

of the National Formulary. Comparative quantitative analyses with thioacetamide and hydrogen sulfide are also planned for chemicals of both the United States Pharmacopeia and the National Formulary where hydrogen sulfide is directed as the quantitative precipitating reagent.

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Kinetics of Reaction between Molecular Oxygen and 2,3-Dimercapto-1-propanol in Aqueous Solution

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Autoxidation of BAL is shown to take place in the absence of metallic catalysts. Although the iodometric sulphydryl concentration in this system was found to decrease linearly with time, the reaction was found to approach true zero order only at higher initial BAL concentration. The observed rate of oxidation was found to decrease with increased hydrogen ion concentration and to be of a mixed order with respect to oxygen tension.

BARRON, *et al.* (1), have suggested that the observed reaction in aqueous solution between 2,3-dimercapto-1-propanol, BAL, and molecular oxygen in absence of added catalyst could be entirely attributed to trace quantities of catalytic copper. In this communication, experimental evidence which supports the existence of the uncatalyzed reaction and kinetic dependencies of this oxidative reaction on pH, oxygen tension, and initial substrate concentration are presented.

EXPERIMENTAL

Reagents and apparatus employed in the work were the same as those reported in previous work on the copper catalyzed oxidation of BAL (2). The procedure used was also essentially the same except that potassium cyanide was added to all reaction solutions at a concentration of $1 \times 10^{-3}M$. While catalase is known to be inhibited by cyanide (3), sufficient catalase was found to remain to destroy satisfactorily any peroxide which may have been formed. The reacting systems were sufficiently stirred (2) so that the solutions were always kept in equilibrium with the gas phase.

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RESULTS AND DISCUSSION

Evidence for Uncatalyzed Reaction.—There appears to be ample evidence which points to reaction in the absence of copper between molecular oxygen and BAL in aqueous solution. As has already been shown (2), significant copper concentration, *ca.* $2 \times 10^{-6}M$, is apparently necessary to account for the observed rate. If such high concentrations of the metal were introduced by chance contamination, it would be expected that variation in sources of buffer salts, water, and substrate would produce significant changes in the observed rates of oxidation. This was not the case. Quite reproducible rates were observed for different lots of buffer salts, BAL, and water.

Further evidence supporting the existence of uncatalyzed reaction is presented in Fig. 1. These plots show the residual sulphydryl concentration of aqueous solutions of BAL as a function of time when exposed to oxygen at 1 atm. The uppermost curve shows the kinetic dependency for a system free of copper and cyanide as far as was possible experimentally. The second line shows essentially the same system but with $1 \times 10^{-3}M$ potassium cyanide added. It is evident that these two functions are quite similar, both qualitatively and quantitatively. The third and fourth curves show the effects of addition of copper, 2×10^{-6} and $5 \times 10^{-6}M$, respectively, on the initial system. The kinetic behavior of these last two runs is obviously quite different from those of the preceding two. Although the data are not shown, addition of $1 \times 10^{-3}M$ cyanide to the latter two systems yielded rate curves identical with the first two.

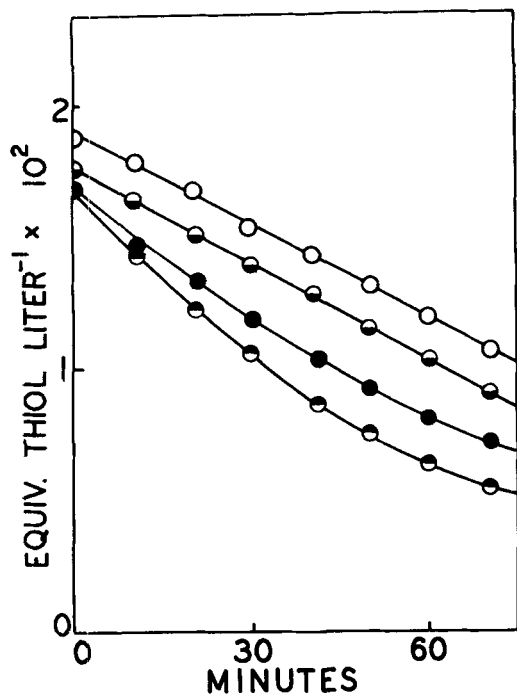


Fig. 1.—Plots contrasting BAL dependency of the oxidation with and without copper. O, essentially copper free; ●, previous system plus 0.001 *M* cyanide; ●, initial system plus 2×10^{-6} *M* copper; ●, initial system plus 5×10^{-6} *M* copper; temp., 20°; pH, 6.66; 0.25 *M* phosphate buffer.

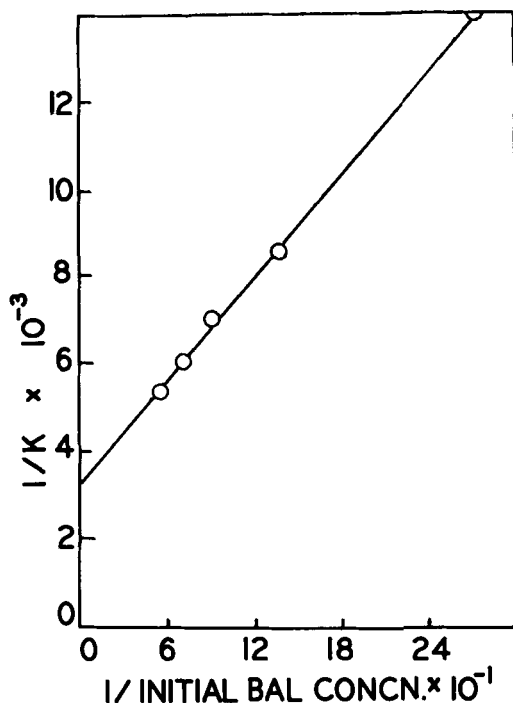


Fig. 2.—Reciprocal zero-order rate constants plotted against the reciprocal initial BAL concentration. The extrapolated value of the limiting rate is 3.06×10^{-4} equiv. per L. per min.; temp., 20°; pH, 6.66; 0.25 *M* phosphate buffer; 1 atm. oxygen.

These facts strongly suggest that BAL can undergo oxidation by molecular oxygen in the absence of copper or any other metallic catalyst. Any catalytic contribution from trace copper appears to be removed effectively by addition of cyanide. Although the part played by the uncatalyzed reaction would be relatively small in the presence of certain metallic ions, it would appear that for simple aqueous systems this would be the important kinetic route of oxidation of the sulfhydryl compound.

Order of the Reaction with Respect to BAL.—The behavior of BAL concentration with respect to time as shown in the upper two plots of Fig. 1 is typical of the uncatalyzed reaction. Essentially straight-line decrease is experienced although the absence of a short initial lag time could not be unequivocally established since readings were difficult to obtain during the first few minutes. Determinations of apparent zero-order rate constants were made over a temperature range of 0 to 24.5° at pH values from 6.50 to 7.14. Individual runs were made with initial molar BAL concentrations of 0.0037, 0.0074, 0.0111, 0.0148, and 0.0185. The apparent zero-order disappearance which was observed through approximately 75 per cent of the reaction suggests a rather complex dependency on the dithiol.

Although individual runs followed apparent zero-order kinetics, marked differences in the rates were found when varying initial concentrations were employed. When the reciprocals of these apparent zero-order rate constants were plotted against the reciprocals of the initial BAL concentrations, a

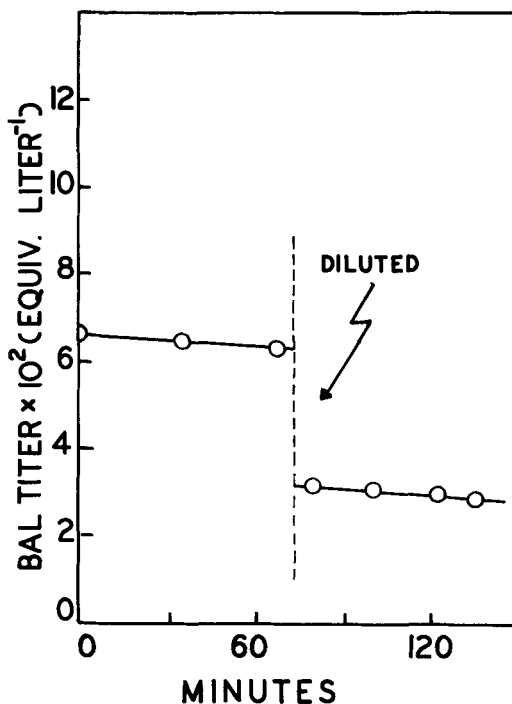


Fig. 3.—Plot of BAL concentration against time showing the effect of dilution of the reaction mixture at a time indicated by the dotted line. Temperature, 0°; 2 atm. oxygen; pH, 6.66; 0.25 *M* phosphate buffer.

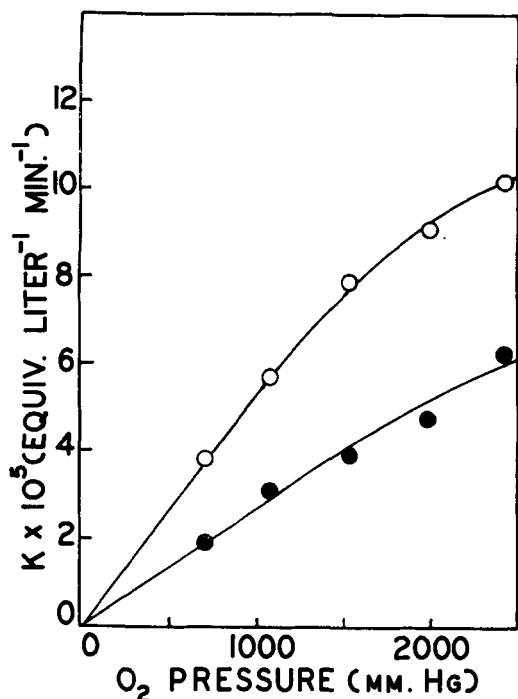


Fig. 4.—Plots of zero-order rate constants of BAL oxidation against oxygen pressure. Initial BAL concentrations: ○, 0.0370; ●, 0.0185; temp., 0°; pH, 6.66; 0.25 *M* phosphate buffer.

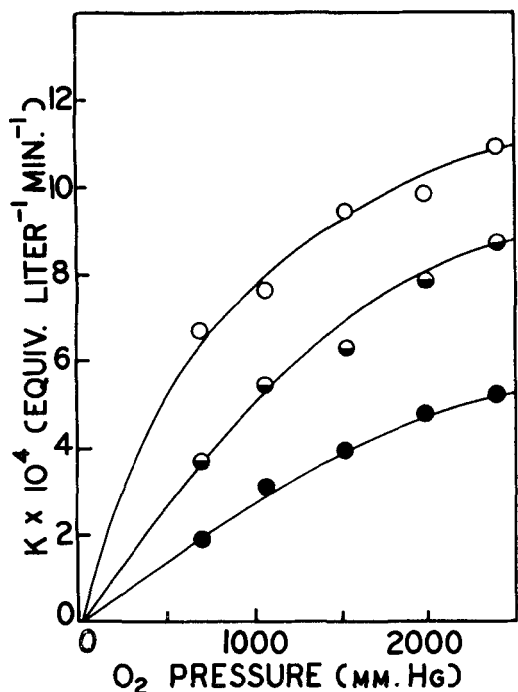


Fig. 5.—Plots of zero-order rate constants of BAL oxidation against oxygen pressure. Initial BAL concentrations: ○, 0.0370; ●, 0.0185; ●, 0.0093; temp., 24.5°; pH, 6.66; 0.25 *M* phosphate buffer.

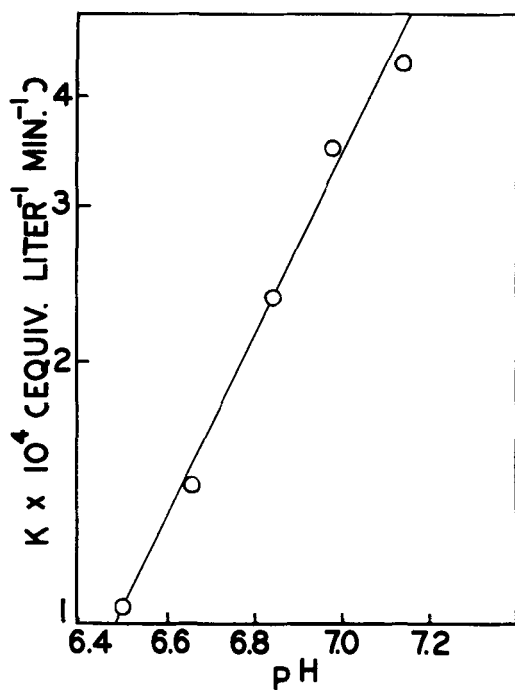


Fig. 6.—Semilog plot of zero-order rate constant against pH determined at 20°. Slope of the line is 1.0; 1 atm. oxygen; 0.25 *M* phosphate buffer.

straight line plot was obtained, as shown in Fig. 2. The intersection of this line with the vertical axis above the origin appears to indicate a finite limiting rate of 3.06×10^{-4} equiv. per L. per min. at theoretically infinite BAL concentration.

Runs were made in which the reaction was allowed to proceed for several minutes, after which the reaction mixture was diluted with an equal volume of solvent solution. A typical run plotted in Fig. 3 shows a change in oxidation rate upon dilution. The rate of oxidation after dilution corresponds to the expected rate, had the reaction been started at half the initial BAL concentration. These observations serve to show that the reaction is truly zero order with respect to BAL only at very high concentrations. Apparent zero-order kinetic behavior at lower concentrations suggests that a rather complex situation exists.

Oxygen Dependency.—Determination of oxygen dependency was carried out at a pH of 6.66, showing oxidation rate as a function of oxygen tension at 0 and 24.5°. Oxygen pressures ranged from 1 to 3 atm. and rate constants are given for initial BAL concentrations of from 0.0093 to 0.0370 *M*. The results of these determinations are plotted in Figs. 4 and 5. Although the oxidation rate appears to be first order with respect to oxygen tension at low oxygen pressures, the dependency at higher oxygen pressures appears to decrease. The rate, however, does not appear to approach a finite limiting value at high oxygen pressures.

Effect of Hydrogen Ion Concentration.—The effect of pH on the rate of BAL autoxidation was determined between pH 6.4 and 7.2 at an initial BAL concentration of 0.0111 *M*. In Fig. 6 the logarithm of the rate in equivalents per L. per min.

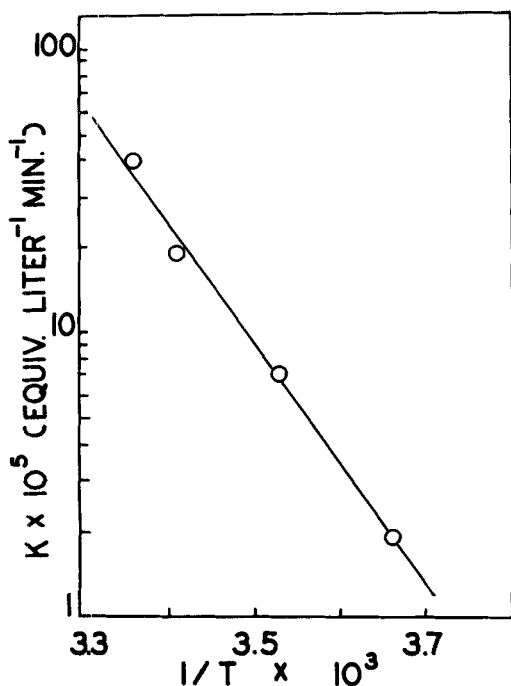


Fig. 7.—Arrhenius plot of zero-order rate constants against reciprocal absolute temperature. Determinations made at pH 6.66 and a pressure of 1 atm. oxygen; 0.25 *M* phosphate buffer.

has been plotted against the pH of solutions buffered by 0.25 *M* potassium phosphate. The isotherm has a positive slope of 1.0, showing more rapid oxidation at the higher pH.

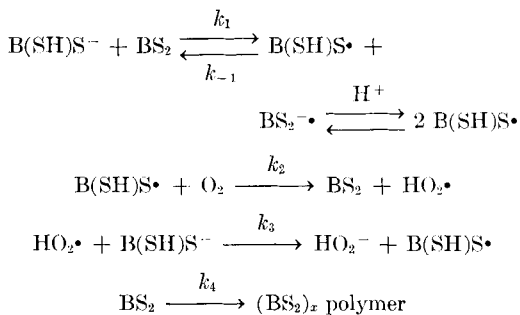
Temperature Dependence.—A semilog plot of apparent zero-order rate constants determined at 0, 10, 20, and 24.5° vs. reciprocal absolute temperature is shown in Fig. 7. The heat of activation, ΔH_a , as calculated from the slope, was 20.1 Kcal. per mole. Since the effect of temperature on oxygen solubility or on the degree of ionization of BAL was not taken into account, the heat of activation given is the apparent heat of activation. This can be corrected by subtracting the heat of solution of oxygen in water (−3.6 Kcal. per mole) to give a corrected ΔH_a of 23.7 Kcal. per mole. The heat of ionization of BAL, however, was not known and no correction has been made for its presumably small effect. The ΔH_a found here was considerably larger than that found for the copper catalyzed oxidation (16.2 Kcal. per mole).

Consideration of the Overall Reaction.—It is evident from the dependency on BAL and oxygen that the reaction mechanism encountered here is probably quite complex. It was suggested by Harrison (4) that the disulfide linkage is essential in the autoxidation of sulfhydryl compounds. Highly reactive cyclic disulfide may possibly play an

important role in BAL oxidation, acting as a source of the radical. The 23.7 Kcal. per mole activation energy found for the overall process is of the magnitude which might be expected for the homolytic cleavage of the disulfide bond (5) of the oxidized BAL monomer. The possibility of heterogeneous catalysis by the glass wall of the reaction vessel was investigated and ruled out.

The overall mechanism which fits the observed facts can be formulated in any number of ways. The apparent linear decrease of sulfhydryl concentration with time may be rationalized, for example, on the basis that a significant buildup of reactive cyclic disulfide may occur. Another rationalization may be based on the assumption that two BAL molecules linked by a disulfide bond may undergo autoxidation more readily than BAL itself. The obvious zero-order character of the reaction with respect to BAL at high BAL concentration appears to suggest, on the other hand, that the dithiol itself may act as an efficient chain stopper at higher concentrations. Such information as is presently available appears to be insufficient to resolve the question.

The following series of reactions may possibly play a part in the oxidative breakdown of BAL in the absence of copper



It offers an explanation of the dilution effect noted previously and the fact that the rate depends upon the initial BAL concentration. Other reactions no doubt take place (6) since this series of equations does not predict mixed-order kinetics with respect to oxygen at higher pressures. It should be emphasized that the chemical reactions suggested here are presented as those which may occur. Because of the apparent complexity of the findings, the overall process in reality may consist of many interacting pathways.

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