[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, WESTERN DIVISION, THE DOW CHEMICAL CO., PITTSBURG, CALIF.]

The Oxidation of Ascorbic Acid by Hydrogen Peroxide. Catalysis by Ethylenediaminetetraacetato-Iron(III)

BY ROBERT R. GRINSTEAD

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The kinetics of the oxidation of ascorbic acid by H_2O_2 in the presence of the iron chelate of ethylenediaminetetraacetic acid have been studied in the *p*H range 3.4–4.5. The direct reduction of the ferric chelate by ascorbic acid has been studied under the same conditions. The rate-determining step of the latter reaction involves a one-electron oxidation of the ascorbic acid to a radical intermediate, the ascorbate radical. This species reacts with a second ferric chelate molecule to complete the process. The H_2O_2 oxidation involves a chain process, being initiated by the reduction step above. The chain itself consists of three steps: (1) reaction of the ferrous chelate with H_2O_2 to produce HO· radicals, (2) reaction of the HO· radicals with ascorbic acid to produce the ascorbate radical, and (3) reduction of ferric chelate by the ascorbate radical. Termination of the chain occurs mainly by the reaction of HO· radicals with ascorbate radicals.

The "model" peroxidase system consisting of the iron chelate of ethylenediaminetetraacetic acid (EDTA) was reported first by Udenfriend and co-workers.[1] It was so named because of its ability under certain conditions to catalyze reactions essentially identical to some reactions catalyzed by the enzyme peroxidase. These reactions involve the addition of a hydroxyl group to an aromatic ring in the presence of oxygen and a reducing agent. The enzyme reaction requires dihydroxyfumaric acid as the reductant,[2] but the model system can utilize ascorbic acid and a number of structurally similar compounds as well. A great deal of information exists concerning the structure of the active site of peroxidase and its mechanism of action in its usual role of catalyzing reactions of hydrogen peroxide with various compounds.[3,4] In such cases it is generally believed that higher oxidation states of iron are involved. The mechanism of the hydroxylation process, on the other hand, has not been established, although the available evidence has been discussed by Mason[3] in terms of an active intermediate involving +4 iron as a ferryl structure, FeO^{++}, where Fe represents the enzymically bound iron ion.

The present work was undertaken in the hope that information about the mechanism of the model system, where the properties of the chelated iron atom are better understood, would shed some light on the nature of the active site in the enzyme and on its mechanism of action. Because of the complexity of the complete model system, it appeared desirable to consider first a simpler system. Udenfriend, *et al.*, had suggested that hydrogen peroxide was an intermediate in the reaction, and they found that it could be substituted for oxygen as the oxidizing agent. They also reported that hydrogen peroxide reacted with ascorbic acid in this system, whether or not the aromatic substrate was present. Accordingly, the reaction of hydrogen peroxide with ascorbic acid in the presence of the iron–EDTA catalyst was selected for the initial study.

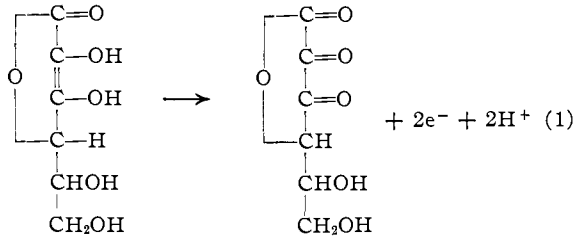
(1) S. Udenfriend, C. T. Clark, J. Axelrod and B. B. Brodie, *J. Biol. Chem.*, **208**, 731 (1954).

(2) B. B. Brodie, J. Axelrod, P. A. Shore and S. Udenfriend, *ibid.*, **208**, 741 (1954); H. S. Mason, I. Onoprienko and D. Buhler, *Biochim. et Biophys. Acta*, **24**, 225 (1957).

(3) H. S. Mason, *Adv. in Enzymol.*, **19**, 76 (1957).

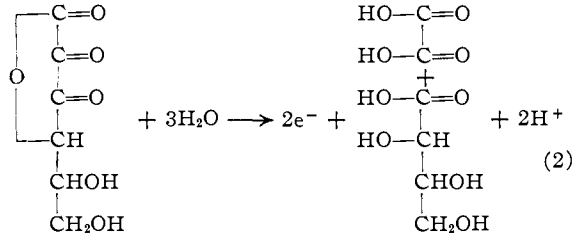
(4) P. George in "Currents in Biochemical Research," D. Green, ed., Interscience Publishers, Inc., New York, N. Y., 1956, p. 338.

The oxidation of ascorbic acid has been the subject of numerous studies, and under mild conditions the first product of the reaction is dehydroascorbic acid (DHA).[5,6]



$$+ 2e^- + 2H^+ \quad (1)$$

Further oxidation of the dehydroascorbic acid can occur in which the chain is split, giving oxalate and threonate (α,β,γ-trihydroxybutyric acid).



$$+ 3H_2O \longrightarrow 2e^- + \quad + 2H^+ \quad (2)$$

The stoichiometry was examined only briefly here in order to check the validity of reaction 1 in the system. The major portion of this work was concerned with the kinetics of this reaction as well as the kinetics of the direct reduction of iron(III)–EDTA by ascorbic acid, as this reaction is an important one in the mechanism of the model system.

Experimental

Materials.—All stock solutions, as well as reaction mixtures, were prepared either with distilled water which was deaerated by boiling and cooling under Matheson prepurified nitrogen, or else by removing oxygen from the solutions by bubbling nitrogen through them. The ascorbic acid was Eastman Kodak Co. white label. Solutions were prepared by weighing and were standardized occasionally by titration with KI_3. The ascorbic acid content as determined by the two methods always agreed to ±0.5%. Reagent grade chemicals were used in all other cases. EDTA solutions were prepared from Fisher reagent grade disodium versenate, and their content was determined by titration with standard zinc solution using Eriochrome Black T indicator. Ferric sulfate solutions were prepared

(5) H. Borsook, H. W. Davenport, C. E. P. Jeffreys and R. C. Warner, *J. Biol. Chem.*, **117**, 237 (1937).

(6) E. S. Guzmann-Barron, R. H. De Meio and F. Klemperer, *ibid.*, **112**, 625 (1936).

by standardization with dichromate. Other solutions were prepared simply by weighing. Hydrogen peroxide solutions were standardized each day against permanganate.

Procedure. Hydrogen Peroxide–Ascorbic Acid Reaction.—For experiments on the stoichiometry known volumes of stock solutions containing: (1) ferric sulfate, EDTA and phosphate buffer; (2) standard ascorbic acid solution; and (3) hydrogen peroxide solution were mixed and diluted to a known volume. When no peroxide remained (determined by absence of yellow color with Ti(IV)), two 1.00-cc. samples were pipetted from the system. To one was added 5 to 10 cc. of 0.5 *M* H_3PO_4. It was then titrated with a dichloroindophenol solution.[7] The second sample was analyzed for dehydroascorbic acid plus ascorbic acid, according to a modification of the method of Roe, *et al.*[8] To this sample was added 10 cc. of 2 *M* citrate buffer of *p*H 4, and the dehydroascorbic acid, which is the immediate oxidation product of the ascorbic acid, was reduced by bubbling H_2S through for 15 minutes. The H_2S was removed with prepurified nitrogen, and the ascorbic acid was titrated as above. Under these conditions, the Fe(III) present was also reduced and was titrated by the indophenol. A correction was applied by running blanks containing no ascorbic acid.

All kinetic runs were made in a thermostated bath at 25.0 ± 0.1°. All solutions were brought up to temperature in the thermostat prior to mixing. The ascorbic acid was added to the Fe–EDTA–phosphate solution a few minutes prior to the addition of H_2O_2. The reaction flask was removed from the thermostat and stirred magnetically while the H_2O_2 was being added. The system was returned to the bath and the run continued. Samples were removed and quenched in 5 to 10 cc. of 0.5 *M* H_3PO_4, and the ascorbic acid was titrated with the indophenol reagent. The initial *p*H was determined with an identical solution prepared without H_2O_2, since it was observed that the addition of H_2O_2 resulted in no immediate *p*H change.

Reduction of Fe(III)-EDTA by Ascorbic Acid.—The Fe(III)-EDTA chelate possesses a strong ultraviolet absorption band with a peak at 260 mμ.[9] The band extends out into the visible region, imparting a yellow color to dilute solutions ($\sim 10^{-3}$ *M*) of the chelate. The ferrous chelate is colorless, and the absorption thus provides a convenient method for measuring the rate of reduction of the iron(III) chelate. Solutions of (1) iron(III) sulfate plus EDTA, and (2) ascorbic acid plus phosphate or perchlorate, both of which had been adjusted to the *p*H of the experiment and deaerated with prepurified nitrogen, were pipetted into spectrophotometer cells under nitrogen. The cells were immediately stoppered and were placed in a Beckman DK 2 spectrophotometer equipped with a time drive, which produced a continuous record of absorbance *versus* time. The temperature was not controlled, but was approximately 23–24°.

Determination of *pK* of Ascorbic Acid.—A solution 0.10 *M* in $NaClO_4$ and 0.02 *M* in ascorbic acid was titrated with standardized NaOH in the absence of oxygen and at a temperature of 24 ± 1°. The *p*H at the point of half-titration was determined to be 4.21. The *pK* for ascorbic acid is therefore 4.21 at an ionic strength of 0.11, corresponding to a dissociation constant of 6.16×10^{-5}.

Results

Oxidation of Ascorbic Acid by H_2O_2.—In Table I are shown the results of these experiments. It will be observed that for any amount of H_2O_2 there is always less dehydroascorbic acid found than ascorbic acid oxidized. If it is assumed that this discrepancy arises from further oxidation of DHA to oxalic and threonic acids, then the amount of H_2O_2 consumed by these two reactions can be calculated and compared with the amount added. This comparison is shown in columns 3 and 4, and was calculated on the basis that each DHA found represents the consumption of one H_2O_2, and each ascorbic acid unaccounted for (difference between columns 1 and 2) represents consumption of two H_2O_2. This assumption appears valid up to an addition of 1.3 moles of H_2O_2 per mole of ascorbic acid, and within this region it appears that reaction 1 accounts for about 90% of the hydrogen peroxide consumption.

TABLE I

STOICHIOMETRY OF H_2O_2–ASCORBIC ACID REACTION

[Fe(III)] = 0.0030 *M*, [EDTA] = 0.0060 *M*, [ascorbic acid] = 0.040 *M*, total phosphate concentration = 0.050 *M*, *p*H 3.7, solution volume = 50 ml.

Absolute amounts, millimoles				
Ascorbate oxidized	Dehydroascorbate found	H_2O_2 accounted for	H_2O_2 added	Fraction of H_2O_2 in reaction 1
0.24	0.20	0.28	0.257	0.86
.46	.41	.51	.514	.90
.66	.57	.75	.771	.88
1.11	.97	1.25	1.270	.89
1.42	1.13	1.71	1 830	
1.68	1.25	2 11	2 311	
1.93	1.23	2.63	3.100	
1.97	1.04	2.90	3.760	

It was observed early in the work that the rate of ascorbic acid disappearance was independent of hydrogen peroxide concentration in the region under study. Figure 1 shows some of the data on the effect of hydrogen peroxide concentration,

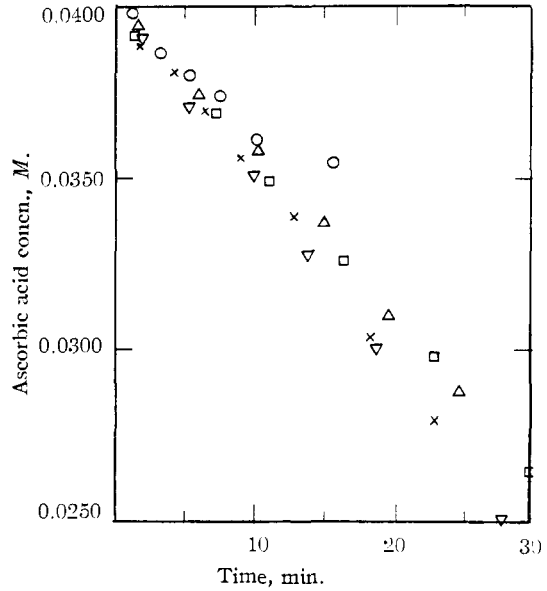


Fig. 1.—Effect of H_2O_2 concentration; [Fe] = 6.0×10^{-4} *M*, [EDTA] = 12.0×10^{-4} *M*, [phosphate] = 0.10 *M*, *p*H 3.73, [H_2O_2]: ○, 0.0063 *M*; □, 0.0189 *M*; △, 0.0317 *M*; ▽, 0.0571 *M*; ×, 0.0716 *M*.

and the independence of the rate on this variable is clearly evident. The last point in the series with (H_2O_2) = 0.0063 *M* was taken after the H_2O_2 had been all used up. The initial reaction rates of these experiments (Table II) were calculated from the slopes of lines (not shown) drawn through the first several points. During the first several minutes of the reaction, there appears to be no substantial difference in rate, although the initial peroxide concentration was varied about twelve-fold. Since

(7) O. A. Bessey, *J. Assoc. Offic. Agr. Chem.*, **27**, 537 (1944).

(8) J. H. Roe, M. B. Mills, M. J. Oesterling and C. M. Damron, *J. Biol. Chem.*, **174**, 201 (1948).

(9) Y. Uzumasa and M. Nishimura, *Bull. Chem. Soc. Japan*, **28**, 88 (1955).

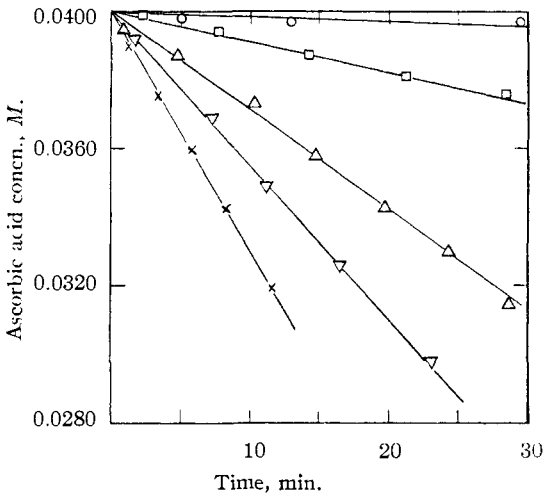


Fig. 2.—Effect of iron concentration; [phosphate] = 0.10 M, pH 3.73, [H_2O_2] = 0.017–0.019 M.

	[Fe], M	[EDTA], M
○	0	5.0×10^{-4}
□	1.50×10^{-4}	3.0×10^{-4}
△	4.0×10^{-4}	8.0×10^{-4}
▽	6.0×10^{-4}	12.0×10^{-4}
×	10.0×10^{-4}	20.0×10^{-4}

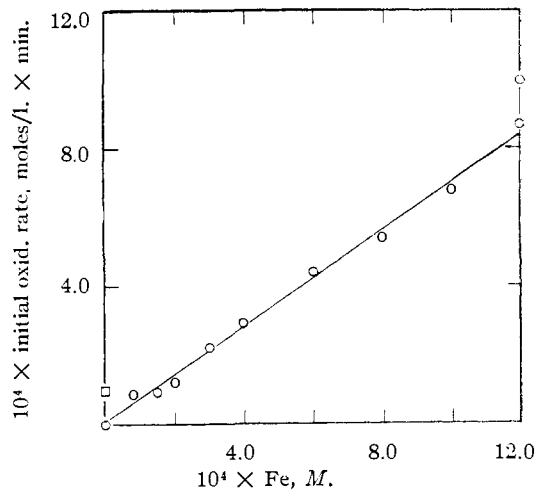


Fig. 3.—Effect of iron concentration; ○, [phosphate] = 0.10 M, [ascorbate] = 0.0400 M, pH 3.73, [H_2O_2] = 0.017–0.019 M, EDTA in twofold excess over Fe, except for [Fe] = 0, where [EDTA] = 5×10^{-4} M; □, same, but no EDTA present.

appreciable fractions of ascorbic acid are consumed during any given experiment and the ascorbic acid concentration is therefore not a constant, it might be concluded from the straight line distribution of points that the rate is independent of ascorbic acid concentration. This is, in fact, not the case, and this aspect will be examined further below.

Some of the data showing the effect of the iron concentration on the rate of ascorbic acid oxidation are shown in Fig. 2. The slopes of the lines in this figure representing the initial rates of reaction are plotted in Fig. 3 and can be fitted with one exception to a straight line nearly intersecting the origin. Experiments 5A, 6A and 7A in Table II show that a

TABLE II

SUMMARY OF KINETIC EXPERIMENTS FOR H_2O_2–ASCORBATE REACTION

	Initial concentrations, M						R = initial rate $\times 10^4$, moles/l. $\times$ min.
Expt.	Phosphate	Ascorbic acid $\times 10^2$	Iron (III) $\times 10^4$	EDTA $\times 10^4$	H_2O_2 $\times 10^2$	pH	
17	0.10	4.00	6.0	12.0	0 63	3.73	~4
13	.10	4.00	6.0	12.0	1.27	3.73	4.4
16	.10	4.00	6.0	12.0	1.89	3.73	4.4
12	.10	4.00	6.0	12.0	3.17	3.73	4.4
15	.10	4.00	6.0	12.0	4.41	3.73	4.6
14	.10	4.00	6.0	12.0	5.71	3.73	5.1
21	.10	4.00	6.0	12.0	7.16	3.73	4.8
5A	.10	4.00	0	0	1.95	3.73	1.0
6A	.10	4.00	0	5.0	1.95	3.73	~0.06
7A	.10	4.00	0	1.6	1.95	3.73	~ .06
4A	.10	4.00	0.80	1.6	1.95	3.73	.8
19	.10	4.00	1.50	3.0	1.87	3.73	.9
1A	.10	4.00	2.0	4.0	1.95	3.73	1.2
18	.10	4.00	3.0	6 0	1.89	3.73	2.2
2A	.10	4.00	4.0	8.0	1.95	3.73	2.9
8A	.10	4.00	8.0	16.0	1.95	3.73	5.4
9A	.10	4.00	10.0	20.0	1.95	3.73	6.8
10A	.10	4.00	12.0	24.0	1.95	3.73	8.7
22	.10	4.00	12.0	24.0	1.85	3.73	10.0
26	.10	4.00	1.5	0	1.74	3.73	>80
24	.10	4.00	6.0	12.0	3.08	3.39	1.2
38	.10	4.00	6.0	12.0	2.63	3.61	2.8
25	.10	4.00	6.0	12.0	3.00	3.92	11
35	.10	4.00	6.0	12.0	2.89	3.92	11
39	.10	4.00	1 5	3.0	2.63	4.11	4.1
36	.10	4.00	1.5	3.0	2.63	4.23	6.2
37	.10	4.00	1.5	3.0	2.63	4.46	18
27	0	4.00	6.0	12.0	2.98	3.71	2.1
31	0.090[a]	8.00	6 0	12.0	2.98	3.71	8.6
30	.100[a]	4.00	6.0	12.0	2.98	3.71	4.6
33	.105[a]	2.00	6.0	12.0	2.89	3.71	2.7
34	.106[a]	1.50	6.0	12.0	2.89	3.71	2.3

[a] No phosphate present; concentration of $NaClO_4$ shown.

reaction does occur even without added iron, but that as long as excess EDTA is present, this rate is essentially negligible. This slow reaction could very well be due to traces of iron, since in experiment 26 where the concentration of iron was 1.5×10^{-4} M and no EDTA was present, the rate was at least a hundred times faster than in the presence of the EDTA. In all other experiments a mole ratio of EDTA:Fe of 2 was provided in order to ensure chelation of all iron. Thus the EDTA is actually an inhibitor under these conditions for the iron-catalyzed oxidation of ascorbic acid by H_2O_2.

Kinetic data showing the effect of ascorbic acid concentration on the oxidation rate are presented in Fig. 4. In all the preceding experiments, the ascorbic acid system was the principal buffer, and the initial pH had been maintained constant by use of constant ratios of ascorbic acid and phosphate solutions. The phosphate acted as a base, partially neutralizing the ascorbic acid. At the stated pH values the phosphate itself was essentially all present as the $H_2PO_4^-$ ion and did not contribute materially to the buffering capacity of the solution. Since it was necessary to vary the ascorbic acid content in these experiments (which would have caused the initial pH to vary), the phosphate was replaced

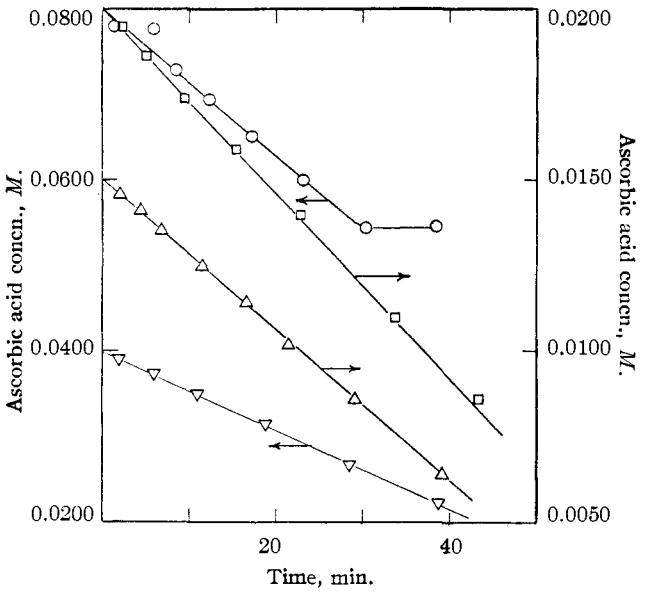


Fig. 4.—Effect of ascorbic acid concentration; [Fe] = 6.0×10^{-4}, [EDTA] = 12.0×10^{-4}, pH 3.71, [H_2O_2] = 0.029–0.030 M.

	[Ascorbic Acid], M	[$NaClO_4$], M
○	0.0800	0.090
▽	.0400	.100
□	.0200	.105
△	.0150	.106

by the non-buffering anion perchlorate. The buffering action was thus reduced to the single species ascorbate, and the initial pH in any experiment was thus independent of the ratio of ascorbate to perchlorate. That the replacement of phosphate by perchlorate had no effect on the reaction is seen by comparing experiments 12 and 30 in Table II. Almost identical initial rates were obtained, whether perchlorate or phosphate was present. The ionic strength was thus maintained at 0.11, which is the value which applied in all preceding experiments. The phosphate, which was essentially all in the form of the $H_2PO_4^-$ ion, contributed about 0.10 M. The ascorbate, which was about 25% ionized, contributed about 0.01 M. The effect of omitting the perchlorate and phosphate giving an ionic strength of about 0.01, is seen by comparing experiments 27 with 12 or 30. The initial rate at $\mu = 0.01$ is about one-half of the rates at $\mu = 0.11$.

Initial rates in the variable ascorbate experiments are plotted in Fig. 5 and appear to adhere fairly well to a straight line, which, however, does not appear to pass through the origin. This suggests the existence of two reactions, one of first order, another of zero order dependence on ascorbate concentration. A clue to the nature of the latter is given by observations on the system in the absence of ascorbic acid. Such experiments were performed, and in these cases the peroxide concentration was followed. While it is possible that some decomposition of H_2O_2 to oxygen and water occurred, no gas evolution was visible. That oxidation of the EDTA occurred was evident from a strong odor of formaldehyde above these solutions. Preliminary experiments have indicated that this reaction

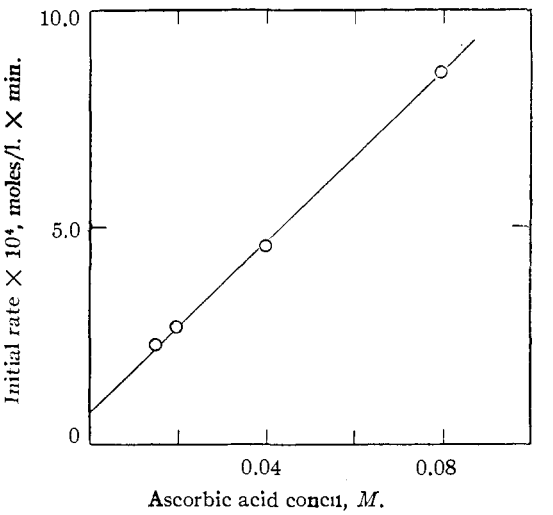


Fig. 5.—Effect of ascorbic acid concentration; [Fe] = 6.0×10^{-4} M, [EDTA] = 12.0×10^{-4}, [H_2O_2] = 0.029–0.030 M, pH 3.71; ionic strength maintained at 0.11 with $NaClO_4$.

is approximately first order in iron concentration, independent of H_2O_2 concentration, and nearly independent of uncomplexed EDTA concentration. This particular reaction appears to involve, therefore, the initial decomposition of the chelate followed by rapid reaction of the products with H_2O_2. The rate of disappearance of H_2O_2 at pH 4.3 and [Fe] = 6.0×10^{-4} M is about 4×10^{-5} mole per liter per minute, which is of the same magnitude as, though lower than, the value of 8×10^{-5} mole per liter per minute for the zero-order rate of ascorbate oxidation shown in Fig. 5. It does not seem unreasonable to assume that in the EDTA–H_2O_2 reaction intermediates may exist which would react readily with ascorbate to give the observed zero point rate.

It was pointed out earlier that the straight line rate curves are incompatible with the participation of ascorbate as a first-order reactant. It was also pointed out that the principal buffer in this system is the ascorbate system, since at the lowest pH encountered, 3.39, only 4% of the phosphate is in the form of undissociated H_3PO_4. The primary reaction occurring in this system is

$$\underset{\text{ascorbic acid}}{C_6H_8O_6} + H_2O_2 \longrightarrow \underset{\text{dehydroascorbic acid}}{C_6H_6O_6} + 2H_2O \qquad (3)$$

Except for the ascorbic acid, none of the components of eq. 3 exhibit acidic properties in the pH range being considered. The stoichiometry of the oxidation thus involves a consumption of undissociated ascorbic acid. In the absence of other buffering substances the concentration of ascorbate ion should there remain constant. The buffer ratio (ascorbate ion)/(ascorbic acid) will rise, and as a consequence the pH will rise also. If now the slow step in the reaction were to involve ascorbate ion rather than ascorbic acid, the rate would tend to remain constant during any given run. A pH dependence would be observed, since at constant total ascorbic acid concentration the concentration of the ion is pH dependent. Actually, the experi-

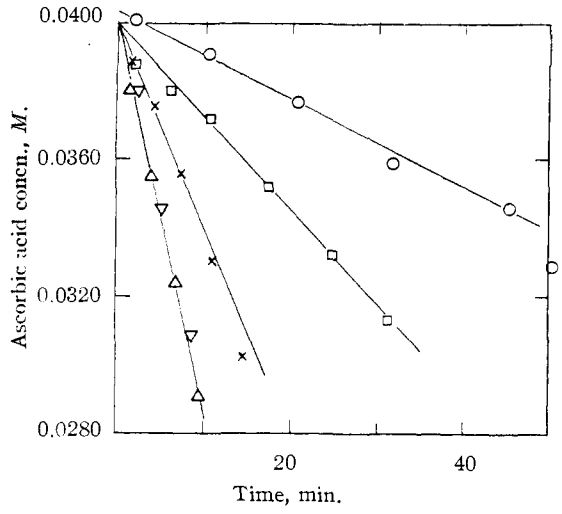


Fig. 6.—Effect of pH; [phosphate] = 0.10 M, [Fe] = 6.0×10^{-4}, [EDTA] = 12.0×10^{-4}, [H_2O_2] = 0.026–0.031 M; initial pH: O, 3.39; □, 3.61; △▽, 3.92 (two expts.); ×, 4.23, Fe = 1.5×10^{-4} M.

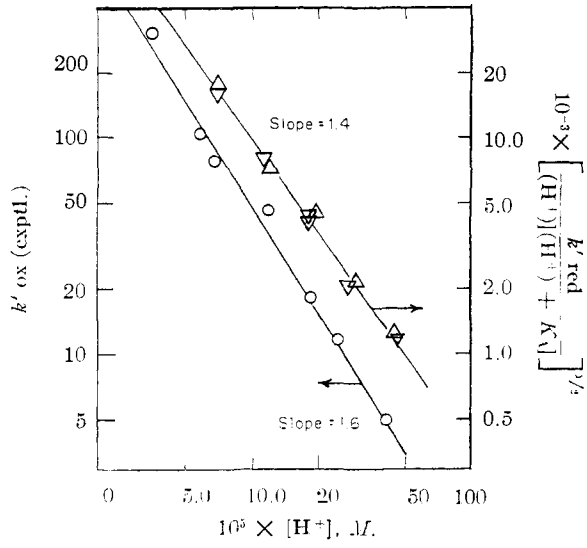


Fig. 7.—Oxidation of ascorbic acid by H_2O_2; effect of H^+ concentration; [phosphate] = 0.10 M, [Fe] = 6.0×10^{-4} M, [EDTA] = 12.0×10^{-4} M, [ascorbic acid] = 4.0×10^{-2} M, [H_2O_2] = 0.026–0.031 M: ⊙, experimental points; ▽, △, calculated from proposed mechanism.

mentally determined pH dependence is greater than can be accounted for by the above mechanism alone, and this would suggest that the rate should increase during a run. In some cases (*e.g.*, Fig. 6, experiment at pH 3.39) this actually occurred, but in most cases the effect was probably balanced out by the occurrence of reaction 4, which produces acid and tends to reduce the rate.

$$C_6H_6O_6 + H_2O_2 + H_2O \longrightarrow H_2C_2O_4 + C_4H_8O_5 \quad (4)$$
dehydroascorbic acid oxalic acid threonic acid

As a matter of fact, the pH does rise during a run by about 0.1 or 0.2 unit. The existence of straight lines is thus apparently fortuitous and does not conflict with the independence of rate on ascorbic acid concentration discussed above. The important feature of the curves, of course, is the initial slope,

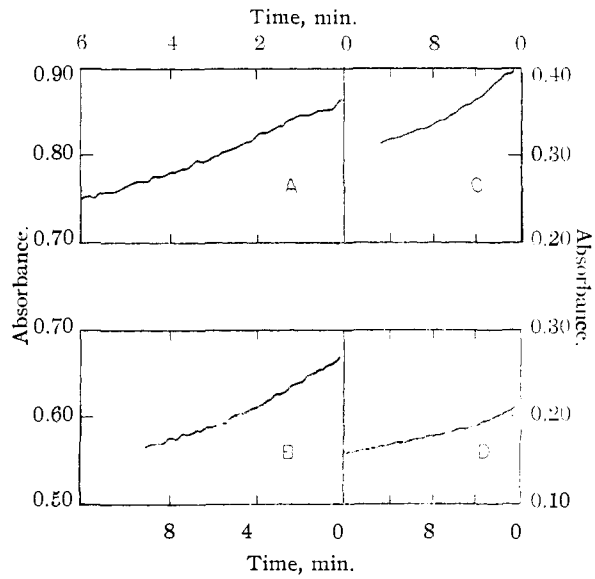


Fig. 8.—Reduction of Fe(III)–EDTA by ascorbic acid; effect of iron concentration; [$NaClO_4$] = 0.050 M, [ascorbic acid] = 0.040 M, pH 3.73; absorbance measured at 364 mμ.

	$10^4 \times$ [Fe], M	$10^4 \times$ [EDTA], M
A	10.0	20.0
B	7.0	14.0
C	4.0	8.0
D	2.0	4.0

and in cases where straight lines are not obtained, only the first few points were used in defining the initial slope.

Rate curves shown in Fig. 6 clearly demonstrate the effect of increasing pH in increasing the reaction rate. These data imply an equation of the form

$$\frac{-\mathrm{d}(AAH_2)_T}{\mathrm{d}t} = k_{ox}\frac{(FeY^-)(AAH_2)_T}{(H^+)^n} = R \quad (5)$$

where Y^- is the ethylenediaminetetraacetate ion, and $(AAH_2)_T$ is the total concentration of ionized and un-ionized forms of ascorbic acid. Taking logarithms and rearranging, we get

$$\log \frac{R}{(FeY^-)(AAH_2)_T} =$$
$$\log k'_{ox} = \log k_{ox} - n \log (H^+) \quad (6)$$

Figure 7 shows a plot of log k'_{ox} *versus* log (H^+) from which the value of n is determined as about 1.6, and the value of k_{ox} as 1.6×10^{-5} in units of mole per liter and minutes.

Reduction of Fe(III)–EDTA by Ascorbic Acid.—In Fig. 8 are shown some of the curves of absorbance *versus* time produced directly by the spectrophotometer. In this case the parameter was the iron concentration. If the reaction is first order in iron, then the integrated kinetic equation will be of the form

$$\log (FeY^-) = (k_F/2.30)t + \text{constant} \quad (7)$$

where the first-order rate constant k_F includes the dependence on concentrations of other species. The absorbance A of a colored solution is related to the concentration of the colored species by the equation

$$A = \epsilon_{FeY^-}(FeY^-) \quad (8)$$

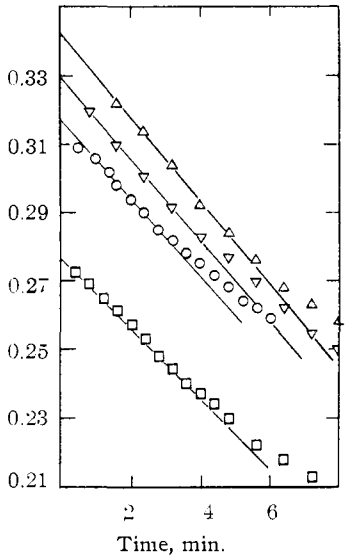


Fig. 9.—Reduction of Fe(III)-EDTA by ascorbic acid; effect of iron concentration; [$NaClO_4$] = 0.050 M, [ascorbic acid] = 0.040 M, pH 3.73:

	Expt.	[Fe], M		[EDTA], M
○	1	10.0×10^{-4}	○	1 20.0×10^{-4}
□	2	7.0×10^{-4}	□	2 14.0×10^{-4}
△	3	4.0×10^{-4}	△	3 8.0×10^{-4}
▽	4	2.0×10^{-4}	▽	4 4.0×10^{-4}

where ϵ_{FeY^-} is the molar extinction coefficient of the colored species. Combining eq. 7 and 8 leads to

$$\log A = (k_F/2.30)t + \text{constant} \qquad (9)$$

In order to determine the first-order rate constants, values of absorbance were read from these curves and replotted as the logarithms. Such a plot is shown in Fig. 9 for the data of Fig. 8. Because the reduction is incomplete, reaching a measurable equilibrium, it was possible to use only the first several points to determine a straight line for which the slope could be taken. While this procedure necessarily reduces the precision of the data, nevertheless the precision appears adequate to establish the kinetics with reasonable assurance. The values of k_F are given in Table III.

Fairly good agreement among the values for the first four experiments confirms the first-order dependence on iron chelate concentration. The effect of ascorbic acid concentration is shown in Fig. 10. The data rather obviously leave a good deal to be desired in the matter of reproducibility, and both the dotted curve and the straight line could be said to be a fair representation of the points. The significance of the curve will be discussed below, and for reasons which will be brought out, a straight line appears the more likely. The reaction is thus first order, or nearly so, in ascorbic acid concentration. As in the H_2O_2 experiments, an equation of the form

$$\frac{-d(FeY^-)}{dt} = k_{red}\frac{(FeY^-)(AAH_2)_T}{(H^+)^m} = k'_{red}(FeY^-)(AAH_2)_T \qquad (10)$$

was assumed where k'_{red} includes only the dependence on H^+. By differentiating eq. 7 and substituting in 10, we have

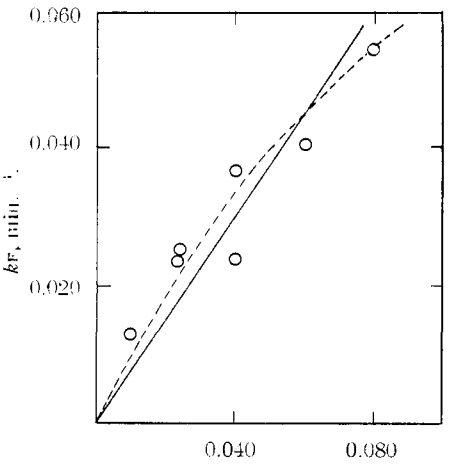


Fig. 10.—Reduction of Fe(III)-EDTA by ascorbic acid; [$NaClO_4$] = 0.10 M, [Fe] = 1.0×10^{-3} M, [EDTA] = 2.0×10^{-3} M, pH 3.73; circles are experimental points; see text for discussion.

$$\frac{k_F}{(AAH_2)_T} = \frac{k_{red}}{(H^+)^m} = k'_{red} \qquad (11)$$

Taking logs we have

$$\log k'_{red} = -m \log (H^+) + \log k_{red} \qquad (12)$$

This equation is plotted in Fig. 2 using the pertinent data from Table III. The slope of the best straight line is about 1.1, and the value of k_{red} is 0.064 in units of mole per liter and minutes.

TABLE III

REDUCTION OF FeY^- BY ASCORBIC ACID

Expt.	Perchlorate, M	Iron(III) $\times 10^4$, M	EDTA $\times 10^4$, M	Ascorbic acid $\times 10^2$, M	pH	k_F, min.$^{-1}$
1	0.05	10.0	20.0	4.0	3.73	0.028
2	.05	7.0	14.0	4.0	3.73	.023
3	.05	4.0	8.0	4.0	3.73	.028
4	.05	2.0	4.0	4.0	3.73	.028
5	.10	10.0	20.0	4.0	3.73	.024
6	.10	10.0	20.0	6.0	3.73	.041
7	.10	10.0	20.0	2.4	3.73	.024
8	.10	10.0	20.0	8.0	3.73	.055
9	.10	10.0	20.0	2.4	3.73	.025
10	.10	10.0	20.0	1.0	3.73	.013
11	.10	10.0	20.0	4.0	3.35	.014
14	.10	10.0	20.0	4.0	3.53	.018
12	.10	10.0	20.0	4.0	3.73	.037
15	.10	10.0	20.0	4.0	3.93	.045
13	.10	10.0	20.0	4.0	4.16	.012
18	.10[a]	10.0	20.0	4.0	3.35	.013
19	.10[a]	10.0	20.0	4.0	3.55	.017
16	.10[a]	10.0	20.0	4.0	3.73	.032
17	.10[a]	10.0	20.0	4.0	3.73	.030
20	.10[a]	10.0	20.0	4.0	3.94	.051
21	.10[a]	10.0	20.0	4.0	4.16	.085

[a] No perchlorate present; total concentration of phosphate shown.

Discussion

Reduction of Fe(III)–EDTA by Ascorbic Acid.—The absence of any dependence on H_2O_2 concentration in the kinetic expression for the oxidation of

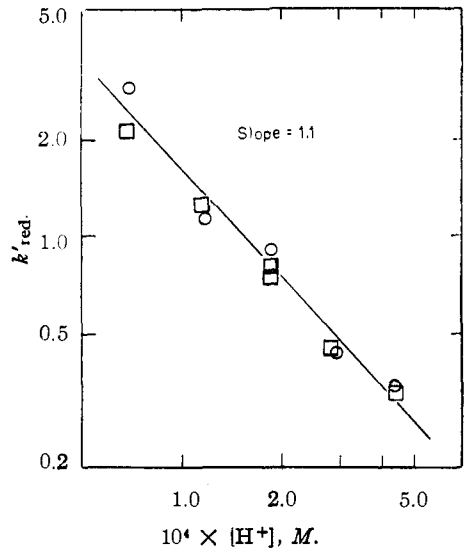


Fig. 11.—Reduction of Fe(III)-EDTA by ascorbic acid; effect of acidity; [Fe] = 1.0×10^{-3} M, [EDTA] = 2.0×10^{-3} M, [ascorbate] = 0.04 M: ⊙, 0.10 M $NaClO_4$; ⊡, 0.10 M NaH_2PO_4.

ascorbic acid suggests that the rate-determining step is the reduction of Fe(III)–EDTA by ascorbic acid. If this were so, both the kinetic expression and the rate constants for the direct reduction and the H_2O_2 oxidation should be identical. The dependence on H^+ concentration is clearly different for the two cases and, while comparison of the rate constants therefore is not meaningful, a comparison of initial rates under identical conditions can be made. In experiment 9A in Table II the initial rate of disappearance of ascorbic acid is seen to be 6.8×10^{-4} mole per liter per minute. In experment 16 in Table III, in which conditions are identical except for the absence of H_2O_2, the initial rate of disappearance of FeY^- species can be calculated from eq. 9 and 10 to be 3.2×10^{-5} mole per liter per minute. The rate of disappearance of ascorbic acid will be half of this, or 1.6×10^{-5} mole per liter per minute. The ascorbate disappears, therefore, some 42 times faster in the presence of H_2O_2. This suggests the existence of a chain reaction for the oxidation wherein the initiation may occur by means of the reduction step. A similar situation was observed by Dekker and Dickinson,[10] who found that the rate of the copper-catalyzed oxidation of ascorbic acid by oxygen was some 50 to 75 times the rate by cupric ion alone. These authors suggested the existence of an intermediate ascorbate free radical which reacted readily with O_2, but they did not attempt to work out the detailed mechanism. The existence of such a radical, which would be a 1-electron oxidation product of ascorbic acid, has been accepted by others[11,12] and is included as part of the mechanism presented here. The kinetics of the reduction step appear to be adequately accounted for by the equations

(10) A. O. Dekker and R. G. Dickinson, THIS JOURNAL, **62**, 2165 (1940).
(11) H. Nord, *Helv. Chim. Acta*, **9**, 442 (1951).
(12) A. Weissberger, J. E. LuValle and D. S. Thomas, Jr., THIS JOURNAL, **65**, 1934 (1943).

$$AAH_2 \overset{K_A}{\rightleftharpoons} AAH^- + H^+ \quad (13)$$

$$FeY^- + AAH^- \overset{K_1}{\rightleftharpoons} FeY^-\cdot AAH^- \quad (14)$$

$$FeY^-\cdot AAH^- \overset{K_2}{\rightleftharpoons} FeY^-\cdot AA^{\cdot -} + H^+ \quad (15)$$

$$FeY^-\cdot AAH^- \xrightarrow{k_{1A}} FeY^- - AAH\cdot \quad (16A)\ (slow)$$

$$FeY^-\cdot AA^{\cdot -} \xrightarrow{k_{1B}} FeY^- + AA^{\cdot -}\cdot \quad (16B)\ (slow)$$

$$AAH \overset{K_3}{\rightleftharpoons} AA^{\cdot -}\cdot + H^+ \quad (17)$$

$$AA^{\cdot -}\cdot + FeY^- \xrightarrow{k_{2A}} AA + FeY^- \quad (18A)\ (fast)$$

$$AAH\cdot + FeY^- \xrightarrow{k_{2B}} AA + FeY^- + H^+ \quad (18B)\ (fast)$$

In this sequence, AA denotes dehydroascorbic acid. Similarly, AAH· and $AA^{\cdot -}$· denote the free radical intermediate oxidation products of ascorbic acid.

Providing $K_1(AAH^-) << 1$ and $K_2 << (H^+)$, *i.e.*, the equilibria in eq. 14 and 15, are to the left, these equations give rise to the following kinetic equation expressing the rate of reduction of the iron(III) chelate

$$\frac{-d(FeY^-)}{dt} = -2\frac{d(AAH_2)_T}{dt} = \frac{(FeY^-)(AAH_2)_T K_A K_1 k_{1A}[(H^+) + (k_{1B}K_2/k_{1A})]}{(H^+)[(H^+) + K_A]} = k'_{red}(FeY^-)(AAH_2)_T \quad (19)$$

This equation exhibits the observed dependence on iron and ascorbate concentrations.

The question of whether the ascorbate dependence was first order was raised previously, and it was pointed out that the curved line of Fig. 10 might also be a valid representation. Such a curve would result if the equilibrium in eq. 14 were such that appreciable concentrations of the iron–versene–ascorbate complex existed. The curve line shown in Fig. 10 was actually calculated using the value of $K_1 = 7.5$, and corresponds to the existence of between about 2 and 30% of the iron chelate in a form complexed with ascorbate. Such a complex would probably have an absorption spectrum somewhat different from the FeY^- species, and could presumably be detected spectrophotometrically. It was observed first that no instantaneous change in the visible spectrum of FeY^- occurred upon addition of ascorbate. Second, when the reaction had reached equilibrium the absorption spectrum was compared with the original spectrum at several points. The data are shown in Table IV.

Except at the lowest absorbance values where blank corrections are quite large, there appears to be no significant change in this portion of the spectrum when ascorbic acid is present. The existence of an ascorbate complex with a different spectrum in appreciable concentrations appears unlikely.

The dependence upon pH will be determined by the relative values of K_A and $(k_{1B}K_2)/k_{1A}$. Since K_A is not negligible compared to (H^+), the observed approximate inverse first-order dependence will be satisfied only if $K_A = (k_{1B}K_2)/k_{1A}$. This is equivalent to saying that in the pH region investigated, both reactions 16A and 16B contribute about equally to the over-all reaction. With this condi-

TABLE IV

COMPARISON OF VISIBLE SPECTRUM OF IRON(III)-EDTA CHELATE IN PRESENCE AND ABSENCE OF ASCORBIC ACID

[Fe] = 1.0×10^{-3} M, [EDTA] = 2.0×10^{-3} M, [$NaClO_4$] = 0.07 M, [Ascorbic acid] = 0.040 M, pH = 3.73

Wave length, mμ	Absorbance of solutions: Ascorbic acid Present	Ascorbic acid Absent	Ratio: present/absent
450	0.017	0.017	1.0
425	.036	.066	0.55
400	.095	.200	.47
390	.144	.315	.46
380	.209	.475	.44
370	.322	.714	.45
360	.478	1.07	.45
350	.688	1.57	.44

tion, eq. 19 thus fits the experimental data satisfactorily, and with the data obtained give $k_{red} = K_A K_1 k_{1A} \sim 0.064$ min.$^{-1}$. The value of K_A was found to be 6.16×10^{-5}, and therefore $K_1 k_{1A} = 1.0 \times 10^{-3}$ liter per mole per minute.

The Ascorbic Acid–H_2O_2 Reaction.—In addition to the above reactions, the possible reactions involved in the oxidation by hydrogen peroxide are

$$AA^{-}\cdot + H_2O_2 \xrightarrow{k_{2A}} AA + OH^- + OH\cdot \quad (20A)$$

$$AAH\cdot + H_2O_2 \xrightarrow{k_{2B}} AA + H_2O + OH\cdot \quad (20B)$$

$$OH\cdot + AAH^- \xrightarrow{k_{4A}} AAH\cdot + OH^- \quad (21A)$$

$$OH\cdot + AAH_2 \xrightarrow{k_{4B}} AAH\cdot + H_2O \quad (21B)$$

$$H_2O_2 + FeY^- \xrightarrow{k_5} FeY^- + OH^- + OH\cdot \quad (22)$$

$$FeY^- + OH\cdot \xrightarrow{k_6} FeY^- + OH^- \quad (23)$$

$$AA^{-}\cdot + OH\cdot \xrightarrow{k_{7A}} AA + OH^- \quad (24A)$$

$$AAH\cdot + OH\cdot \xrightarrow{k_{7B}} AA + H_2O \quad (24B)$$

The existence of two separate chains is possible, depending upon the fate of the ascorbate radical produced in 16 and 21. If it reacts only according to eq. 18, the chain transfer steps will consist of 18, 21 and 22. If the ascorbate radical reacts instead with H_2O_2, a chain reaction consisting only of steps 20 and 21 will exist. Termination in either case should occur *via* 23 or 24. Kinetic rate equations were derived for both conditions by setting up the steady state equation for the intermediates. The contribution of initiation and termination steps was neglected, in view of the fact that the chain oxidation is some 42 times the speed of the reduction step. In the case of the chain involving eq. 20 and 21, derivation of the kinetic expression leads to a zero or half-order dependence on chelate concentration, and also leads to either a first-order or half-order dependence on H_2O_2 concentration. This is contrary to the experimentally determined kinetics, and reaction 20 is therefore excluded as part of the scheme. On the other hand, reaction 18, 21 and 22 lead to the kinetic expression

$$-\frac{d(AAH_2)_T}{dt} = (AAH_2)_T(FeY^-)\frac{K_A}{K_A + (H^+)}\left[\frac{K_1 k_1 k_2 k_4}{k_7}\right]^{1/2} = k'_{ox}(AAH_2)_T(FeY^-) \quad (25)$$

providing $k_6 k_2(FeY^-) << k_5 k_7(H_2O_2)$. In this equation and in the inequality expression, the individual rate constants include the pH dependence expressed by their respective a and b reactions as

$$k_1 = k_{1A} + (k_{1B}K_2/(H^+)$$
$$k_2 = k_{2A} + (k_{2B}(H^+)/K_2$$
$$k_4 = k_{4A} + (k_{4B}(H^+))/K_A$$
$$k_7 = k_{7A} + (k_{7B}(H^+)/K_2$$

The condition imposed by the inequality is that the termination must proceed *via* reaction 7 rather than 6. The observed dependence on both iron and ascorbic acid concentration is satisfied by these kinetics, and it remains to inquire about the pH dependence. The greatest possible inverse pH dependence will occur if reactions 2A, 4A and 7B predominate over 2B, 4B and 7A, respectively. This simplification leads to

$$(k'_{ox})^2 = \frac{K_2 K_A k_{2A} k_{4A}}{k_{7B}} \times \frac{1}{(H^+)[(H^+) + K_A]} \times \frac{[(H^+) + ((k_{1B}K_2)/k_{1A})]\,K_A K_1 k_{1A}}{[(H^+) + K_A][(H^+)]}$$

The third factor can be replaced by k'_{red} (eq. 19), leaving

$$k'_{ox} = \left[\frac{K_2 K_A k_{2A} k_{4A}}{k_{7B}}\right]^{1/2}\left[\frac{k'_{red}}{(H^+)[(H^+) + K_A]}\right]^{1/2} \quad (26)$$

The pH dependence now resides in the second bracket, values of which can be calculated from the data in Table III and the aid of eq. 11. A plot of log (H^+) *vs.* log of the pH dependent part of eq.26 is shown in Fig. 7. A fair straight line is obtained, the slope of which is about 1.4. In other words, the proposed chain mechanism, in conjunction with the observed kinetics of direct reduction, implies an inverse power dependence of k'_{ox} on (H^+) of about 1.4. The observed dependence, also shown in Fig. 7, is still higher, being about 1.6.

Considering the difficulty experienced in obtaining precise results in the direct reduction experiments, this agreement is probably fairly good, and suggests that the gross features of the mechanism are correct. Minor modifications in the mechanism could be proposed which would increase the pH dependence (*e.g.*, participation of the iron chelate as a partially hydrolyzed species). However, in the absence of positive evidence for such species, it would appear prudent to first refine the measurements on the pH effect before attempting to explain the discrepancies by postulating additional (and less likely) species.

The oxidation mechanism thus involves:

initiation: about equally by reactions 16A and 16B
propagation: by a cyclic process consisting of reactions 18A, 21A, and 22
termination: reaction 24B

The predominance of 21A over 21B, even though $(AAH_2) > (AAH^-)$ in most experiments, means that the OH· radical evidently prefers to react with the ionized ascorbate ion, AAH^-. In the termination reaction (24) the OH· radical would be expected to react with the most numerous species, which is therefore the AAH· radical rather than its ionized form, $AA^{-}\cdot$. The predominance of reaction 18A involving the $AA^{-}\cdot$ radical over 18B must then indicate a strong preference of the iron chelate for the ionized species, $AA^{-}\cdot$.