feces of two subjects. It is obvious that the vitamins in this component of the product were largely wasted and that the sphere could not exert desired effects *in vivo*. The results obtained do not support claims that the product provides improved nutritional benefits with less loss of vitamins, and are in agreement with similar findings obtained previously with other sustained release vitamin preparations (4, 5). Furthermore, the data again (4) emphasize that it is most difficult to formulate vitamin preparations in true sustained-release form.
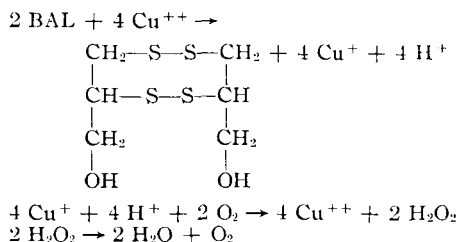
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Kinetics of Copper Catalyzed Oxidation of 2,3-Dimercapto-1-propanol by Molecular Oxygen

By EDWARD G. RIPPIE† and TAKERU HIGUCHI‡

The rate of disappearance of the sulfhydryl groups of 2,3-dimercapto-1-propanol (BAL) in aqueous solutions in the presence of molecular oxygen and catalytic quantities of copper has been shown to depend directly on hydroxyl ion and BAL concentrations and on oxygen pressure. The concentration of BAL decreases logarithmically following a short induction period. The dependency on copper concentration was found to be approximately linear at low concentrations but showed an apparent limiting zero-order dependency at high levels of the metal. These findings suggest that some mechanism other than that proposed by Barron, *et al.* (1), must be responsible for the overall reaction.

BARRON, *et al.* (1), studying the oxidation of (BAL) by dissolved molecular oxygen in aqueous solutions in the presence of copper, manometrically, have reported that BAL and and similar dithiols apparently do not undergo oxidation by free oxygen in the absence of certain metal ions and that any observed oxidation in the apparent absence of these catalysts is due to residual trace quantities of metals (probably copper). They suggest a mechanism involving three stoichiometric reactions

$$2 \text{ BAL} + 4 \text{ Cu}^{++} \rightarrow$$

$$\begin{array}{ll} CH_2\!-\!S\!-\!S\!-\!CH_2 & + 4 \text{ Cu}^+ + 4 \text{ H}^+ \\ | \qquad\qquad | \\ CH\!-\!S\!-\!S\!-\!CH \\ | \qquad\qquad | \\ CH_2 \qquad\quad CH_2 \\ | \qquad\qquad | \\ OH \qquad\quad OH \end{array}$$

$$4 \text{ Cu}^+ + 4 \text{ H}^+ + 2 \text{ O}_2 \rightarrow 4 \text{ Cu}^{++} + 2 \text{ H}_2O_2$$
$$2 \text{ H}_2O_2 \rightarrow 2 \text{ H}_2O + O_2$$

Every dithol molecule oxidized, according to this mechanism, involves reaction with copper. Present findings based on iodometric determination of residual sulfhydryl groups appear to be in partial conflict with these suggestions. A possible alternate route is presented.

EXPERIMENTAL

Reagents.—Reagent grade mono- and dipotassium phosphate; 0.1 *N* iodine solution; 0.1 *N* thiosulfate solution; oxygen U.S.P.; catalase (Worthington); water redistilled from all glass apparatus; 2,3-dimercapto-1-propanol containing not more than 0.93% 1,2,3-trimercaptopropane.

Apparatus.—A water-jacketed, glass reaction vessel was fitted with a water-sealed stirrer, manometer, and leveling bulb for maintenance of oxygen pressure, and a tube dipping below the level of the thiol solution for removal of samples at various time intervals. This apparatus was used in the experiments which were run with the oxygen pressure equal to atmospheric pressure.

A glass pressure vessel was used at elevated oxygen pressures. The bomb was fitted with a heavy rubber diaphragm through which samples could be withdrawn periodically by means of a hypodermic syringe. A Beckman model G pH meter equipped with a thermostated cell was used to determine the pH of reaction mixtures at the various temperatures. Titrations were carried out with a 2-ml. microburet with an immersion tip.

Procedure.—All studies were carried out in solutions thermostated to ±0.1°. The ionic strength was maintained constant at 0.5 by phosphate buffer to eliminate salt effects. Measured quantities of BAL were dissolved in the buffer solution containing the appropriate quantity of copper sulfate. The system was then flushed with oxygen, sealed, and stirred with the stirrer, or shaker in the case of the pressure bomb, with sufficient violence to assure saturation with respect to oxygen. Samples were withdrawn periodically for analysis.

Residual sulfhydryl content was determined iodometrically. An aliquot of the mixture was quenched by pipetting a sample of the reaction mixture into 0.1 N iodine solution, the excess iodine being back-titrated with 0.1 N thiosulfate. The insoluble disulfide present during the titration did not interfere with the end point, which was taken as the disappearance of the iodine color since starch indicator did not work well in the presence of oxidized BAL. A precision of ±1 μl. was obtained with a microburet fitted with an immersion tip.

Since in preliminary experiments it was found that hydrogen peroxide formed during the reaction interfered with the iodine titration, catalase was added (200 units per ml.) to all subsequent reaction solutions. Keilin (2) has shown that although both blood and liver catalase preparations increase the rate of thiol oxidation, the concentration necessary is 10⁵ times higher than that required for the catalytic decomposition of hydrogen peroxide at an approximately similar rate. While Webb (3) found up to 90% inhibition of catalase by BAL, the amount remaining was presumed to be sufficient to decompose any peroxide formed. The addition of catalase did not appreciably alter the chemical kinetics of BAL oxidation but permitted us to follow the reaction closer to completion without interference by peroxide.

RESULTS AND DISCUSSION

Order of the Reaction with Respect to BAL.—The rate of disappearance of sulfhydryl groups in the presence of copper from stirred aqueous solutions of BAL under constant oxygen tension appeared to be complex functions of time as is evident from data presented in Fig. 1. There seemed to be an initial autocatalytic lag phase followed by an apparently first-order disappearance of SH groups from the solution. The exact form and extent of the induction phase could not be established easily since the
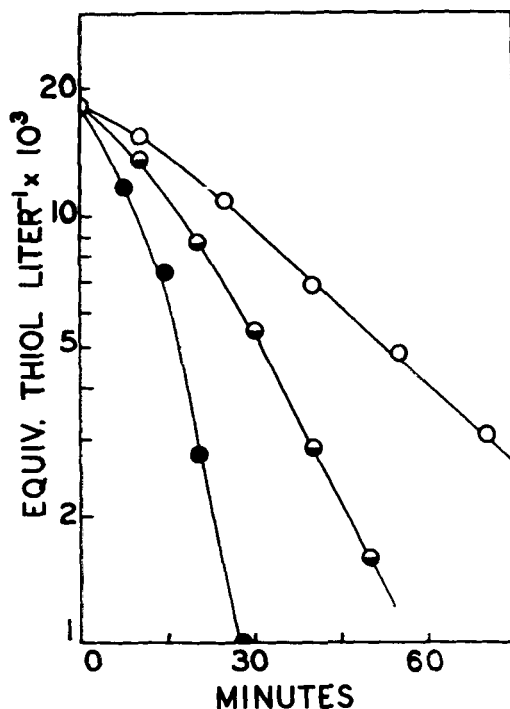

Fig. 1.—Semilog plots of thiol titer *vs.* time showing lag time. O, 15°; ◑, 25°; ●, 35°; 2×10^{-5} M Cu(II); 760 mm. O_2 pressure; pH = 6.66.

experimental procedure employed did not permit determinations of an unambiguous zero time for the reaction. The induction period was also noted for systems followed manometrically, although Barron, *et al.* (1), do not report this.

The first-order phase of the observed reaction rate seemed to be truly first order. The slopes of the straight line portions such as are shown in Fig. 1 were found to be independent of initial BAL concentration within experimental error over a wide range of concentrations. This relationship was also found to be valid at different oxygen tensions as is evident from Fig. 2. For these runs, readings were taken after the initial phase so that only that part of the reaction which follows the first-order dependency is shown.

Effect of Oxygen Pressure.—The rate of the copper catalyzed oxidation of BAL appeared to vary directly with oxygen tension. This is evident in Fig. 3 where the first-order reaction rate constants calculated from the straight line portion from plots such as shown in Fig. 2 were plotted against pressure to yield a straight line passing through the origin. Numerical values of the rate constants and some of the experimental conditions are given in Table I.

For these runs, solutions of BAL were shaken violently in a glass reaction bomb under various oxygen pressures; aliquots were withdrawn and titrated periodically. Drop in oxygen pressure, due to consumption by the reaction and withdrawal of aliquots, introduced an estimated error of 2%. Pressures of oxygen were assumed to be partial pressures since the vapor pressure of water was negligible in this case.
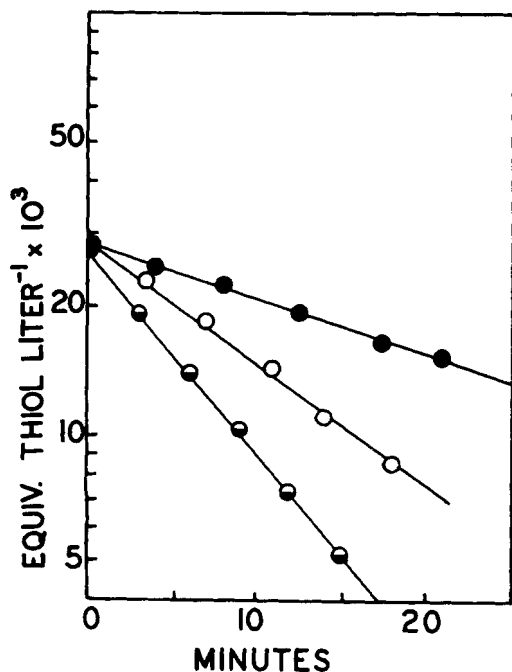
Fig. 2.—Plot showing first-order dependence on BAL after lag phase at several oxygen pressures; time "O" was taken arbitrarily at approximately 5 minutes following reaction initiation. ●, 760; ○, 1580; ◓, 2400 mm. O₂ pressure; 24.5°; pH = 6.66; 1 × 10⁻⁵ M Cu(II).

TABLE I.—EXPERIMENTAL RATES AT VARIOUS COPPER CONCENTRATIONS AND OXYGEN PRESSURES AT pH 6.66

Tempera-ture, °C.	Cu(II) Concn., M	O₂ Pressure, mm. Hg	$k \times 10^{-2}$ min.$^{-1}$
20.0	2×10^{-6}	760	1.07
20.0	5×10^{-6}	760	1.61
20.0	1×10^{-5}	760	2.65
20.0	2×10^{-5}	760	3.96
20.0	4×10^{-5}	760	5.21
24.5	1×10^{-5}	760	3.25
24.5	1×10^{-5}	1170	5.21
24.5	1×10^{-5}	1580	7.26
24.5	1×10^{-5}	1990	9.63
24.5	1×10^{-5}	2400	11.27

Effect of Hydrogen Ion Concentration and Temperature.—The effect of pH on the rate of BAL oxidation was determined at 15, 20, and 25°. Figure 4 shows the collected results of these determinations in which the logarithm of the rate in reciprocal minutes has been plotted against the pH determined at the individual temperatures. The lines, which have an average positive slope of 0.73, are essentially parallel.

Figure 5 shows the results of heat of activation determinations at three pH's. The average value of the heat of activation, ΔHa, as determined from the slopes, was 12.6 Kcal. per mole. No account was taken of the effect of temperature on oxygen solubility or on the degree of ionization of BAL
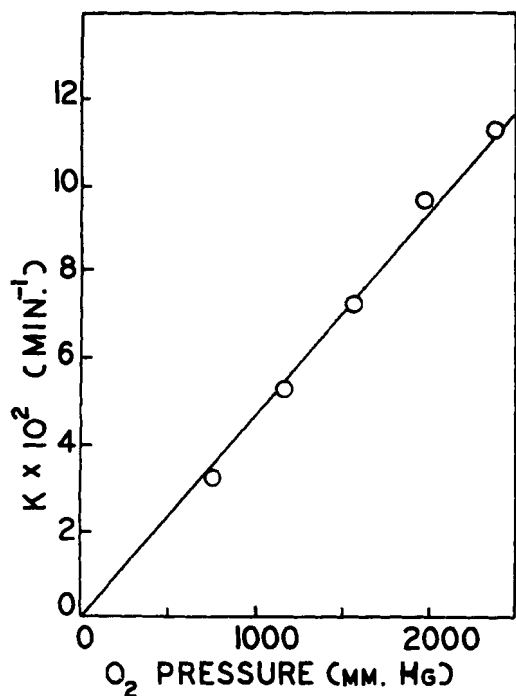

Fig. 3.—Plot showing first-order nature of BAL oxidation with respect to oxygen pressure in the presence of copper. Readings were taken at 24.5°; pH = 6.66; 1 × 10⁻⁵ M Cu(II).
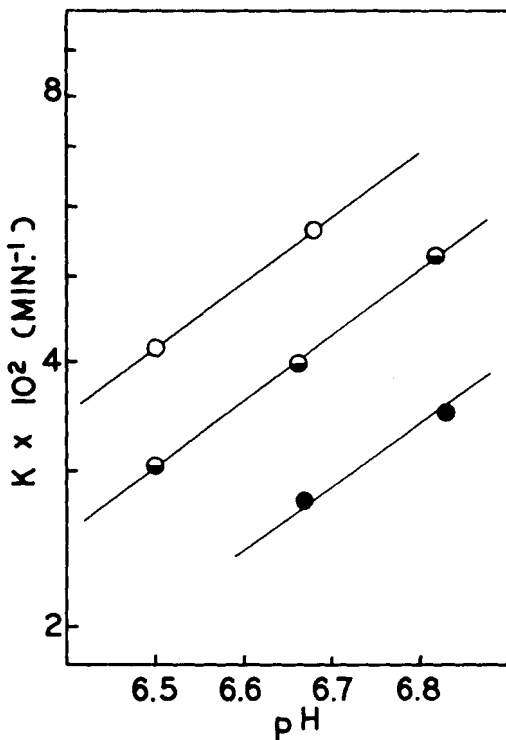

Fig. 4.—Semilog plots of first-order constants of BAL oxidation in reciprocal minutes against pH. The average slope of the lines is 0.73. Readings were taken at ○, 25°; ◓, 20°; ●, 15°; 1 atm. O₂ pressure; 2 × 10⁻⁴ M Cu(II).
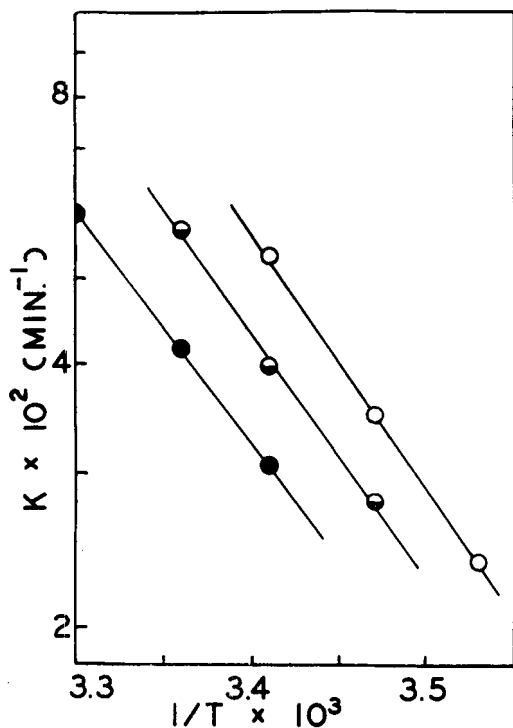
Fig. 5.—Typical Arrhenius plots obtained at different pH values. Solutions contained 2×10^{-5} M Cu(II); runs were made at O, pH = 6.83, ◑, pH = 6.66, ●, pH = 6.50, under 1 atm. O₂.

Fig. 6.—Plot showing linear copper dependency of first-order reaction rate constants at low copper concentration. Runs were made at 20°, pH = 6.66, under 1 atm. O₂.

in this calculation; therefore, the ΔHa given is the apparent heat of activation. The subtraction of the heat of solution of oxygen in water, -3.6 Kcal. per mole, from the apparent ΔHa yields a corrected value of 16.2 Kcal. per mole for the process. The heat of ionization of BAL, however, was not known and no correction has been made for its presumably small effect. Results of these experiments are given in Table II.

TABLE II.—EXPERIMENTAL RATES AT VARIOUS TEMPERATURES AND pH VALUES[a]

Temperature, °C.	pH	Rate constant × 10², min.⁻¹
10	6.83	2.36
15	6.83	3.49
15	6.67	2.77
20	6.82	5.29
20	6.66	3.96
20	6.50	3.05
25	6.68	5.63
25	6.50	4.14
30	6.48	5.88

[a] Cu(II) = $2 \times 10^{-5} M$; O₂ pressure = 760 mm. Hg.

Effect of Copper Concentration on Reaction Rate.—Rate determinations made in the presence of varying copper concentrations yielded data shown plotted in Fig. 6, which indicate a nonlinear relationship between the two. The extrapolated value of the rate constant at zero copper concentration agreed with the experimentally determined
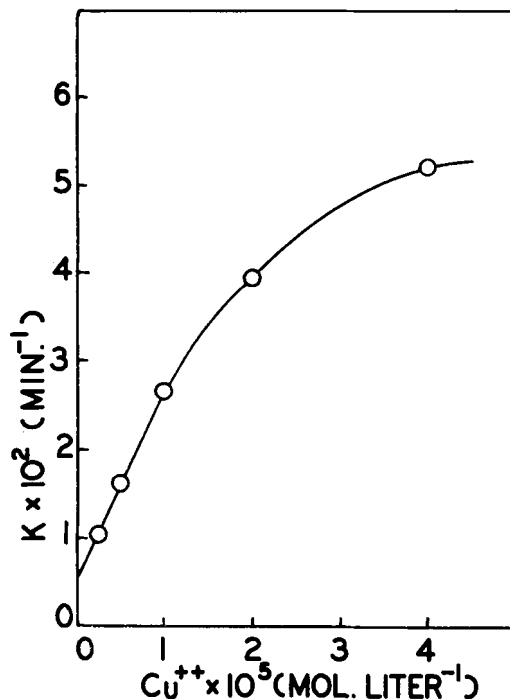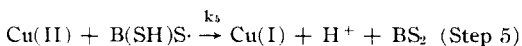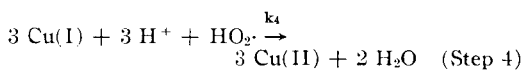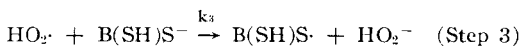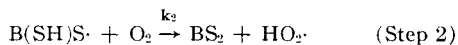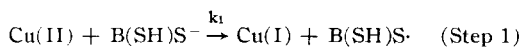
rate of oxidation when a copper-free solution of BAL was oxidized in the presence of 1×10^{-3} molar potassium cyanide. In Fig. 7, the reciprocal of the observed rate constant has been plotted against the reciprocal of the copper concentration. Extrapolation of the resulting straight line plot to infinite copper concentration indicates a limiting rate constant equal to 7.81×10^{-2} min.⁻¹.

The saturation effect has been previously noted in studies on the cysteine-cystine system by Elliott (4) who found that the rate of oxidation by molecular oxygen was proportional to cupric ion at low concentrations, after which the rate was found to approach a limiting value. No mention was made of a possible cause of this phenomenon.

Consideration of the Overall Copper Catalyzed Reaction.—No limiting oxidation rate at high copper concentration, as is actually observed, would be expected from a totally copper-mediated mechanism such as has been proposed (1), as any increase in copper concentration would accelerate the oxidation further. One might expect, moreover, a limiting rate with respect to BAL, especially since the overall rate is directly dependent on oxygen. At BAL concentrations substantially in excess of the copper concentration, a BAL-copper complex could be expected to limit the breakdown rate because its concentration would not change appreciably with a change in BAL concentration. It is also apparent that if oxidation of the dithiol was totally mediated by copper, the overall rate would be rate-limited by either the rate of reduction of Cu(II) or its regeneration. In the former case, the observed rate would be substantially independent of oxygen tension;

in the latter, the rate should be largely zero order with respect to BAL.

As a result of the present studies, the following alternate reactions are proposed as possible components of the overall reactions. We are at present unable to write the total mechanism because of the apparent great complexity of the system. These reactions are, however, suggested on the basis of the present studies. Since the exact forms of cupric and cuprous copper have not been established, copper is shown only as to its oxidation state in the following reactions

$$Cu(II) + B(SH)S^- \xrightarrow{k_1} Cu(I) + B(SH)S \cdot \quad \text{(Step 1)}$$

$$B(SH)S \cdot + O_2 \xrightarrow{k_2} BS_2 + HO_2 \cdot \quad \text{(Step 2)}$$

$$HO_2 \cdot + B(SH)S^- \xrightarrow{k_3} B(SH)S \cdot + HO_2^- \quad \text{(Step 3)}$$

$$3\ Cu(I) + 3\ H^+ + HO_2 \cdot \rightarrow$$
$$3\ Cu(II) + 2\ H_2O \quad \text{(Step 4)}$$

$$Cu(II) + B(SH)S \cdot \xrightarrow{k_5} Cu(I) + H^+ + BS_2 \quad \text{(Step 5)}$$

If these reactions are considered to be representative of the principal reactions occurring after lapse of the lag time, several observations can be rationalized.

It can be seen that, when the copper concentration is such that step 5 occurs at a rate still insignificant compared with step 2, the rate is apparently independent of copper concentration. This was the case experimentally. Nord (5) in his studies on the autoxidation of cuprous chloride, reported rapid air oxidation of the cuprous ion at hydrogen ion concentrations of 0.1 M and above which, if extrapolated to neutrality, would predict extremely slow reaction rates. He also reported a pH-independent oxidation of cuprous ion at hydrogen ion concentrations below 0.1 M. If this rather slow reaction were assumed to occur at neutrality and if cupric ion were the only oxidant of BAL, it would also fail to account for the rapid oxidation of the sulfhydryl compound noted in the present work.

The illustrated partial mechanism agrees with the experimentally observed first-order dependency on BAL. However, a discrepancy may be noted between the expected and observed slopes when the overall rate constants are plotted *vs.* pH. If the dissociation of the B(SH)S· species to BS_2^- is necessary before the reaction with cupric ion, the reaction 5 will be a function of the pH and result in a negative deviation from slope 1.0, as observed. Hydrogen peroxide as such was not thought to contribute significantly to the reaction rate since no detectable
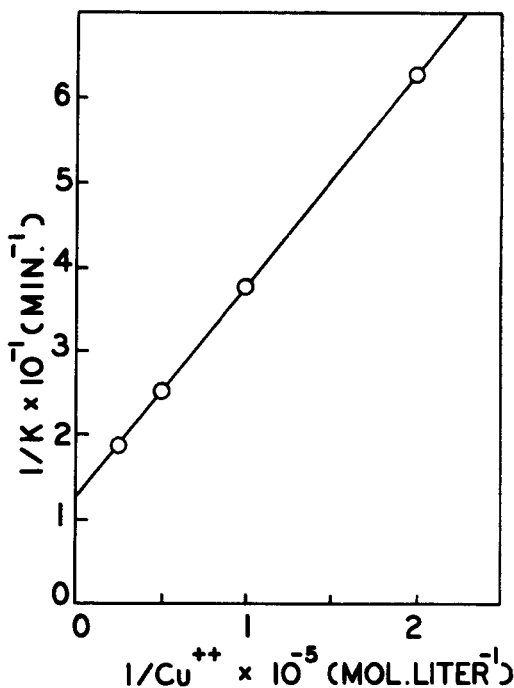

Fig. 7.—Plot of reciprocal first-order rate constant against reciprocal copper concentration, indicating limiting rate constant of 7.81×10^{-2} (min.$^{-1}$). Runs were made at 20°, pH = 6.66, under 1 atm. O_2.

change in the overall rate constant was found upon addition of catalase to the system.

Although this mechanism appears to be consistent with the experimental evidence, it is not necessarily unique since alternate mechanisms may be equally successful in explaining the experimental findings. Moreover, the chemical equations suggested here should not be considered as the only ones occurring. There is a possibility that reactions, kinetically indistinguishable from those suggested, may take part in the oxidative breakdown.

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