THE PRODUCTION OF HYDROGEN PEROXIDE BY HIGH OXYGEN PRESSURES*

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ABSTRACT

Hydrogen peroxide is formed in solutions of glutathione exposed to oxygen. This hydrogen peroxide or its precursors will decrease the viscosity of polymers like desoxyribonucleic acid and sodium alginate. Further knowledge of the mechanism of these chemical effects of oxygen might further the understanding of the biological effects of oxygen. This study deals with the rate of solution of oxygen and with the decomposition of hydrogen peroxide in chemical systems exposed to high oxygen pressures. At 6 atmospheres, the absorption coefficient for oxygen into water was about 1 cm./hour and at 143 atmospheres, it was about 2 cm./hour; the difference probably being due to the *modus operandi*. The addition of cobalt (II), manganese (II), nickel (II), or zinc ions in glutathione (GSH) solutions exposed to high oxygen pressure decreased the net formation of hydrogen peroxide and also the reduced glutathione remaining in the solution. Studies on hydrogen peroxide decomposition indicated that these ions act probably by accelerating the hydrogen perioxide oxidation of glutathione. The chelating agent, ethylenediaminetetraacetic acid disodium salt, inhibited the oxidation of GSH exposed to high oxygen pressure for 14 hours. However, indication that oxidation still occurred, though at a much slower rate, was found in experiments lasting 10 weeks. Thiourea decomposed hydrogen peroxide very rapidly. When GSH solutions were exposed to high oxygen pressure, there was oxidation of the GSH, which became relatively smaller with increasing concentrations of GSH.

When reduced glutathione solutions are exposed to high oxygen pressures, there is a significant production of hydrogen peroxide (1). If sodium desoxyribonucleic acid or sodium alginate were present in these solutions there was a decrease in viscosity which was attributed to reaction of these polymers with the hydrogen peroxide formed or its precursors (free radicals). These prooxidative effects of glutathione are inhibited by ethylenediaminetetraacetic acid disodium salt (EDTA) and thiourea. It has been found that agents

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such as glutathione, thiourea, cobalt (II) ions, and manganese (II) ions have protected against the biological effects of high oxygen pressures (51, 21, 19). In order to gain some insight into the mechanism by which these modifying agents act, an investigation was made to determine some of their chemical effects in *in vitro* systems exposed to oxygen. Part of these experiments have previously been reported (2).

Methods

A volume of 12 ml. of the solution studied was exposed to oxygen for a designated period of time in 15 ml. gauze-stoppered glass vials. For the experiments at 2 and 6 atmospheres, the chambers were flushed with oxygen and brought up to pressure in 1 minute. For the experiments at 130 or 143 atmospheres, the chambers were brought up to pressure within 5 seconds without flushing because of technical convenience. At these high pressures, flushing was considered an unnecessary complication. The rate of oxygen absorption into the liquid phase was assumed to be exponential (3–6) and was expressed as $\frac{dw}{dt} = K_L \frac{A}{V} (100 - w)$ in which w = per cent saturation, A = area, V = volume, t = time, and K_L = absorption coefficient. The time at which the oxygen reached 50 per cent of the saturation value was designated as the half-time. The relation between the half-time and absorption coefficient can be obtained by integration of the previous equation and by using the definition of the half-time. This relation is expressed as $T_{\frac{1}{2}} = \frac{0.693V}{K_L A}$. The saturation value was determined using Henry's law (1.26 mM O_2/atmosphere at room temperature) up to 70 atmospheres' pressure (7), but above this pressure, less oxygen becomes dissolved than is predicted by Henry's law (8). From the data of Zoss *et al.* (8), it was estimated that the saturation value of oxygen at 6 atmospheres was 7.56 mM O_2 and at 143 atmospheres it was 138 mM O_2. Throughout these experiments a volume of solution of 12 $cm.^3$ with a surface area of water-gas interphase of 2.66 $cm.^2$ was used. After exposure to the gas, samples of the liquid were collected into 1 ml. tuberculin syringes as rapidly as possible (within 20 seconds). The samples were then analyzed for oxygen gas in the Van Slyke apparatus.

Hydrogen peroxide was determined by the peroxytitanic acid test as previously described (1). The ratio of the normality of the hydrogen peroxide found to the oxygen was expressed as a per cent. (For hydrogen peroxide, one mole is equal to two equivalents; whereas for reduced glutathione, one mole is equal to one equivalent.)

An iodometric titration was utilized for the estimation of reduced glutathione (GSH) (Eastman Organic Chemicals or for some samples, Nutritional Biochemicals) (1). Glutathione concentrations were expressed as the per cent of reduced glutathione remaining unoxidized.

The complexing ability of ethylenediaminetetraacetic acid disodium salt (EDTA) (Eastman Organic Chemicals) was determined by the removal of calcium ions, and the complexing ability of diethyldithiocarbamic acid sodium salt (DEDTC) (Eastman Organic Chemicals) was determined qualitatively by the color of the copper DEDTC in chloroform (9).

For the experiments on the decomposition of hydrogen peroxide, peroxide without

any stabilizer was used (hydrogen peroxide (30 per cent), Baker Chemical Co., meets American Chemical Society specifications). The time at which 50 per cent of the hydrogen peroxide was destroyed was designated the half-time. If the half-time was longer than 1 day, the per cent of hydrogen peroxide remaining after 1 day was recorded.

All the solutions used in these studies were unbuffered.

TABLE I

*The Per Cent Oxygen Saturation Values (w) of Water Exposed to High Oxygen Pressures**

Duration of exposure	6 atmospheres O_2		143 atmospheres O_2	
	Observed *w*	Calculated *w*	Observed *w*	Calculated *w*
min.				
5			0.8	3.8
10			6.5	7.4
20	7.4	6.7	13.6	14.3
30			22.8	20.6
60	18.5	18.8	32.0	37.0
60			36.1	37.0
60‡	31.6	34.0	52.8	60.3
75			33.8	43.8
120	35.7	34.0		
180	56.8	46.4	77.6	75.0
240	56.1	56.4		
480	77.3	81.0		
600	83.0	87.5	121.8	99.0
960	84.2	96.4		
1020	86.2	97.1		
1800			120.3	100.0
2120			89.6	100.0
2760	95.3	100.0		

w = per cent saturation.

* Surface area equal to 2.66 $cm.^2$ and volume equal to 12 ml.

‡ 6 ml. sample instead of 12 ml. sample.

RESULTS

Rate of Absorption of Oxygen.—From the per cent saturation values, it was calculated that the half-time was 3.3 hours at 6 atmospheres of oxygen and 1.5 hours at 143 atmospheres of oxygen for a 12 $cm.^3$ volume with a surface area of 2.66 cm^2. Absorption coefficients which should be independent of the volume and area, were calculated to be 0.936 cm./hour at 6 atmospheres and 2.08 cm./hour at 143 atmospheres. Table I shows the comparison between the observed per cent saturation values and the calculated ones using the absorption coefficients. Because the temperature could not be critically controlled, and because of the effects of convection currents, the observed values agree only approximately with the calculated ones. In spite of the errors involved

TABLE II

*Effect of Ions on the Net Formation of H_2O_2 and on GSH Oxidation in Solution Containing 3.26 mM GSH Exposed to 6 Atmospheres of Oxygen**

Ion added	No. of determinations	$\frac{\text{mN } H_2O_2 \text{ found}}{\text{mN original GSH}}$	GSH remaining unoxidized	$\frac{\text{Calculated mN } H_2O_2 \text{ produced}}{\text{mN original GSH}}$
		per cent	*per cent*	*per cent*
—‡	6	12 ± 2§	72 ± 3	20
1 mM NaCl	2	10 ± 1	75 ± 6	18
10 mM NaCl	2	9 ± 2	69 ± 3	20
100 mM NaCl	1	12	65	24
1 mM KCl	2	14 ± 1	49 ± 6	32
10 mM KCl	2	11 ± 1	59 ± 6	26
100 mM KCl	1	9	53	28
1 mM K_2SO_4	1	12	81	16
10 mM K_2SO_4	1	14	80	17
1 mM $CaCl_2$	2	14 ± 1	66 ± 7	24
10 mM $CaCl_2$	2	9 ± 1	71 ± 4	19
100 mM $CaCl_2$	1	3	56	24
1 mM $MgCl_2$	2	15 ± 1	67 ± 7	24
10 mM $MgCl_2$	3	19 ± 2	40 ± 7	40
100 mM $MgCl_2$	1	10	39	36
10 mM $CoCl_2$	1	9 ± 2‖	57	26
100 mM $CoCl_2$	1	0	32	34
1 mM $MnSO_4$	1	11 ± 1‖	80	16
10 mM $MnSO_4$	2	1 ± 1	5 ± 5	48
100 mM $MnCl_2$	1	0	0	50
1 mM $NiCl_2$	1	14	69	22
10 mM $NiCl_2$	1	5	32	36
100 mM $NiCl_2$	1	0	0	50
1 mM $ZnSO_4$	1	14	72	21
10 mM $ZnSO_4$	1	7	44	32
100 mM $ZnSO_4$	1	0	16	42

* Exposed to oxygen for about 14 hours.

‡ Previously reported (1).

§ No. of determinations for H_2O_2 = 29.

‖ No. of determinations for H_2O_2 = 2.

with this method, it would be reasonable to consider them accurate at least within one order of magnitude.

Effect of Metals and Metal-Complexing Agents on the Production of Hydrogen Peroxide in GSH Solutions Exposed to High Oxygen Pressures.—Addition of

TABLE III

*Effect of Metal-Complexing Agents on the Net Formation of H_2O_2 and on GSH Oxidation in Solutions Containing 3.26 mM GSH Exposed to High Oxygen Pressures**

Complexing agent added‡	Atmospheres O_2	No. of determinations	mN H_2O_2 found / mN original GSH	GSH remaining unoxidized	Calculated mN H_2O_2 produced / mN original GSH
			per cent	*per cent*	*per cent*
—§	6	6	12 ± 2‖	72 ± 3	20
—§	130	6	58 ± 12¶	0 ± 0	79
0.3 mM DEDTC	6	3	8 ± 2	84 ± 8	12
3.0 mM DEDTC	6	2	1 ± 0	100 ± 0	0
0.3 mM DEDTC	130	2	65 ± 2**	0 ± 0	82
3.0 mM DEDTC	130	1	3	98	2
0.003 μM EDTA	6	1	14	77	18
0.03 μM EDTA	6	2	15 ± 0	64 ± 10	26
0.3 μM EDTA	6	2	6 ± 6	94 ± 6	6
3 μM EDTA	6	1	0	100	0
30 μM EDTA	6	2	1 ± 0	100 ± 1	0
300 μM EDTA	6	2	1 ± 0	100 ± 1	0
0.003 μM EDTA	130	1	68	0	84
0.03 μM EDTA	130	2	61 ± 9	0 ± 0	80
0.3 μM EDTA	130	2	21 ± 20	53 ± 47	34
3 μM EDTA	130	1	1	100	0
30 μM EDTA	130	1	1	100	0

* Exposed to oxygen for about 14 hours.

‡ DEDTC = diethyldithiocarbamic acid sodium salt and EDTA = ethylenediaminetetraacetic acid disodium salt.

§ Previously reported (1).

‖ No. of determinations = 29.

¶ No. of determinations = 11.

** No. of determinations = 3.

divalent ions to GSH solutions exposed to 6 atmospheres of oxygen diminished the resulting concentration of hydrogen peroxide, and the amount of reduced glutathione remaining (Table II). The determination of hydrogen peroxide or GSH was not affected by the presence of these ions. Sodium chloride and potassium chloride had very little effect on decreasing the concen-

tration of either hydrogen peroxide or reduced glutathione. Calcium chloride and magnesium chloride had a slight effect. Cobalt (II) chloride, manganese (II) chloride or manganese (II) sulfate, nickel (II) chloride, and zinc chloride had a marked effect.

The inhibition of GSH oxidation by two metal-complexing agents, DEDTC and EDTA, was investigated. Table III shows that 3 mM DEDTC or 0.3 μM EDTA resulted in both a decrease of hydrogen peroxide and an increase of reduced glutathione present after exposure to 6 or 130 atmospheres of oxygen.

TABLE IV

Effect of Various Substances on H_2O_2 Decomposition

Substance	No. of determinations	Mean mM H_2O_2 at zero time	$T_{\frac{1}{2}}$ in min.	Per cent of H_2O_2 after 1 day
Control	5	1.98 ± 0.15		98 ± 1
50 mM $MnCl_2$	3	2.15 ± 0.08		78 ± 5
50 mM $CoCl_2$	2	2.26 ± 0.12		80 ± 0
50 mM $NiCl_2$	2	2.20 ± 0.12		98 ± 0
50 mM $ZnSO_4$	2	2.18 ± 0.10		98 ± 1
50 mM $MgCl_2$	2	2.18 ± 0.10		100 ± 0
50 mM $CaCl_2$	2	2.18 ± 0.10		100 ± 0
0.3 mM DEDTC	4	1.55 ± 0.27		79 ± 7
0.003 mM EDTA	2	2.20 ± 0.08		99 ± 0
0.3 mM EDTA	2	1.43 ± 0.04		100 ± 0
3.26 mM thiourea	3	2.13 ± 0.03	42 ± 9	0
100 mM thiourea	2	2.15 ± 0.05	1 ± 0	0

Control studies of decomposition of hydrogen peroxide (approximately 2 mM, prepared from 30 per cent hydrogen peroxide) by metal ions and by metal-complexing agents showed only very slight changes (about 2 per cent) after 1 day of standing in room air (Table IV). From the impurities in the stock solution, the diluted solution was calculated to contain about 0.001 μM of heavy metals (as Pb) and about 0.002 μM of iron. When manganese (II) chloride (50 mM) or cobalt (II) chloride was added to the hydrogen peroxide, its decomposition proceeded slightly faster, so that after 1 day only about 80 per cent of the hydrogen peroxide remained. Nickel (II), zinc, magnesium, or calcium chloride had no observable effects on hydrogen peroxide decomposition upon 1 day of standing. DEDTC (0.3 mM) and EDTA (0.3 and 0.003 mM) had very little effect on hydrogen peroxide decomposition.

In the presence of 3.26 mM GSH only 62.3 per cent of the hydrogen peroxide remained after 1 day (Table V). When nickel (II) chloride (50 mM),

manganese (II) chloride, zinc sulfate, or cobalt (II) chloride was added to the system, 50 per cent or more of the hydrogen peroxide was destroyed within 10 hours. Magnesium or calcium chloride had little effect in this system. A concentration of 0.3 mM DEDTC did not seem to have any effect, but when increased tenfold, it accelerated the decomposition of hydrogen bringing down the half-time to only 6 minutes. However, the chelating agent, EDTA, had an inhibiting effect at 0.3 mM which was still present at 0.003 mM.

TABLE V

Effect of Various Substances on H_2O_2 Decomposition in the Presence of 3.26 mM Reduced Glutathione

Substance	No. of determinations	Mean mM H_2O_2 at zero time	$T_{\frac{1}{2}}$ in min.	Per Cent of H_2O_2 after 1 day
Control	6	1.87 ± 0.15		62 ± 3
50 mM $NiCl_2$	2	1.80 ± 0.20	120 ± 40	
50 mM $MnCl_2$	2	1.80 ± 0.20	230 ± 10	
50 mM $ZnSO_4$	2	1.80 ± 0.20	320 ± 210	
50 mM $CoCl_2$	3	1.90 ± 0.16	583 ± 297	
50 mM $MgCl_2$	3	1.89 ± 0.13		56 ± 8
50 mM $CaCl_2$	2	1.80 ± 0.26		63 ± 10
0.3 mM DEDTC	3	1.67 ± 0.23		68 ± 6
3.0 mM DEDTC	3	1.37 ± 0.12	6 ± 1	0
0.003 mM EDTA	2	1.80 ± 0.30		97 ± 1
0.3 mM EDTA	2	1.45 ± 0.04		98 ± 2
3.26 mM thiourea	2	1.85 ± 0.25	70 ± 9	0

Even after exposure for 14 hours to oxygen at 6 atmospheres, DEDTC (0.3 and 3.0 mM) still possessed complexing ability, but addition of 3.26 mM GSH destroyed this ability even in air.

Yet, if glutathione solutions were exposed to oxygen at 130 atmospheres for a very prolonged period (3 to 4 weeks), a slight but significant oxidation was found as evidenced both by a formation of hydrogen peroxide and by a decrease in the concentration of reduced glutathione (Table VI). The rate of oxidation did not appear to change during the 70 days of exposure to oxygen. On the 70th day of exposure of the system, the chelating ability of the EDTA (as measured by calcium removal) was not found to be changed.

Effect of Thiourea on the Decomposition of Hydrogen Peroxide.—Thiourea rapidly decomposed hydrogen peroxide either in the absence (Table IV) or in the presence of 3.26 mM GSH (Table V).

Effect of Varying Oxygen Pressures, and GSH Concentration on the Oxidation

of GSH.—Table VII shows that if the concentration of glutathione was kept constant, the formation of hydrogen peroxide was increased with increasing oxygen pressures. If the oxygen tension was kept constant, there was a de-

TABLE VI

Effect of 130 Atmospheres O_2 on 3.26 mM GSH Containing 0.3 mM EDTA

Duration o exposure	$\frac{\text{mN } H_2O_2 \text{ found}}{\text{mN original GSH}}$	GSH remaining unoxidized	Calculated $\frac{\text{mN } H_2O_2 \text{ produced}}{\text{mN original GSH}}$
days	*per cent*	*per cent*	*per cent*
3	0	100	0
9	0	100	0
21	1	97	2
28	1	89	6
42	2	80	11
70	4	38	33

TABLE VII

Effect of Varying O_2 Pressures and GSH Concentrations on H_2O_2 Production Expressed in $\frac{mN\ H_2O_2}{mN\ original\ GSH}\ 100$

Duration of exposure	Original GSH in mN	O_2 pressure in atmospheres			
		1	2	6	130
hrs.					
2	1	0	6	0	40
	3.26	0.0	1.8	0.6	43.0
	10	0.0	0.6	0.2	18.8
	100	0.00	0.10	0.12	2.52
16	1	4	6	16	62
	3.26	1.8	3.1	8.0	58.9
	10	0.6	1.4	2.8	58.0
	100	0.20	0.20	0.40	18.00
144	1	6	16	30	64
	3.26	8.6	9.2	23.3	66.3
	10	1.4	4.0	10.4	59.6
	100	0.12	0.20	0.88	10.40

crease in the relative hydrogen peroxide formation with increasing concentrations of glutathione. The oxidation of glutathione was generally increased with duration of exposure to oxygen as judged by hydrogen peroxide formation (Table VII).

DISCUSSION

Absorption of Oxygen in Liquid Systems.—Since it is to be expected that both the *in vitro* and *in vivo* effects of oxygen would be directly dependent upon the concentration of oxygen in the liquid phase, the factors influencing the rate of absorption are of paramount importance in determining the effects of oxygen. The importance of correlating the effects of oxygen tension with the concentration of oxygen in the liquid phase has been discussed by others (10, 11). Factors which can influence the concentration of oxygen in the liquid phase are: (1) the time of exposure, absorption coefficient, volume, and surface area of the liquid (see equation in Methods); (2) the solubility (saturation value) of the oxygen, which can be modified by pressure (Henry's law); (3) chemical reactions of oxygen; (4) diffusion and convection currents. Other variables such as solutes and temperature can influence these factors. Misleading conclusions from experiments dealing with the effects of oxygen can result if these factors are not adequately taken into account. For instance, in our experiments when the liquid volume was decreased from 12 to 6 cm.[3] (Table I), the rate of absorption was increased. The influence of temperature is illustrated in the experiments of Malamed (12) who found that at a given pressure of oxygen, the toxic effect on frog embryos was augmented if the temperature was lowered; he explained this on the basis of increased solubility of the gas. The role of chemical reactions as a factor in determining gas absorption has been quantitatively studied by Davis and Crandall (13). Convection currents are difficult to assess quantitatively, but their significance is certainly not to be overlooked. Hutchinson and Sherwood (14) found that the absorption coefficients for oxygen in their system were 0.41 cm./hour when there was no stirring of the liquid and increased to 7.64 cm./hour when the stirring speed was increased to 1025 R. P. M. In our systems, the absorption coefficient at 6 atmospheres of oxygen was 0.936 cm./hour and at 143 atmospheres was 2.08 cm./hour. The difference between these two values is most easily explained on the basis of an increased convection factor due to the *modus operandi*: the 6 atmospheres were attained in 1 minute and the 143 atmospheres in 5 seconds. Since the values are within the range reported by Hutchinson and Sherwood using 1 atmosphere of oxygen, there is no reason to attribute any important influence to pressure *per se*. It is evident that in organisms containing circulatory systems, an increased blood flow, for instance, would be analogous to stirring liquids in *in vitro* systems. Because of the experimental difficulties, the absorption coefficient for oxygen could change perhaps within an order of magnitude. Therefore, no attempt was made to describe mathematically the kinetics of the glutathione oxidation.

In the experiments reported by Barron (15), several systems were exposed to high oxygen pressures for only 20 minutes, but there was no mention of

the volume to area ratio. However, if the ratio was not much smaller than the one used in the present study, it becomes evident that his systems were no where near equilibrium at the time of his measurements. Barron, himself, noted that stirring his system did increase the amount of oxidation. In our previous study (1) and in the present one, the solutions were exposed to oxygen for about 14 hours after which they were almost saturated with oxygen. Thus, the exposure of solutions to high oxygen pressures might show only minimal changes unless enough time is allowed for sufficient oxygen to enter solution. The duration of the induction period for oxidation of fatty acids (16) may be influenced by this factor.

Effects of Metals and Metal-Complexing Agents on the Production of Hydrogen Peroxide in GSH Solutions and Exposed to High Oxygen Pressure.—(*a*) Effect of metals on hydrogen peroxide production in GSH solutions exposed to high oxygen pressure. It has been reported that manganese (II) ions (17–19) and cobalt (II) ions (17–21) protect biological systems against the toxic effects of high oxygen pressure. Thus, it became of interest to investigate the possibility that these and other ions might modify the amount of hydrogen peroxide formed in solutions of GSH exposed to high oxygen pressures. It was found that, in general, divalent cations not only decreased the net amount of hydrogen peroxide found after exposure to high oxygen pressure, but also decreased the amount of reduced glutathione remaining after exposure. Although the actual total production of hydrogen peroxide could not be measured, an estimate of this value was attempted. GSH oxidation by oxygen can be summarized by the following equations (1):

$$X\ \mathrm{GSH} + \tfrac{1}{2}X\ \mathrm{O_2} \rightarrow \tfrac{1}{2}X\ \mathrm{H_2O_2} + \tfrac{1}{2}X\ \mathrm{GSSG} \qquad (1)$$

$$Y\ \mathrm{GSH} + \tfrac{1}{2}Y\ \mathrm{H_2O_2} \rightarrow Y\ \mathrm{H_2O} + \tfrac{1}{2}Y\ \mathrm{GSSG} \qquad (2)$$

$$(X + Y)\ \mathrm{GSH} + \tfrac{1}{2}X\ \mathrm{O_2} \rightarrow \tfrac{1}{2}(X - Y)\ \mathrm{H_2O_2} + Y\ \mathrm{H_2O} + \tfrac{1}{2}(X + Y)\ \mathrm{GSSG} \qquad (3)$$

If there are no further appreciable oxidations, then the net formation of hydrogen peroxide in equivalents would be equal to $(X - Y)$ [(in moles, it would be $\frac{1}{2}(X - Y)$ and the equivalents (or moles) of GSH oxidized would equal $(X + Y)$]. The actual total production of hydrogen peroxide in equivalents should be equal to X and should correspond to one-half the net formation of hydrogen peroxide in equivalents plus one-half the equivalents of GSH oxidized. The total production of hydrogen peroxide was calculated on this basis and included in Tables II, III, and VI. The maximum production of hydrogen peroxide would occur when all the GSH is oxidized by the oxygen as in Equation 1, and when no GSH is oxidized by the hydrogen peroxide as in Equation 2. A tendency to this maximum production occurred in our experiments at 130 atmospheres of oxygen for which the calculated total production of hydrogen peroxide was 79 per cent (Table III). The calculations in Table II show

that the divalent cations increased the total production of hydrogen peroxide, even though the net amount actually diminished. These apparently paradoxical results could be plausibly explained by postulating an acceleration of hydrogen peroxide decomposition by these divalent ions. The experiments discussed below were designed to test this possibility.

(*b*) *Effects of Metals on the Decomposition of Hydrogen Peroxide Solutions.*— We found that pure solutions of hydrogen peroxide were quite stable as previously reported by Rice (22). The decomposition of hydrogen peroxide was not affected by nickel (II), zinc, magnesium, or calcium ions, and was only slightly accelerated by manganese (II) and cobalt (II) ions. Reports in the literature mention that cobalt (II) and nickel (II) ions can catalyze hydrogen peroxide decomposition (23), but only slowly (24). Other reports indicate that calcium (25–27), magnesium (26, 27), zinc (27), cobalt (II) (27), manganese (27), and nickel (II) (27) ions have little or no effect on the rate of hydrogen peroxide decomposition.

Since the metal ions alone had practically no effect on the decomposition of hydrogen peroxide, these experiments were repeated with the addition of GSH to the system. In this connection it should be mentioned that GSH can be oxidized by hydrogen peroxide (28), and this would explain the acceleration of its decomposition by GSH in our experiments. In the presence of GSH, magnesium and calcium ions had hardly any effect, but nickel (II), manganese (II), zinc, and cobalt (II) ions greatly accelerated the peroxide decomposition. An explanation for these results might be that the divalent cations would form complexes with GSH and that these complexes, except in the case of magnesium or calcium, would be easily oxidized by hydrogen peroxide. Glutathione has been reported to complex with calcium (29) and with zinc (30). The oxidation of GSH by oxygen has been reported to be catalyzed by copper (II) ions (31–33) and to a slight extent by small amounts of cobalt (II) ions (31). No effect on GSH oxidation was obtained with small amounts of manganese (II) and nickel (II) ions, but an inhibition was obtained with zinc ions (31). It is surprising to note that iron will catalyze GSH oxidation by hydrogen peroxide (28) but not by molecular oxygen (31).

The above considerations suggest that in the presence of divalent ions a part of the hydrogen peroxide formed by GSH in high oxygen pressure is used to oxidize part of the GSH to GSSG, and is itself destroyed in the process. The over-all effect would result in less reduced glutathione and less hydrogen peroxide present. The action of these ions cannot be explained by catalysis, since large concentrations were necessary to obtain maximum effects. Further work with zinc would be of interest since it had the same effect as the other ions in spite of its reported inhibition of GSH oxidation (31). In plant tissues, cobalt (II) inhibited and manganese accelerated peroxigenesis (20, 34). The influence of cobalt (II) and manganese (II) ions on hydrogen

peroxide decomposition may play some role in the effect of these agents in oxygen poisoning. In this connection, it is of special interest to mention that obligate bacteria have been made to grow successfully in air, if small amounts of cobalt (II) ions (35) were present in the nutrient solution. Significant production of hydrogen peroxide was reported to occur in anaerobic bacteria exposed to air (36–38).

(*c*) *Effect of Metal-Complexing Agents on Hydrogen Peroxide Production in GSH Solutions Exposed to High Oxygen Pressures.*—Adequate concentrations of DEDTC and EDTA inhibited the oxidation of GSH by high oxygen pressure. These agents decreased not only the net, but also the total hydrogen peroxide produced. These results would also explain why EDTA inhibited the viscosity decrease produced by high oxygen pressure in desoxyribonucleic acid solutions containing GSH (1). EDTA also prevented the decomposition of hydrogen peroxide solutions by GSH. A concentration of 0.3 μM EDTA was sufficient to produce inhibition of GSH oxidation. Traces of copper present in the system were detected by mass spectrography (analysis kindly performed by Dr. L. T. Steadman) and were estimated to be in the order of 0.1 μM. It would seem reasonable to conclude that EDTA chelated the copper and thus prevented its catalytic action. Since DEDTC loses its complexing ability in solutions of low pH (39), it is not surprising to find that the complexing ability of DEDTC was inhibited in 3.26 mM GSH solutions, which possess a pH of about 3.5. Thus, more DEDTC was required to remove trace metals which can catalyze GSH oxidation. It might also be interesting to mention here that Alexander *et al.* (40) found that metal-complexing agents, including EDTA and DEDTC, decreased the x-ray degradation of polymethacrylic acid.

From a theoretical point of view, it is important to mention that prolonged exposure to oxygen of GSH solutions in an excess of the metal-chelating agent, EDTA, resulted in a very slow but nevertheless measurable non-catalyzed oxidation, a fact which is consistent with the idea that catalysts will change the rate only, but not the reaction itself. Since the rate of oxidation of GSH did not increase appreciably up to 70 days, it would seem that the reaction proceeds without a chain utilization of oxygen. In this connection, it is interesting to note that Barr and King (41) reported an absence of chain utilization of oxygen by ascorbic acid, and suggested that the protective effect against radiation damage in biological systems by substances like ascorbic acid, thiourea, tocopherol, and hydroquinone might be due to their action of limiting extended chain utilization of oxygen. These authors also mentioned that protective substances of this class are characterized by their ease of oxidation and by their ability to form one-electron oxidation products. Glutathione also fits into this class of substances in the presence of metal catalysts.

Effect of Thiourea on the Decomposition of Hydrogen Peroxide.—Because

thiourea has been reported to give a positive sulfhydryl test (42), it was surprising to find that this substance acted differently from GSH when submitted to high oxygen pressure. Thiourea inhibited hydrogen peroxide production in the presence of GSH and oxygen, and prevented the viscosity decrease of desoxyribonucleic acid in the presence of GSH and oxygen (1). Thiourea has also been reported to inhibit the decrease in desoxyribonucleic acid viscosity caused by x-irradiation (43). Thiourea protected mice against 6 atmospheres of oxygen (21). Part of these actions might be explained by the acceleration of hydrogen peroxide decomposition caused by thiourea (Tables IV and V). This observation is in agreement with the results reported by Randall (44).

Effect of Glutathione Concentration on Hydrogen Peroxide Production.—Our results show that the oxidation of GSH is decreased as the GSH concentration is increased. Oxidation by oxygen of ascorbic acid (45) or of sodium sulfite (46) is also decreased as the concentration of these chemicals is increased. Fuller and Crist (46) showed that at least part of this decreased oxidation of sodium sulfite was due to the limiting rate of solution of oxygen. At least part of the decreased oxidation of GSH and ascorbic acid could be attributed to the limiting rate of solution of oxygen. Our experiments indicated that a substance like GSH, which is considered to be an antioxidant, can also act as a prooxidant. This is not surprising since it is known that antioxidants can act as prooxidants (47–49) depending on the condition. A toxic effect of GSH *in vivo* has been attributed to a prooxidative property (50). On the other hand, GSH has been shown to lengthen the survival time of mice exposed to 6 atmospheres of oxygen (21, 51), but to decrease it at 1 atmosphere (21). The latter can be interpreted as an activation of oxygen by glutathione, an effect which can be described as prooxidative. Yet, *in vivo* GSH could act as a chain breaker, and also be oxidized in preference to essential cell constituents and by doing so act as an antioxidant. A pertinent discussion on how substances can act as either antioxidants or prooxidants depending upon the circumstances has been recently published (49). The removal of metal catalysts by chelating agents such as EDTA, and the inhibition of oxidation by inclusion (52) should also be taken into account when analyzing the effects produced by substances which can influence systems exposed to oxygen.

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