Acknowledgment.—Financial support from the U. S. Public Health Service and from the Louis and Maud Hill Family Foundation is gratefully acknowledged. Preliminary work on the development of some of the procedures has been carried out by Dr. R. K. Neogy.

We are indebted to Dr. R. W. Lumry and Dr. B. R. Willeford for their interest in our work.

MINNEAPOLIS, MINN.

[CONTRIBUTION FROM THE RADIATION BRANCH NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE, U. S. DEPARTMENT OF HEALTH, EDUCATION AND WELFARE]

A Mechanism for the Reaction of Fe^{++}, H_2O_2 and Glycine in an Aerobic Aqueous Solution

BY CHARLES R. MAXWELL AND DOROTHY C. PETERSON

RECEIVED APRIL 11, 1957

The kinetics and stoichiometry of the action of Fenton's reagent in aerobic aqueous solutions of glycine are presented and a mechanism is proposed to describe the observations. The reaction is initiated and the rate controlled by the production of an OH free radical from a H_2O_2 molecule. The stoichiometry, which varies as the reaction proceeds, is the result of two pairs of competing steps: the first between Fe^{++} and glycine for the OH free radical and the second between Fe^{++} and Fe^{+++} for an intermediate organo-peroxy free radical. The Fe^{++}–peroxy reaction regenerates the H_2O_2 used in the initial step and thus accounts for the small $\Delta H_2O_2/\Delta Fe^{++}$ ratios observed in the early phase of the reaction. The Fe^{+++}–peroxy reaction regenerates the Fe^{++} used in the initial step and thus accounts for the large $\Delta H_2O_2/\Delta Fe^{++}$ ratios observed in the late phase of the reaction. HCOCOOH and NH_3 are formed by both reactions. The effects of H^+ concentration and of glycine concentration have been measured and are discussed. The ratios of the reaction rate constants of the competing steps have been evaluated for various conditions.

Introduction

A mixture of H_2O_2 and Fe^{++}, commonly referred to as Fenton's reagent, has been used since before the turn of the century to study the oxidation of organic compounds in aqueous solution. More recently, as the mechanism of the reaction began to be more fully understood[1-3] it has been used extensively to study the effect of OH free radicals. We became interested in the action of this reagent upon glycine as a result of a study[4] of the effect of ionizing radiation upon solutions of this amino acid. Since ionizing radiation in aqueous solution reacts indirectly[5] through a complex mixture of H and OH free radicals and H_2O_2, it was believed that an investigation of the Fenton reaction would provide a mechanism which would be useful in the interpretation of radiation data.

Dakin[6] has reported that the products of the oxidation of amino acids by Fenton's reagent are in general ammonia, a keto acid, an aldehyde and carbon dioxide with the yield of the aldehyde usually greater than the yield of the keto acid. However, with glycine he found the yield of HCOCOOH greater than the yield of HCHO. Merz and Waters,[7] on the other hand, have listed only HCHO as the product of the oxidation. The authors,[4] working with ionizing radiation rather than Fenton's reagent, found HCOCOOH, HCHO, NH_3, CO_2, and in addition observed that the ratios between the yields of HCOCOOH, HCHO and NH_3 varied with the dose of radiation. These observations were interpreted in terms of secondary reactions which oxidized the HCOCOOH and HCHO but did not affect the NH_3.

Merz and Waters[7] have proposed a general scheme for the action of OH radicals from Fenton's reagent upon organic substrates in which many compounds are oxidized by a chain reaction involving the substrate and H_2O_2. Glycine and alanine are listed among these compounds. The rationale for this chain, which is initiated by an OH radical formed from Fe^{++} and H_2O_2 and terminated by the reaction of an OH radical with a Fe^{++} ion, appears to be based solely upon large H_2O_2 consumption/Fe^{++} oxidation ratios. No quantitative measurements were reported for the yield of products or consumption of substrate nor of the rate of the reaction.

Kolthoff and Medalia[8] have suggested another scheme for the action of Fenton's reagent upon organic substrates. In the presence of dissolved oxygen (which Merz and Waters did not consider) this scheme includes a different chain reaction which is also initiated by the action of an OH radical upon the substrate. This chain which involves oxygen (not H_2O_2), substrate and Fe^{++} provides for the oxidation of many molecules of substrate and Fe^{++} ions for each OH radical formed (H_2O_2 consumed). However, here again the mechanism appears to be based solely upon H_2O_2 consumption/Fe^{++} oxidation ratios. No data were reported for substrate oxidation, product yield or rates of reaction.

From the above it is apparent that data as to the extent of secondary reactions, the actual substrate oxidation or product yield, and the rate of the reaction is needed. We, therefore, first studied the yields of products as a function of "OH exposure" and differentiated the initial and secondary products. The study of the reaction was then limited to conditions which eliminated or minimized the

(1) F. Haber and J. Weiss, *Proc. Roy. Soc.* (*London*), **A147**, 332 (1934).

(2) A. I. Medalia and I. M. Kolthoff, *J. Polymer Sci.*, **4**, 377 (1949).

(3) W. G. Barb, J. H. Baxendale, P. George and K. R. Hargrave, *Trans. Faraday Soc.*, **47**, 462, 591 (1951).

(4) C. R. Maxwell, D. C. Peterson and N. E. Sharpless, *Rad. Research*, **1**, 530 (1954).

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(6) H. D. Dakin, *J. Biol. Chem.*, **1**, 171 (1905).

(7) J. H. Merz and W. A. Waters, *J. Chem. Soc.*, S15, 2427 (1949).

(8) I. M. Kolthoff and A. I. Medalia, THIS JOURNAL, **71**, 3777, 3784 (1949).

secondary reactions. In this study the concentrations of Fe^{++}, H_2O_2 and HCOCOOH (a product of the OH–glycine reaction) were each followed as a function of time. The $\Delta H_2O_2/\Delta Fe^{++}$ ratios were observed to vary widely and in a range and manner similar to the observations of other investigators for other compounds. The observed kinetics is, however, incompatible with either of the general schemes heretofore proposed and a new mechanism is presented to describe our observations. Although tested only for glycine, this mechanism suggests an alternate general scheme for the explanation of the stoichiometric observations of the other investigators.

Experimental

Procedure.—Two beakers of glycine, one containing H_2O_2 and the other Fe^{++}, were rapidly poured together and aliquots for analysis removed by pipet. The reaction was stopped by blowing each aliquot into a solution of either *o*-phenanthroline or tetravalent titanium.

The *o*-phenanthroline stops the reaction by complexing the ferrous ion; the titanium stops the reaction by complexing the H_2O_2.[9] In some cases where the NH_3 yield was to be measured the reaction was stopped in the late slow stages of the reaction by the addition of catalase which destroyed the H_2O_2 without effect upon the substrate. In general the reaction was followed until 75–90% of the Fe^{++} was oxidized with time intervals between pairs of aliquots in the early rapid phase of the reaction of the order of 15 seconds.

***p*H.**—The *p*H of the reaction mixture was adjusted by the addition of 1 *N* H_2SO_4 to the glycine solution and was measured with a Beckman *p*H meter and glass electrode. The kinetic study was limited to *p*H levels between 2.8 and 4.6. At levels greater than 4.6 the reaction was too fast to follow with accuracy and at levels less than 2.8 the reaction was not stopped immediately by addition to *o*-phenanthroline.

Temperature.—When the effect of temperature upon the rate of reaction was being studied the reaction mixture was immersed in a regulated water-bath, otherwise the reaction was carried out on the laboratory bench at room temperatures between 22 and 25°.

Analytical.—The Fe^{++} and H_2O_2 concentrations were determined by the method described elsewhere.[9] This method restricted the investigation to mixtures in which the H_2O_2 concentration was greater than one-half the Fe^{++} concentration and, with no buffer in the *o*-phenanthroline reagent, to *p*H levels above 2.8.

Ammonia was determined colorimetrically with Nessler reagent after an overnight microdiffusion in a Conway dish into dilute H_2SO_4 from an aliquot of sample made basic with NaOH. Any H_2O_2 in the sample was destroyed with catalase prior to the addition of NaOH in order to prevent a "dark" reaction which produced additional ammonia.

HCOCOOH was determined colorimetrically by a modification of the Bamburger test[10] for keto-acids, using ethyl acetate as the organic solvent and filtering the final basic solution to remove turbidity caused by traces of Ti^{++++} carried (mechanically?) through the procedure. The procedure was standardized with twice recrystallized $NaOOCCHO \cdot H_2O$ prepared by the periodic oxidation of tartaric acid according to a method described by Weissbach and Sprinson.[11]

HCHO was determined colorimetrically with chromotropic acid using a modification of the method described by Kleinert and Srepel.[12] Appropriate knowns and controls were measured simultaneously in order to minimize the interference of large amounts of Fe^{+++} and HCOCOOH.

HCOOH was also determined colorimetrically with chromotropic acid, after reduction to HCHO with Mg and HCl by a modification of the method described by Grant.[13] Prior to reduction, the HCOOH was removed from the reaction mixture by steam distillation and concentrated as the sodium salt.

Reagents. Water.—All water was distilled from a tin lined still, redistilled in Pyrex from basic $KMnO_4$ and again from dilute H_2SO_4 and stored for short periods in Pyrex bottles.

H_2O_2.—99.9% inhibitor-free H_2O_2 was used in all experiments reported here. Merck 30% Superoxal gave identical results in other experiments not reported.

Fe^{++}.—Both reagent grade $FeSO_4$ and reagent grade $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ were used without further purification.

Fe^{+++}: reagent grade $FeNH_4(SO_4)_2 \cdot 12H_2O$.

Glycine.—Glycine from Nutritional Biochemicals Corporation was recrystallized twice; once from distilled water by the addition of C.P. absolute methanol followed by vacuum drying at 50° and again thermally from distilled water with a second vacuum drying.

Results

The Reaction.—Ammonia, glyoxalic acid, formic acid and formaldehyde have been found in glycine solutions treated with Fenton reagent where the initial Fe^{++} and H_2O_2 concentrations were of the order of 10^{-3} to 10^{-2} *M*. However, the ratios among the Fe^{++} oxidation, H_2O_2 consumption and the yields of the various products varied with the initial concentrations of Fe^{++} and H_2O_2 and with the time the reaction was allowed to proceed. Pertinent portions of the data are shown in Table I. The lack of a constant ratio among the products has been interpreted as the result of secondary reactions which oxidize some of the primary products.

TABLE I

STOICHIOMETRY OF FENTON'S REACTION IN 0.1 *M* GLYCINE—*p*H 3.5—AIR SATURATED

Reactants, μM				Products, μM				
Fe_0^{++}	H_2O_{20}	ΔFe^{++}	ΔH_2O_2	HCOOH	NH_3	HCHO	HCOOH	Time, min.
450	1030	440	190	300	500	..	19	5
920	1810	910	760	480	940	..	60	7
1800	5700	1790	3410	490	1980	..	110	12
120	320			145		0		10
480	1280			260		≈2		2
480	1280			305		≈3		10
1920	5120			560		≈5		2
1920	5120			500		≈4		10
132	83	113	45	88	93			10
200	104	176	68	124	135			10
330	167	300	130	197	231			12
400	210	360	170	236	292			12

In order to determine the initial ratios before secondary reactions or oxygen depletion became important the data were analyzed on the assumption that ammonia is a stable product which is a measure of the glycine consumption. A plot of the $HCOCOOH/NH_3$ ratio against the NH_3 yield for the last four lines of data in Table I is a straight line which extrapolates to a ratio of one at zero NH_3 yield. This indicates that initially NH_3 and HCOCOOH are formed in equal amounts but that a secondary reaction or oxygen depletion has distorted the ratio by the time the NH_3 yield is large enough to be measured accurately. The fact that this distortion is due to a secondary reaction rather than oxygen depletion has been clearly demonstrated by measuring the ratio in an oxygen-free

(9) C. R. Maxwell and D. C. Peterson, to be published.

(10) H. J. Fister, "Manual of Standardized Procedures for Spectrophotometric Chemistry," Standard Specific Supply Corp., New York, N. Y., 1950.

(11) A. Weissbach and D. B. Sprinson, *J. Biol. Chem.*, **203**, 1023 (1953).

(12) T. Kleinert and E. Srepel, *Mikrochem. Mikrochim. Acta*, **33**, 328 (1948).

(13) W. M. Grant, *Anal. Chem.*, **20**, 267 (1948).

system. The data, which will be presented in a subsequent paper, yields $HCOCOOH/NH_3$ ratios which also extrapolate to one at zero NH_3 yield and which incidentally are much less distorted by secondary reactions than the ratios in air.

A similar examination of the HCOOH yields indicates that HCOOH is a secondary product. It is almost certainly the result of the oxidation of HCOCOOH. The low yield of HCHO has prevented a detailed study of this product and its role in the initial reaction has been assumed to be insignificant.

In order to minimize the distortion resulting from secondary reactions the investigation of the kinetics of the glycine oxidation was made using initial Fe^{++} and H_2O_2 concentrations of less than 10^{-4} M which produced final concentrations of HCOCOOH of less than 50 μM. Under these conditions only Fe^{++}, H_2O_2 and HCOCOOH could be measured with precision.

The analysis of data from many experiments in this region has led to the following general observations.

(1) Throughout the course of any experiment the rate of glyoxalic acid production is proportional to the product of the ferrous and hydrogen peroxide concentrations.

(2) The *initial* rate of Fe^{++} oxidation is equal to twice the *initial* rate of the HCOCOOH production.

(3) The rate of Fe^{++} oxidation, $\Delta Fe^{++}/[Fe^{++}][H_2O_2]\Delta t$, approaches zero as the reaction proceeds.

(4) The *initial* rate of H_2O_2 consumption, $\Delta[H_2O_2]/[Fe^{++}][H_2O_2]\Delta t$, is small but the rate increases as the reaction proceeds and approaches the rate of HCOCOOH production.

(5) The presence of Fe^{+++} reduces the *initial* rate of Fe^{++} oxidation.

(6) The presence of both F^- and Fe^{+++} produces no change in the *initial* rate of Fe^{++} oxidation.

(7) The removal of dissolved oxygen increases the *initial* rate of H_2O_2 consumption.

The Proposed Mechanism.—Equations 1 through 6 are proposed as a mechanism for the action of Fenton's reagent on glycine as described above. Equation 6 is not a truly mechanistic equation but an over-all equation which for convenience is used in lieu of eq. 11 and 12 developed later in the section on the effect of H^+ concentration. The species reacting with the Fe^{+++} is believed to be the anion of the ionized peroxy radical rather than the peroxy radical itself. However, in experiments where the H^+ concentration is essentially constant the concentration of the anion will be a constant fraction of the concentration of the radical and it is equivalent and more expedient mathematically to work with the effective equation as presented.

$$Fe^{++} + H_2O_2 \longrightarrow Fe^{+++} + OH^- + \dot{O}H \quad k_1 \quad (1)$$

$$\dot{O}H + Fe^{++} \longrightarrow Fe^{+++} + OH^- \quad k_2 \quad (2)$$

$$\dot{O}H + NH_2CH_2COOH \longrightarrow H_2O + NH_2\dot{C}HCOOH \quad k_3 \quad (3)$$

$$NH_2\dot{C}HCOOH + O_2 \longrightarrow NH_2CH(\dot{O}_2)COOH \quad k_4 \quad (4)$$

$$NH_2CH(\dot{O}_2)COOH + Fe^{++} \longrightarrow Fe^{+++} + (NH_2CH(O_2)COOH)^- \quad k_5 \quad (5)$$

$$(NH_2CH(O_2)COOH)^- \longrightarrow HN{=}CHCOOH + HO_2^- \quad (5a)$$

$$HO_2^- + H^+ \longrightarrow H_2O_2 \quad (5b)$$

$$HN{=}CHCOOH + H_2O \longrightarrow NH_3 + HCOCOOH \quad (5c)$$

$$NH_2CH(\dot{O}_2)COOH + Fe^{+++} + H_2O \longrightarrow Fe^{++} + O_2 + HCOCOOH + NH_3 + H^+ \quad k_6 \quad (6)$$

The reaction is initiated and the rate controlled by reaction 1 which produces an OH free radical. Fe^{++} and glycine then compete for the OH free radical according to eq. 2 and 3. However, as is shown later, at $[Glycine]/[Fe^{++}] > 2000$ reaction 2 occurs less than 5% of the time and may be neglected in a gross analysis.

Reaction 3 produces a glycine free radical which reacts in the presence of dissolved oxygen by 4 to form a peroxy free radical. The reaction is then terminated by either 5 or 6 where Fe^{++} and Fe^{+++} compete for the peroxy radical. The net reaction measured at any moment is thus the sum of two reaction sequences 1, 3, 4 and 5 and 1, 3, 4 and 6 whose relative contribution to the sum changes from moment to moment as the Fe^{++}/Fe^{+++} ratio changes. The first sequence, summed as A, describes the initial phase of the reaction when the

$$2Fe^{++} + O_2 + NH_2CH_2COOH + 2H^+ \longrightarrow 2Fe^{+++} + HCOCOOH + NH_3 + H_2O \quad (A)$$

$$H_2O_2 + NH_2CH_2COOH \longrightarrow HCOCOOH + NH_3 + H_2O \quad (B)$$

Fe^{+++} concentration is small. The second sequence, summed as B, describes the late phases of the reaction when the Fe^{++} concentration is small. Thus, on the basis of this model there should be no simple constant ratio between any two of the measured quantities, Fe^{++} oxidation, H_2O_2 consumption, and glyoxalic production but there should be a simple relation between the three. The glyoxalic acid production should be equal to the sum of the H_2O_2 consumption and one-half the Fe^{++} oxidation. Figure 1 shows how the observed data from two experiments carried out under widely different conditions satisfy this prediction.

Inherent in this model are other more complex relationships between Fe^{++}, H_2O_2 and HCOCOOH concentrations and time which provide methods for the evaluation of k_1, the ratio k_6/k_5 and the ratio k_2/k_3. These relationships provide additional methods for testing the model against the observed data. The development of such relationships and the testing of them by the observed data follow.

Evaluation of k_6/k_5.—An equation describing the H_2O_2 concentration as a function of the Fe^{++} concentration, the initial concentrations of the reactants and the ratio k_6/k_5 for the reaction rate constants of 5 and 6 may be derived in the following manner: (I) follows from the fact that the rate of Fe^{++} oxidation by A is twice the rate of 5, and (II) follows from the fact that the rate of H_2O_2 consumption by B is equal to the rate of 6. When the Fe^{+++} concentration is expressed as a function of the Fe^{++} concentration and I is divided by II to re-

$$\frac{d[Fe^{++}]}{2\,dt} = k_5[Fe^{++}][NH_2CH(\dot{O}_2)COOH] \quad (I)$$

$$\frac{d[H_2O_2]}{dt} = k_6[Fe^{+++}][NH_2CH(\dot{O}_2)COOH] \quad (II)$$

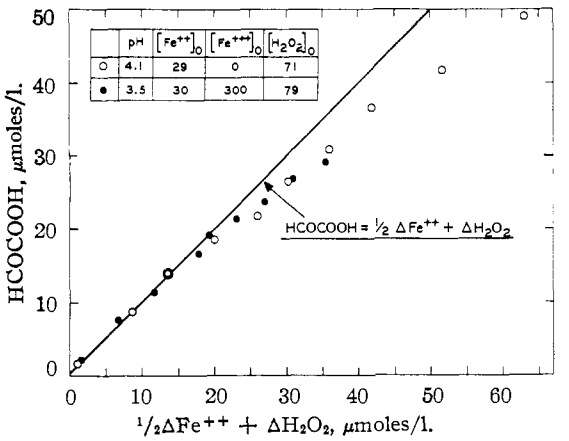

Fig. 1.—The HCOCOOH yield as a function of the Fe^{++} oxidation and H_2O_2 consumption. See equations A and B.

move the unknown peroxy radical concentration and the time factor dt, a differential equation is obtained which, when integrated yields III or the equivalent form III'. A plot of the H_2O_2 consumption against Z should yield a straight line with slope of $k_6/2k_5$. Figure 2 shows such a plot for the data previously used in Fig. 1. The difference in the k_6/k_5 ratio for the two sets of conditions is, as will be

$$[H_2O_2]_0 - [H_2O_2] = \frac{k_6}{2k_5}\left\{\left([Fe^{+++}]_0 + [Fe^{++}]_0\right)\ln\frac{[Fe^{++}]_0}{[Fe^{++}]} - ([Fe^{++}]_0 - [Fe^{++}])\right\} \quad \text{(III)}$$

$$\Delta H_2O_2 = \frac{k_6}{2k_5}Z \quad \text{(III')}$$

shown later, primarily due to the difference in H^+ concentration. The deviations from linearity observed in the late stages of the reaction in the presence of a large Fe^{+++} concentration are almost surely the result of a secondary "dark" reaction[14] which we have observed between Fe^{+++} and HCOCOOH.

Evaluation of k_1.—With III and a value for the ratio k_6/k_5 it is now possible to derive an expression for the Fe^{++} concentration as a function of time and to evaluate k_1. With an underlined integer defined as the rate of the corresponding reaction and p_5 and p_6 defined as the probabilities of any particular peroxy radical reacting by either 5 or 6, formulae IV → IX follow from the proposed mechanism.

$$\frac{-d[Fe^{++}]}{dt} = \underline{1} + \underline{5} - \underline{6} \quad \text{(IV)}$$

$$\frac{p_5}{p_6} = \frac{k_5[Fe^{++}]}{k_6[Fe^{+++}]} \quad \text{(V)}$$

$$p_5 + p_6 = 1 \quad \text{(VI)}$$

$$\underline{5} = \underline{1}\,p_5 \quad \text{(VII)}$$

$$\underline{6} = \underline{1}\,p_6 \quad \text{(VIII)}$$

$$\underline{1} = k_1[Fe^{++}][H_2O_2] \quad \text{(IX)}$$

(14) Unpublished data. Measurements of the Fe^{++} formation and HCOCOOH depletion as a function of time in both water and 0.1 M glycine brought to a pH of 3.5 with H_2SO_4 have shown the following reaction sequence to proceed in the absence of H_2O_2.

$$Fe^{+++} + HCOCOOH \longrightarrow Fe^{++} + X^{\cdot} \quad \text{(a)}$$

$$Fe^{+++} + X^{\cdot} \longrightarrow Fe^{++} + X \quad \text{(b)}$$

The kinetics of the initiating reaction (a) can be described by a second order rate constant of 0.1 liter $mole^{-1}$ $sec.^{-1}$ at a pH of 3.5.

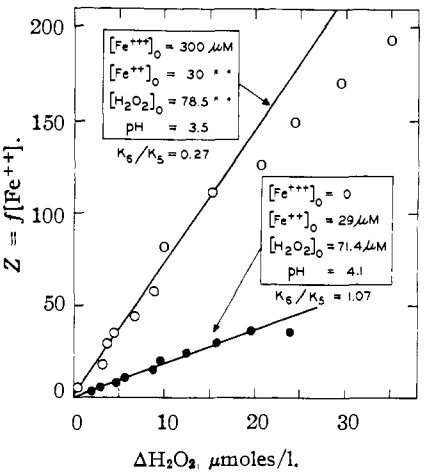

Fig. 2.—The evaluation of the ratio of the reaction rate constants k_5 and k_6 by equation III.

The differential equation X follows mathematically

$$k_1\,dt = \frac{KC + (1-K)x}{\left(2B + KA - Kx - KC\ln\frac{A}{x}\right)x^2}\,dx \quad \text{(X)}$$

where

$$x = [Fe^{++}]$$
$$A = [Fe^{++}]_0$$
$$B = [H_2O_2]_0$$
$$C = [Fe^{+++}]_0 + [Fe^{++}]_0$$
$$K = k_6/k_5$$

In principle this can be integrated between limits to yield XI and k_1 evaluated as the slope of the plot of Δt *vs.* ΔI. In practice we were unable to

$$k_1(t_0 - t) = I_0 - I \quad \text{(XI)}$$

find an analytical expression for I and had to resort to numerical integrations. Plots of such values of ΔI *vs.* the observed time are shown in Fig. 3 for data from the two experiments previously shown in Figs. 1 and 2. The difference in the values of k_1 is believed to be caused primarily by the difference in pH.

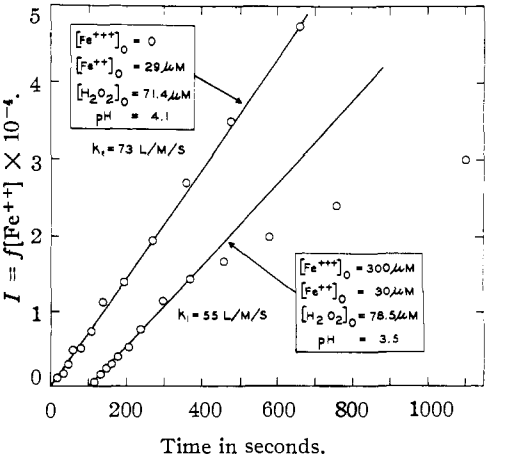

Fig. 3.—The evaluation of the reaction rate constant k_1 by a numerical integration of equation X. One group of data has been displaced by 100 seconds along the abscissa.

A comparison between the observed concentrations of Fe^{++} and H_2O_2 as a function of time and curves calculated using the values of k_1 and k_6/k_5 from Figs. 2 and 3 are shown in Figs. 4 and 5. The agreement is considered good in view of the "dark" reaction between Fe^{+++} and HCOCOOH.

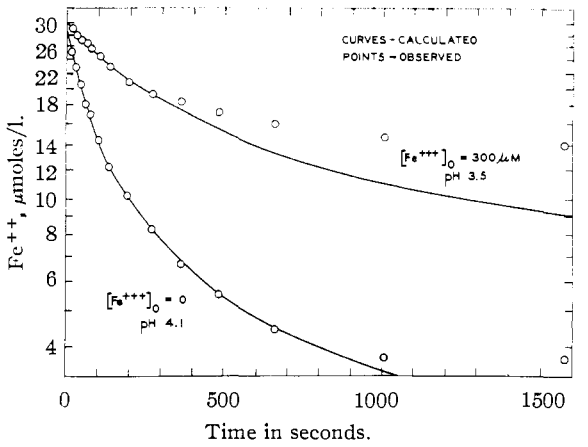

Fig. 4.—Calculated and observed Fe^{++} concentrations as a function of time. The curves were calculated by a numerical integration of eq. X using the constants evaluated in Figs. 2 and 3.

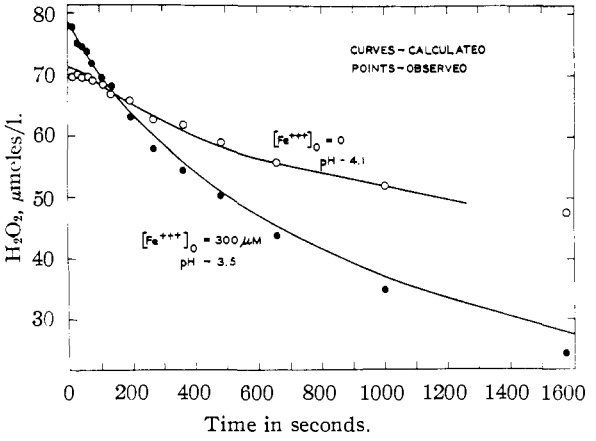

Fig. 5.—Calculated and observed H_2O_2 concentrations as a function of time. The curves were calculated by a numerical integration of eq. X, using the constants evaluated in Figs. 2 and 3 and eq. III.

Evaluation of k_2/k_3.—Since HCOCOOH is produced by both 5 and 6 the yield of this product is a measure of 3. There is no direct measure of 2 but it may be evaluated as the difference between the OH production by 1 and the HCOCOOH production. Thus, XII follows from the mechanism

$$\frac{k_2}{k_3} = \left(\frac{\text{OH—HCOCOOH}}{\text{HCOCOOH}}\right)\left(\frac{[\text{Glycine}]}{[Fe^{++}]}\right) \quad \text{(XII)}$$

k_2/k_3 was evaluated from measurements of HCOCOOH production and calculations of the OH production using glycine concentrations from 0.0005 to 0.01 M at pH 3.5 with an initial ferrous and H_2O_2 concentration of 60 μM. The HCOCOOH yield was measured at the end of 140 seconds and the Fe^{++} and H_2O_2 concentrations were measured at 15 second intervals for 180 seconds. The OH production was evaluated by a numerical integration of the rate equation for 1 using a value of 50 l. mole^{-1} sec.$^{-1}$ for k_1 and the measured values of Fe^{++} and H_2O_2 concentrations. The results are shown in Table II. The average value of 80 for k_2/k_3 is of the same order of magnitude as the 30 reported by Merz and Waters.[7] A potentially more accurate method of evaluating k_2/k_3 by complexing the ferric ion with F^- so as to suppress 6 and using the H_2O_2 consumption as a measure of 2 was unsuccessful. The F^- complexed the Ti^{++++} to such an extent that the remaining Ti^{++++} concentration was too small to complex all of the H_2O_2 and stop 1.

TABLE II

THE EVALUATION OF k_2/k_3

pH 3.5; $[H_2O_2]_0$ = 60 μM; $[Fe^{++}]_0$ = 60 μM

[Glycine], M	$[Fe^{++}]_{140}$, μM	HCOCOOH, μmoles	OH, μmoles	k_2/k_3[a]
0.010	35	15.3	19.0	48
.003	35	9.0	20.0	73
.002	34	6.26	18.9	81
.001	31	3.19	18.3	95
.0005	30	1.97	18.8	85

[a] From XII using an "average" $[Fe^{++}]$ of 50 μM.

The Influence of Glycine Concentration.—The mechanism as proposed in 1 through 6 provides no explanation of the large influence of both glycine concentration and H^+ concentration upon values for both k_1 and the ratio k_6/k_5 and is thus obviously a simplification of a more complex mechanism. Figure 6 portrays the data used in the evaluation of k_1 at various glycine concentrations at constant pH. The linearity of the data for each of these concentrations indicated the validity of the general mechanism over this concentration range.

Column 2 in Table III lists the values in Fig. 6 after a correction for small temperature differences.

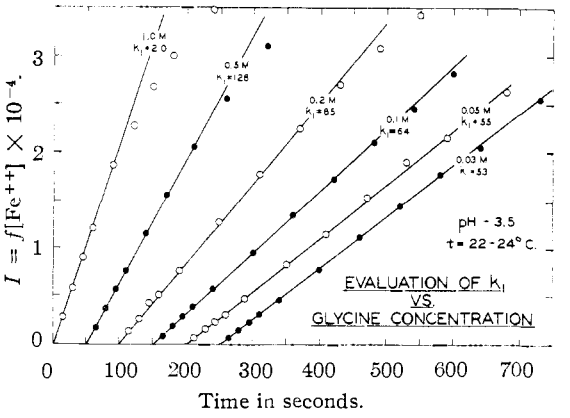

Fig. 6.—The evaluation of the reaction rate constant k_1 for various glycine concentrations by numerical integrations of eq. X. Successive groups of data have been displaced by successive multiples of 50 seconds along the abscissa.

In column 3 are listed approximate values of k_1 based upon only the first three measurements of Fe^{++} and H_2O_2 concentrations. The agreement between these two sets of values eliminates the possibility that the dependence shown in columns 1 and 2 is a virtual one arising from a flaw in the proposed mechanism and the associated formulas.

TABLE III

INFLUENCE OF GLYCINE CONCENTRATION UPON k_1 AT 22°

Glycine, moles/l.	k_1, l. mole^{-1} sec.$^{-1}$ By eq. X	Approximate[a]
0.03	53	47
.05	55	47
.10	64	57
.20	81	81
.50	125	121
1.00	205	187

[a] $k_1 \cong \frac{1/2\,\Delta\,[Fe^{++}]}{[Fe^{++}][H_2O_2]\Delta t}$.

When the data in columns 1 and 2 are plotted a straight line is obtained which has an intercept of 49 l. mole^{-1} sec.$^{-1}$ at zero glycine concentration and a slope of 156 l.^{2} moles^{-2} sec.$^{-1}$. The intercept value agrees well with a measured value of 50 in dilute H_2SO_4 at pH 3.5 obtained in this Laboratory and a value of 48.6 calculated from the data of Barb, *et al.*,[3] for $HClO_4$ solutions. The value is slightly less but still in reasonable agreement with the value of 56.8 calculated from the data of Rigg, *et al.*,[15] and the value of 57.3 calculated from the data of Baxendale,[16] *et al.*, for H_2SO_4 solutions.

The fact that Barb, *et al.*, found k_1 independent of $HClO_4$ concentrations from 0.5 N to $\approx$0.002 N (pH 2.65), that Rigg, *et al.*, found k_1 independent of H_2SO_4 concentrations from 0.8 to 0.05 N and that both these values are in reasonable agreement with the values of Baxendale, *et al.*, in 1 N H_2SO_4, and of this Laboratory in $\approx$0.0003 N H_2SO_4 (pH 3.5) indicates that this dependence of k_1 upon glycine concentration is not a simple dependence upon ionic strength. We have interpreted this as the formation of a ferrous glycine complex C by eq. 7, which then reacts with H_2O_2 *via* 8.

$$Fe^{++} + G\ (\text{glycine}) \rightleftarrows C \qquad (7)$$

$$K_7 = \frac{[C]}{[Fe_t^{++} - C][G]} \qquad (XIII)$$

Fe_t^{++} = total ferrous concentration.

$$C + H_2O_2 \longrightarrow OH + Fe^{+++} + \ldots \qquad k_8 \quad (8)$$

The observed k_1 listed in Table III is then given by XIV where k_1' is reaction rate constant for the Fe^{++} not complexed by 7. XV follows from XIII and XIV.

$$k_1 = \frac{[Fe_t^{++} - C]}{[Fe_t^{++}]}\,k_1' + \frac{[C]}{[Fe_t^{++}]}\,k_8 \qquad (XIV)$$

$$k_1 = k_1' + \frac{[Fe_t^{++} - C]}{[Fe_t^{++}]}\,K_7(k_8 - k_1)\,[G] \qquad (XV)$$

XV will approach the form of the straight line exhibited by the data in Table III if $C << Fe_t^{++}$, *e.g.*, if K_7 is small and k_8 large. Taking the intercept value of 49 l. mole^{-1} sec.$^{-1}$ as correct for k_1' and assuming a reasonable value of 10% for the probable error in the k_1 measured in 1 M glycine it can be calculated that $K_7 \leqq 0.1$ and $k_8 \geqq 1500$ l. mole^{-1} sec.$^{-1}$. Since both these values are reasonable the proposed model can be considered in agreement with the data.

(15) T. Rigg, W. Taylor and J. Weiss, *J. Chem. Phys.*, **22**, 575 (1954).

(16) J. H. Baxendale, M. G. Evans and G. S. Park, *Trans. Faraday Soc.*, **42**, 155 (1946).

Such a dependence of k_1 is not unique to glycine. Very rough measurements in sodium acetate from 0.02 to 1 M at pH 3.5 exhibit the same type dependence with a slope of approximately 100 l.^{2} mole^{-2} sec.$^{-1}$. Similar measurements in phosphate buffer at this pH show a non-linear dependence with an initial slope of the order of 4000 and a slope of approximately 1000 at 1 M. However, k_1 is apparently independent of $(NH_4)_2SO_4$ concentration from 0.02 to 1.0 M.

The variation of k_6/k_5 with glycine concentration at constant pH as shown in Fig. 7 can be described in terms of the formation of a ferric–glycine complex, C', by 9, if it is assumed that the complex C' does not react with the peroxy radical. With this model the observed values of k_6/k_5 shown in Fig. 7 are given by XVII where D is the value of the ratio

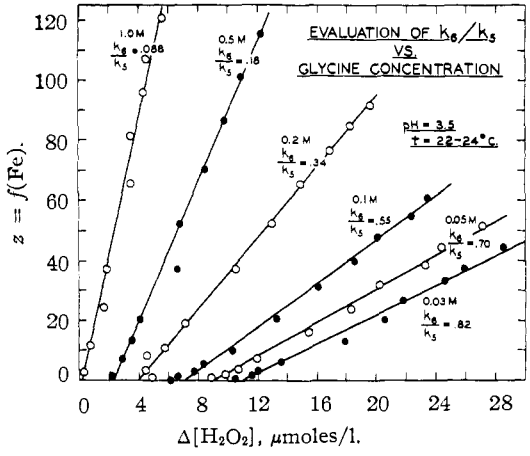

Fig. 7.—The evaluation of the ratio of the reaction rate constants k_5 and k_6 at various glycine concentrations by eq. III. Successive groups of data have been displaced by successive multiples of 2 μmole/l. along the abscissa.

at zero glycine concentration. XVIII which follows from XVI and XVII predicts that the reciprocal of these values for k_6/k_5 should fall upon a straight line with an intercept of $1/D$ and a slope of K_9/D. A plot of the data shows that, with the exception of the 1 M value which is 24% high the values do fall quite accurately upon such a straight line with a value of 1.1 for D and 10.2 l. mole^{-1} for K_9. Independent experiments now in progress confirm the existence of the ferric–glycine complex and indicate a value for K_9 of this magnitude.

$$Fe^{+++} + G \longrightarrow C' \qquad (9)$$

$$K_9 = \frac{[C']}{[Fe_t^{+++} - C'][G]} \qquad (XVI)$$

$$\frac{k_6}{k_5} = \frac{[Fe^{+++} - C']}{[Fe_t^{+++}]}\,D \qquad (XVII)$$

$$\frac{k_5}{k_6} = \frac{1}{D} + \frac{K_9}{D}\,[G] \qquad (XVIII)$$

The Influence of H+ Concentration.—The influence of the H^+ concentration upon the ratio k_6/k_5 is shown in Fig. 8. Again the linearity of the observed data support the validity of the general mechanism. These data can be described in terms of the hydrolysis of the ferric ion, 10; and the ionization of the glycine–peroxy radical, 11, if the overall eq. 6 is now replaced by 12. Formula XXI

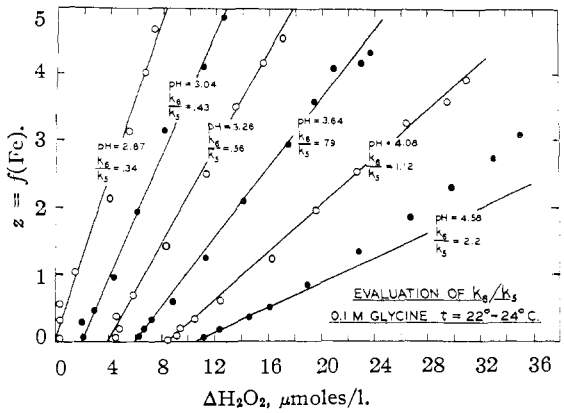

Fig. 8.—The evaluation of the ratio of the reaction rate constants k_5 and k_6 by eq. III. Successive groups of data have been displaced by successive multiples of 2 μmole/l. along the abscissa.

which follows from 5, 12, XIX and XX, predicts that a plot of k_5/k_6 should be a straight line function of the H^+ concentration. When such a plot is made the values for H^+ concentrations between 2.2×10^{-4} and 13.7×10^{-4} N fall quite accurately upon a straight line with a slope and intercept corresponding to values of 6×10^{-4} and 6.5×10^{-4}, respectively, for K_{10} and K_{11}. In view of the

$$Fe^{+++} + H_2O \rightleftharpoons FeOH^{++} + H^+ \quad (10)$$

$$K_{10} = \frac{[FeOH^{++}][H^+]}{[Fe^{+++} - FeOH^{++}]} \quad (XIX)$$

$$NH_2CH(O_2)COOH \rightleftharpoons (NH_2C(O_2)COOH)^- + H^+ \quad (11)$$

$$K_{11} = \frac{[H^+][(NH_2C(O_2)COOH)^-]}{[NH_2CH(O_2)COOH]} \quad (XX)$$

$$(NH_2C(O_2)COOH)^- + Fe^{+++} \longrightarrow (NH_2C(O_2)COOH) + Fe^{++} \quad (12)$$

$$(NH_2C(O_2)COOH) \longrightarrow NH{=}CHCOOH + O_2 \quad (12a)$$

$$NH{=}CHCOOH + H_2O \longrightarrow NH_3 + HCOCOOH \quad (12b)$$

$$k_5/k_6 = \frac{K_{10}}{K_{11}} + \frac{1}{K_{11}}[H^-] \quad (XXI)$$

marked dependence of ionic equilibrium constants upon the ionic strength of the solution, and in many cases the specific composition of the solution, the observed value for K_{10} is considered in satisfactory agreement with other evaluations of this constant. Rabinowitch and Stockmayer[17] have estimated, from the values 3.5×10^{-3} and 6×10^{-3} given, respectively, by Lamb and Jacques[18] and Bray and Hershey[19] for K^0_{10} at zero ionic strength, that K_{10} is of the order of 7 or 12×10^{-4} for ionic strengths of the order of 0.2.

The observed ratios of k_5/k_6 at H^+ concentrations of 2.6×10^{-5} and 8.3×10^{-5} are much less than those predicted by this model. No adequate explanation has been found for this departure. The data suggest the inhibition of reaction 5 by the removal of Fe^{++} cation by some reaction favored by a low H^+ concentration and the above model was expanded to allow for the removal of Fe^{++} cation by hydrolysis. Formulas were developed which quite adequately described the experimental data. However, the value of 2×10^{-5} for the ferrous hydrolysis constant required by this model for agreement with the data is so inconsistent with the values of 1.2×10^{-6}, 1.2×10^{-8}, and 5×10^{-9} reported by Lindstrand,[20] Gayer and Wootner,[21] and Leussing and Kolthoff,[22] respectively, that it seems this model must be discarded.

The influence of the H^+ concentration upon k_1 is shown in Fig. 9 and Table IV. The data in column

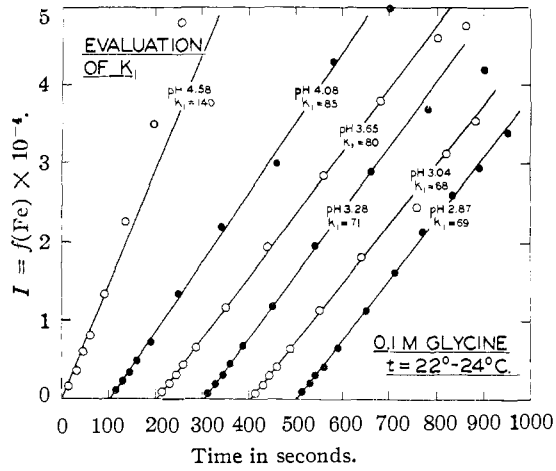

Fig. 9.—The evaluation of the reaction rate constant k_1 at various pH levels by numerical integrations of X. Successive groups of data have been displaced by successive multiples of 100 seconds along the abscissa.

2 are the data in Fig. 9 corrected for small temperature differences with the temperature coefficient presented later. The data in column 3 are approximate values of k_1 based upon data for the first few measurements of the Fe^{++} and H_2O_2 concentrations. The dependence of k_1 upon pH is not due to the presence of glycine. The same general type dependence in this pH range is observed in dilute unbuffered H_2SO_4, acetate buffer and phosphate buffer.

TABLE IV

EFFECT OF H^+ CONCENTRATION UPON k_1 IN 0.1 M GLYCINE AT 22°

H^+, moles l. $\times 10^5$	k_1, l. mole^{-1} sec.$^{-1}$ By eq. X	Approximate[a]
0.5		240
1.0		160
2.6	140	100
8.3	85	81
23	80	81
53	71	72
91	68	60
135	69	65

[a] $k_1 \cong \frac{1/2\ \Delta\ [Fe^{++}]}{[Fe^{++}][H_2O_2]\ \Delta t}$.

No explanation is offered for the increase in k_1 with decreasing H^+ concentration. However, it is

(17) E. Rabinowitch and W. H. Stockmayer, THIS JOURNAL, **64**, 335 (1942).
(18) A. B. Lamb and A. G. Jacques, *ibid.*, **60**, 967, 1215 (1938).
(19) W. C. Bray and A. V. Hershey, *ibid.*, **56**, 1889 (1934).
(20) F. Lindstrand, *Svensk Kem. Tidskr.*, **56**, 282 (1944).
(21) K. H. Gayer and L. Wootner, THIS JOURNAL, **78**, 3944 (1956).
(22) D. L. Leussing and I. M. Kolthoff, *ibid.*, **75**, 2476 (1953).

interesting to note that the range of H^+ concentrations where there is a marked increase in k_1 is the range where there is a marked decrease in the empirical values of k_5/k_6 from those predicted by the proposed model. One can hardly fail to speculate upon a common denominator in which the Fe^{++} cation apparently not available for reaction 5 is available for reaction with H_2O_2 in a reaction paralleling reaction 1. In fact the data in column 2 can be described adequately by assuming a reaction rate constant for this latter reaction and the discarded ferrous hydrolysis model with its hydrolysis constant of 2×10^{-5}.

The Influence of Temperature.—The effect of temperature upon k_1 in 0.1 M glycine at pH 3.5 is shown in Table V. The data may be described by the equation

$$k_1 = 7.08 \times 10^9 \exp(-10{,}800/RT)$$

The indicated activation energy of 10,800 cal./mole is in approximate agreement with the values of 10,100, 9,400 and 8,460 given, respectively, by Baxendale, *et al.*,[16] Barb, *et al.*,[3] and Rigg, *et al.*[15] The difference probably arises from both a difference in the activation energies of 1 and 8 and a temperature effect upon K_7.

TABLE V

EFFECT OF TEMPERATURE UPON k_1 AND k_6/k_5 IN 0.1 M GLYCINE AT pH 3.5

Temp., °C.	k_1	k_6/k_5
1.0	17.3	0.88
14.5	45	.68
23	78	.58
29	113	.53
36	144	.60

Discussion

Although this mechanism seems to be entirely consistent with the observed data, the marked dependence of the over-all reaction rate upon glycine concentration requires a careful consideration of the general mechanism proposed by Kolthoff and Medalia.[3] Working with a variety of substrates, these investigators measured the total ferrous oxidation and H_2O_2 consumption after the reaction was completed by the exhaustion of either the Fe^{++} or H_2O_2 but they made no kinetic measurements. $\Delta Fe^{++}/\Delta H_2O_2$ ratios greater than two often were observed and a chain reaction involving the ferrous ion and an organo-peroxy radical formed according to our eq. 4 was proposed as an explanation. The net result of this chain was the oxidation of several Fe^{++} ions for each OH radical produced by reaction 1. This model suggests that rate of H_2O_2 consumption should be unaffected by the organic substrate and that the rate of ferrous oxidation should increase with increasing substrate concentration. Our data are consistent with this last prediction but inconsistent with the first. In the case of 1 M glycine a simple analysis of the gross data showed that the measured H_2O_2 consumption for the first 60 seconds of the reaction was only one-half the value calculated using the measured Fe^{++} and H_2O_2 concentrations and a limiting value of 49 l. mole^{-1} sec.$^{-1}$ for k_1. Hence, we conclude that the chain reaction is not an explanation for the glycine data. It is interesting to note that the mechanism proposed here, if general, is an equally plausible explanation for the stoichiometric data obtained by these authors. Kinetic measurements are now in progress to provide data upon which a selection may be made.

A correlation with the works of Merz and Waters[7] is made difficult by the fact that Fe^{++} and H_2O_2 concentrations were used which in many cases must have completely exhausted the dissolved oxygen in the system. Hence, since the observed data were probably the sum of both an aerobic and an anaerobic mechanism, any attempt at a correlation should be deferred until the kinetics of anaerobic systems have been studied.

It should be pointed out that the explanation offered here for the influence of H^+ concentration upon this mechanism exactly parallels the explanation offered by Barb, *et al.*,[3] for a similar effect observed in a study of Fenton's reaction where a large excess concentration of H_2O_2 served as a substrate. They postulated the ionization of the simpler hydrogen peroxy radical and the hydrolysis of the Fe^{+++}. If this mechanism should be general, it may well offer a method for at least an approximate evaluation of the ionization constant for a host of organo-peroxy radicals. Although it was not specifically suggested in their mechanism, it follows from their equation—$Fe^{++} + HO_2 \rightarrow Fe^{+++} + HO_2^-$—that they were, in an acid solution, also postulating the regeneration of H_2O_2.

Summary

The kinetics and stoichiometry of the action of Fenton's reagent in aerobic aqueous solutions of glycine are presented and a mechanism is proposed to describe the observations. The pertinent features of the mechanism are: 1. Glycine is oxidized to HCOCOOH and NH_3. Other products found after extensive oxidation are believed to arise from secondary reactions. 2. The over-all rate of the reaction is controlled by the rate of production of the OH free radical which in turn depends upon both the glycine concentration and the H^+ concentration. 3. The course of the reaction is determined first by competition between Fe^{++} and glycine for the OH radical. The ratio of the reaction rate constants for the competing reactions has been evaluated as $k_{Fe}/k_{Gly} = 80$. 4. The OH radical reacts with glycine to form an organo-free radical which combines with dissolved oxygen to form an organo-peroxy radical. 5. The final path of the reaction is determined by competition between Fe^{++} for the organo-peroxy radical and Fe^{+++} for the anion of the organo-peroxy radical. The first path regenerates the H_2O_2 consumed in the original production of the OH radical and the second path regenerates the Fe^{++} oxidized in the OH production. 6. The mechanism when generalized is potentially an explanation for the large array of Fe^{++} oxidation/H_2O_2 consumption ratios which have been reported for other systems studied with Fenton's reagent.

BETHESDA, MD.