On the oxidation of 2,3-dimercaptopropanol

During an investigation of the role of the sulfhydryl groups of enteroviruses in the interaction with cells[1,2] it was found that British anti-Lewisite (BAL) inactivated the viruses rapidly and that an oxidized form of the compound was responsible for this inactivation[3]. In order to get a better understanding of this process the oxidation of dithiol compounds has been investigated. BARRON *et al.*[4] have previously suggested that the oxidation of BAL results in the formation of an insoluble disulfide comprising two molecules of BAL. These authors measured the oxidation of BAL by oxygen uptake. The present investigation concerns the oxidation of dithiol compounds as determined by the disappearance of sulfhydryl groups. Estimation of the infrared pattern and molecular weight as well as elementary analysis has also been carried out on the insoluble product formed after oxidation of BAL.

Determination of sulfhydryl groups of dithiols: The spectrophotometric technique of BOYER[5] was used. Commercial preparations of dithiols and a fixed concentration of *p*-chloromercuribenzoate were mixed in 0.02 M phosphate buffer (pH 7.0) and reacted for 2 min at 20° after which the absorption at 255 mμ was measured in a Beckman DU spectrophotometer. The concentration of *p*-chloromercuribenzoate was based on a molar extinction coefficient of $1.69 \cdot 10^4$ at 232 mμ. When dithiols in concentrations ranging from 1 to $6 \cdot 10^{-5}$ M were mixed with fixed amounts of *p*-chloromercuribenzoate the typical concentration dependence of increase in absorbancy at 255 mμ, as shown for other sulfhydryl compounds[5], was found. Fig. 1 shows the data for three different dithiols and the mean figures of 2.0 + 0.1, 1.9 ± 0.1 and 1.6 ± 0.15 was found for the number of -SH groups per mole of the thiols, 2,3-dimercaptopropanol, 1,3-dimercaptoheptane and 1,5-dimercaptoheptane, respectively. The low value for 1,5-dimercaptoheptane may be due to the low solubility of this compound in water. Recently HOCH AND VALLEE[6] have reported similar results with dithiols.

The oxidation of BAL: This reaction was performed by shaking 2 ml of dithiols at concentrations of $2 \cdot 10^{-4}$ M in 0.02 M phosphate buffer or other buffers as indicated

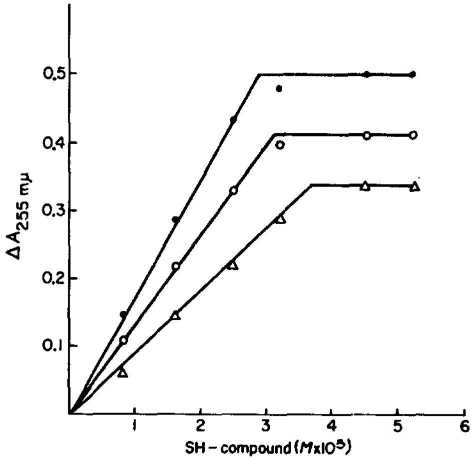

Fig. 1. Increase in absorbancy of *p*-chloromercuribenzoate at 255 mμ with different concentrations of dithiols. ●—●, 2,3-dimercaptopropanol; ○—○, 1,3-dimercaptopropane; △—△, 1,5-dimercaptoheptane.

in open test tubes. The oxidation of BAL, as followed by disappearance of sulfhydryl groups, was investigated at different pH values. As can be seen in Fig. 2 there was essentially no oxidation at pH 5.0 but the rate of oxidation increased with increasing pH up to pH 8.2. At higher pH the disappearance of sulfhydryl groups was even more rapid than at pH 8.2. The insoluble component which is formed during oxidation of BAL is rapidly formed only in the pH range of 6–9, but a much longer time is required for this component to form on either side of this pH range. A similar disappearance of sulfhydryl groups when 1,3-dimercaptopropane was oxidized by air was observed, but the rate of the reaction is substantially slower than that of BAL.

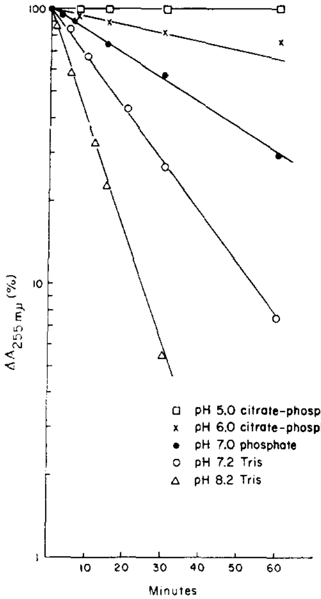

Fig. 2. Rate of disappearance of sulfhydryl groups upon oxidation of 2,3-dimercaptopropanol in air at different H^+ concentrations.

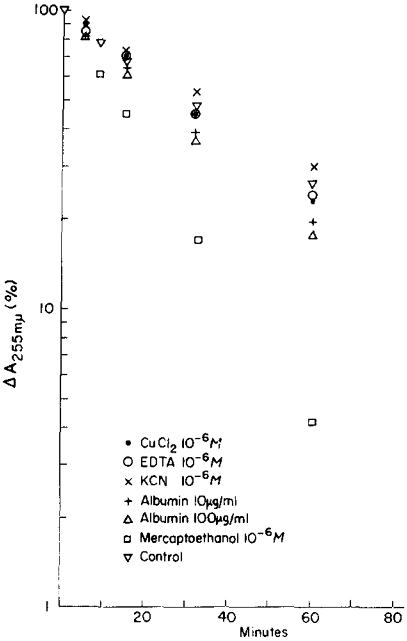

Fig. 3. The effect of different reagents on the disappearance of sulfhydryl groups from 2,3-dimercaptopropanol.

The effect of different reagents on the oxidation of BAL: Since BARRON *et al.*[1] showed that the uptake of oxygen by BAL was strongly catalyzed by Cu^{2+} and inhibited by cyanide, it was of interest to compare the rate of disappearance of sulfhydryl groups of BAL in the presence of various reagents affecting an oxidation process. Fig. 3 gives the rates observed in the presence of a number of compounds that might affect the rate of oxidation. Of the compounds tested, mercaptoethanol increased the oxidation rate slightly, but Cu^{2+}, chelators and albumin had no effect.

Characteristics of the oxidized product of BAL: To gain some information about the different steps in the oxidation cycle of BAL, the final product of BAL oxidation, a white insoluble compound, was investigated as to composition and molecular weight, and was also examined by infrared spectroscopy. Two analyses gave an average of 4.31% H, 28.8% C and 58.2% S, which would correspond to an approximate formula of $C_4H_xOS_3$. In comparison the reduced BAL has 6.49% H, 29.0% C and 51.6% S, and a formula of $C_3H_8OS_2$. The oxidation product of BAL was not soluble in water, 5 *N* HCl, or 5 *N* NaOH. It was soluble in pyridine and conc. $HClO_4$. Equilibrium centrifugation in an Analytical E centrifuge of the pyridine solution indicated a molecular weight between 550–600 which would correspond to a pentamer of the original BAL. The infrared spectrum in a Perkin Elmer Model 137 NaCl or Model 221 NaCl–Pl of this product showed no distinct features and was without a characteristic pattern suggesting a mixed "primary" or highly disordered "secondary" structure. The original BAL showed an infrared pattern which could be used to detect both –SH and S–S groups.

The oxidation of dithiols has not been extensively investigated. It has been hypothesized that during oxidation, BAL forms an insoluble disulfide comprising two molecules[4]. Oxidation of L-cysteinyl-L-cystine has been shown to yield either a cyclic or a dimeric form of the compound depending on H^+ concentration[7].

The present investigation furnished evidence that the insoluble product formed in the course of oxidation by BAL is not a dimeric disulfide, but rather a rigid polymer structure without a characteristic pattern in the infrared. Further, the rapid disappearance of sulfhydryl groups indicates that the –SH groups are involved in binding this polymer. When Couitauld models of BAL were made, it was found that a polymer structure with one S–S bond between the molecules was sterically possible. The increased rate of disappearance of sulfhydryl groups from BAL in the presence of mercaptoethanol may indicate that mixed disulfide formation is involved in the mechanism of oxidation of BAL. However, it is interesting that the rate of disappearance of sulfhydryl groups was not substantially affected by Cu^{2+}. The rate of oxygen uptake of BAL during oxidation is, however, increased in the presence of Cu^{2+} (ref. 4). This raises the possibility that the oxygen uptake and the disappearance of sulfhydryl groups measure different steps of BAL oxidation.

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