concentration of Cu; and (3) the pH of the solution. Unlike the effect of Cl^- on the aerobic oxidation of ascorbic acid, there is no reversal of the effect of the Cl^- with increasing concentration of the latter; an increase in concentration of the halide ion produces a progressive acceleration in the rate of reaction of ascorbic acid with H_2O_2 in the presence of Cu.

4. Chloride ions accelerate the rate of reduction of Cu^{++} by ascorbic acid, but inhibit the oxidation of the Cu^+ by O_2; the oxidation of the Cu^+ by H_2O_2 is not affected.

5. It is concluded that the influence of Cl^- on the Cu-catalyzed aerobic oxidation of ascorbic acid and on the reaction with H_2O_2 may possibly be explained by its influence on the cupric-cuprous system.

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