THE TOXIC ACTION OF OXYGEN ON GLUCOSE AND PYRUVATE OXIDATION IN HEART HOMOGENATES*

By NIELS HAUGAARD, MARILYN E. HESS, AND HAROLD ITSKOVITZ

(From the Laboratory of Pharmacology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania)

(Received for publication, December 18, 1956)

The experiments reported in this paper are concerned with the inhibitory action of oxygen at 1 atmosphere pressure on the carbohydrate metabolism of heart muscle studied *in vitro*.

It has been observed by several investigators that oxygen at 1 atmosphere pressure may have a harmful effect on the metabolism of mammalian tissue *in vitro* or on individual enzyme systems (1–5). Dickens (6, 7) and Stadie and coworkers (8, 9) have studied systematically the effect of oxygen at elevated tension on tissue respiration and on the activity of various enzymes. The conclusions arrived at by the two groups of investigators were similar: Many enzymes, particularly those dependent upon sulfhydryl groups for activity, are more or less easily inactivated by oxygen pressures of 1 atmosphere or greater and many are completely resistant to the toxic action of oxygen. The enzymes inactivated by oxygen *in vitro* usually require 1 or more hours of exposure to 1 atmosphere of oxygen before inhibition becomes apparent. Even at oxygen pressures as high as 8 atmospheres, tissue respiration *in vitro* is only slowly inhibited. It has also been a general finding that enzymes in tissue extracts or homogenates are more readily inactivated than the same enzymes when present in the intact cells of tissue slices. For these and other reasons, it has been difficult to relate the effects of oxygen observed *in vitro* to the severe symptoms of oxygen poisoning produced within 15 to 30 minutes in animals exposed to 3 to 8 atmospheres of oxygen (5, 10, 11). The review by Dickens (5) contains an excellent critical evaluation of the work that has been carried out on the toxic action of oxygen on enzymes and tissue metabolism. From recent work on the mechanism of oxygen poisoning in the intact animal, it appears that there is a close similarity between the toxic effects of excess radiation and of oxygen at high pressure (12, 13).

In the present work, the effect of 1 atmosphere of oxygen on the multienzyme systems involved in the oxidation of glucose and pyruvate has been studied. Rat heart homogenates fortified with KCl and diphos-

* This work was supported by a research grant (No. H-1813) from the National Heart Institute of the National Institutes of Health.

phopyridine nucleotide (DPN) were used. Such homogenates have the ability to catalyze at a rapid rate the complete oxidation of glucose and pyruvate to carbon dioxide and water (14, 15). It was found that, compared to controls in air, the rate of oxidation of both glucose and pyruvate was consistently depressed by 1 atmosphere of oxygen and that the inhibitory effect of oxygen was profoundly influenced by metal ions in the reaction medium. A study of this toxic action of oxygen and its modification by metal ions is presented in this paper.

Methods

Enzyme System—Male albino rats, weighing 150 to 200 gm., were used and fed *ad libitum*. For each experiment an animal was killed by decapitation and the heart was placed in cold medium of the following composition: 0.040 M sodium phosphate, 0.095 M NaCl, pH 7.2. After being blotted on filter paper, the heart was cut into small pieces and ground by hand in a glass homogenizer in 3 ml. of chilled medium of the same composition. The suspension was filtered through cheesecloth to remove gross particles. An additional 5 ml. of chilled medium were added to the homogenate. In later experiments, the heart was weighed before homogenization and the total volume of medium added was 1.5 ml. per 100 mg. of wet weight. In these later experiments, we also substituted KCl for NaCl in the homogenization medium, with which the enzyme system is more stable. The homogenates may be cleared of large particles and cellular debris by centrifugation at about 200 $\times$ g for 5 minutes with little or no loss of activity.

Incubation—The homogenates oxidized glucose and pyruvate vigorously when incubated at 37° in the presence of DPN and KCl or $MgCl_2$. The composition of the reaction system varied in different experiments as explained. In a typical experiment, the system consisted of the following: 0.5 ml. of homogenate (corresponding to 30 to 40 mg. of tissue), 0.05 ml. of 5 per cent glucose (or 0.1 ml. of 0.1 M sodium pyruvate), 0.1 ml. of 3 per cent DPN, 0.5 ml. of 0.3 M sorbitol, and 0.5 ml. of 0.15 M KCl. The reaction mixtures were incubated at 37° in Warburg flasks containing alkali in the side compartments. In the absence of added substrate, there was considerable oxygen uptake for the first 20 to 40 minutes caused by the oxidation of substrates initially present in the homogenate. With *added* glucose or pyruvate, activity continued for 2 to 3 hours or more. Under optimal conditions, the oxygen uptake of the system with glucose as substrate corresponds to 400 to 450 μmoles per gm. of wet tissue per hour. This is approximately 5 times the respiration of rat heart slices incubated *in vitro* with glucose as substrate. Utilization of glucose by the heart homogenate was determined by measuring the initial and final glucose

concentration in the medium by the method of Miller and Van Slyke (16). Activity was determined with air, 4 per cent oxygen (96 per cent nitrogen), 7.4 per cent oxygen (92.6 per cent nitrogen), or 100 per cent oxygen in the gas phase. $CoCl_2$, $CuSO_4$, $MgCl_2$, or the chelating agent ethylenediaminetetraacetic acid (EDTA or Versene) was added in some of the experiments as indicated.

Chemicals—DPN was obtained from the Pabst Laboratories and solutions of it were neutralized with NaOH before use. Sodium pyruvate was prepared from pyruvic acid and crystallized.

EXPERIMENTAL

Inhibition by 100 Per Cent Oxygen—When homogenates were incubated with glucose or pyruvate as substrates at 37°, the rates of oxygen uptake were initially linear with time and the same in air and oxygen. However, in all experiments, the subsequent decline in the rates of reaction of the systems in oxygen began sooner and was more pronounced than in the controls in air (Table I). Typical experiments illustrating the effect of oxygen on the oxidation of glucose and pyruvate are recorded in Fig. 1.

Table I contains the results of a series of experiments in which oxidation of glucose by rat heart homogenate was studied with air or 100 per cent oxygen in the gas phase. Total oxygen uptake and utilization of glucose are recorded. In all of the experiments, glucose was present in excess so that at least one-half of the added glucose remained at the end of the period of incubation. The experiments in Table I show that both oxygen uptake and glucose utilization were depressed in 100 per cent oxygen compared to the controls in air.

It was shown in a previous paper (15) that glucose oxidation by heart homogenate is highly dependent upon the cations present in the reaction medium. When sodium ions are the only cations added, there is no activity. Addition of KCl, $MgCl_2$, or both will activate the system. KCl was present in most of the experiments reported in Table I. However, we have also included some experiments in which $MgCl_2$ or both KCl and $MgCl_2$ were added. Inactivation by 100 per cent oxygen was found under all of these experimental conditions. The results (Table I) show that the oxygen uptake was approximately 6 times the utilization of glucose, indicating that most of the glucose used during the experiment was completely oxidized. The rate of reaction during the 70 to 100 minute period in per cent of the initial rate is recorded in the seventh column. It is seen that the activity of the system decreased considerably during incubation in air, but that in all experiments the decline of the rate of reaction was greater in oxygen than in air. The times at which the rates of oxygen uptake of the samples in oxygen had declined to 50 per cent of the rates of the con-

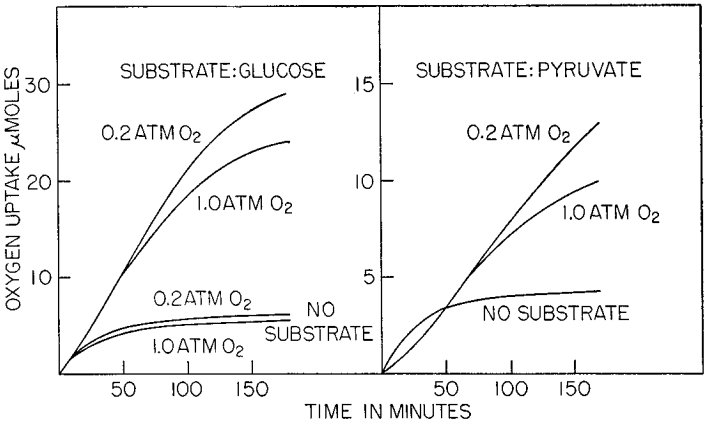

FIG. 1. Oxidation of glucose and pyruvate by rat heart homogenate in air and oxygen. The reaction system was as described under "Methods." Glucose when present, 0.008 M; sodium pyruvate when present, 0.0065 M. ATM = atmosphere.

TABLE I

Glucose Oxidation by Rat Heart Homogenate in Air and Oxygen

The reaction system had the following composition: 0.028 M NaCl; 0.012 M sodium phosphate; 1.8 mg. of DPN per ml.; 0.008 M glucose; KCl, when added, 0.045 M; $MgCl_2$, when added, 0.0012 M; sorbitol added to constant osmolarity (0.270); pH 7.2; 40 to 50 mg. of tissue. Total volume, 1.70 ml. Each figure is the mean of duplicate determinations.

Experiment No.	Activatng ions	Gas phase	Time	Oxygen uptake	Glucose utilization	Oxygen uptake, $\frac{70\text{–}100\text{ min.}}{10\text{–}40\text{ min.}} \times 100$	Time for 50 per cent inhibition*
			min.	*μmoles*	*μmoles*	*per cent*	*min.*
1	K^+	Air	120	17.5	2.9	66	
		Oxygen	120	13.6	1.3	24	85
	K^+	Air	120	17.4	1.8	42	
		Oxygen	120	12.5	0.6	14	70
3	K^+	Air	150	21.0	3.5	90	
		Oxygen	150	15.8	3.0	69	105
4	K^+	Air	210	25.1	3.8	82	
		Oxygen	210	19.3	3.4	78	115
5	Mg^{++}	Air	120	22.3	3.8	39	
		Oxygen	120	19.9	3.2	17	75
6	K^+, Mg^{++}	Air	120	15.6	3.5	29	
		Oxygen	120	12.6	1.8	17	85
7	K^+, Mg^{++}	Air	120	28.7	5.7	45	
		Oxygen	120	25.8	5.1	20	80

* The time, *t*, at which the rate of oxygen uptake of the sample in oxygen, during the interval $t \pm 5$ minutes, had decreased to 50 per cent of the rate of the control in air during the same interval of time.

trols in air are given in the eighth column of Table I. It is apparent that the time necessary to reach a certain degree of inactivation varies widely from experiment to experiment. Apparently, both the extent and the time of onset of oxygen toxicity vary from one tissue preparation to another.

Effect of Cupric Ions—The variation in the time of onset of oxygen poisoning of the system made it likely that some factors, so far unrecognized, influenced the toxic action of oxygen in these experiments. One possibility was that trace metals present in the tissue or introduced by the reagents affected the results. Cupric ions are known to catalyze the oxidation by oxygen of compounds containing sulfhydryl groups (17), ascorbic acid (18), and possibly other essential tissue components. The cupric ion is a normal tissue constituent in trace amounts. The oxidation of glucose by the rat heart homogenate was, therefore, studied in air and oxygen in the presence and absence of small amounts of $CuSO_4$. It was shown in separate experiments that Na_2SO_4 in high concentrations (compared to the concentrations of $CuSO_4$ used) had no effect on the system, so that any effects of $CuSO_4$ could be assumed to be caused by the cupric ions. $CuSO_4$ in concentrations above 2×10^{-5} M was found to be highly toxic to the enzyme system when studied with air or with oxygen in the gas phase. At somewhat lower concentrations, the system in oxygen was affected to a much greater extent than was the control in air, and at concentrations of $CuSO_4$ in the range of 5×10^{-6} M to 10^{-5} M the copper ion had little or no effect on the enzyme system incubated in air but strongly inhibited the system incubated in oxygen. These findings are illustrated in Fig. 2. Similar effects of cupric ions were observed when pyruvate was the substrate.

The inhibitory action of the cupric ion could be abolished by the addition to the medium of the chelating agent EDTA (Table II).

These observations constitute indirect evidence for the hypothesis that the toxic effect of oxygen on metabolism is brought about by an oxidation of one or more essential metabolites or enzymes to an inactive form.

Dickens (6) demonstrated with brain slices and homogenates that certain cations, particularly Co^{++} and Mn^{++}, when present in small concentrations, protected against the toxic action of oxygen at increased tension. The effect of the addition to the medium of a small amount of $CoCl_2$ was studied in the presence and absence of added cupric ions. These experiments are reported in Table III.

In air, neither $CuSO_4$ nor $CoCl_2$ in the concentrations used had any effect on the enzyme system. Oxygen uptake and glucose utilization were significantly depressed in 100 per cent oxygen. Traces of cupric ions greatly increased the toxic effect of oxygen, and cobalt ions had no significant effect in the absence of added $CuSO_4$, but did significantly diminish the

inhibition by oxygen in the presence of added $CuSO_4$. The nature of this effect of cobalt ions may be similar to the action of cobalt and manganese

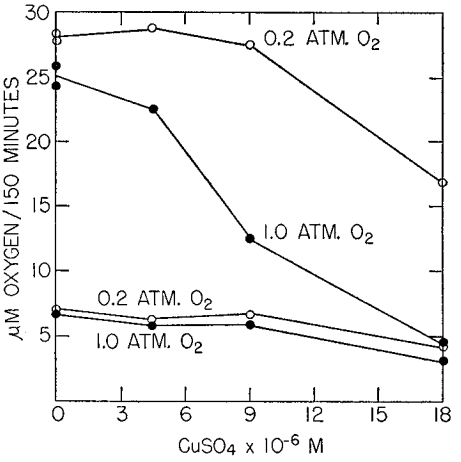

FIG. 2. The effect of copper ions on glucose oxidation by rat heart homogenate in air and oxygen. The reaction system was as described under "Methods." The abscissa is the concentration of $CuSO_4$ and the ordinate is the total oxygen uptake during 150 minutes of incubation at 37°. Two upper curves were obtained with glucose as substrate, and the two lower curves without substrate added. ATM = atmosphere.

TABLE II

Glucose Oxidation by Rat Heart Homogenate; Effect of $CuSO_4$ and EDTA

The reaction system was as described under "Methods." $CuSO_4$ and EDTA were added as indicated below. Time of experiment, 130 minutes. Each figure is the mean of duplicate determinations.

Gas phase	$CuSO_4$	EDTA	Oxygen uptake	Glucose utilization
	M	*M*	*μmoles*	*μmoles*
Air	0	0	24.9	4.2
Oxygen	0	0	23.1	3.2
Air	1.5×10^{-5}	0	22.0	3.5
Oxygen	1.5×10^{-5}	0	13.3	1.7
Air	1.5×10^{-5}	6×10^{-5}	28.9	3.4
Oxygen	1.5×10^{-5}	6×10^{-5}	28.4	3.9

ions observed by Dickens (6, 7) and may consist of an antagonism to ions that accelerate oxygen poisoning. These ions may be naturally present in the tissue or, as in our experiments, they may be added to the reaction medium. It should be pointed out that the concentration of cupric ions

added in these experiments is actually lower than the concentration naturally present in human plasma (19).

TABLE III

Effect of Copper and Cobalt Ions on Oxidation of Glucose by Rat Heart Homogenate in Air and Oxygen

Five experiments were performed with separate heart homogenates. In addition to the regular components of the reaction mixture, described under "Methods," replicates contained (1) no extra added ions; (2) $CuSO_4$; (3) $CoCl_2$; (4) $CuSO_4$ + $CoCl_2$ as indicated below; gas phase was air or 100 per cent oxygen. In each experiment (120 minutes of incubation at 37°) the total oxygen uptake and glucose utilization were measured and the mean activity of the controls in air (and without $CuSO_4$ or $CoCl_2$) was designated 100 per cent. The activities of the other samples were expressed in per cent of the control. The figures below for each experimental category are the means of the per cent differences from the controls ± the standard error of the mean. The figures in parentheses represent the number of individual determinations.

Category No.	Ions added	Oxygen uptake		Glucose utilization	
		In air	In oxygen	In air	In oxygen
		*per cent**	*per cent**	*per cent**	*per cent**
I	None	0 (9)	−14 ± 1.6 (9)	0 (9)	−14 ± 3.3 (9)
II	9×10^{-6} M $CuSO_4$	0 ± 2.3 (4)	−46 ± 3.7 (9)	−4 ± 6.1 (4)	−62 ± 6.9 (9)
III	3.8×10^{-5} M $CoCl_2$	+1 ± 1.7 (4)	−7 ± 1.0 (7)	−8 ± 4.1 (4)	−18 ± 5.9 (7)
IV	9×10^{-6} M $CuSO_4$ +3.8×10^{-5} M $CoCl_2$	0 ± 1.1 (5)	−31 ± 3.2 (8)	+4 ± 3.2 (5)	−44 ± 2.5 (8)
	Effect of cobalt in presence of copper (Categories IV–II)	0 ± 2.5	+15 ± 4.9 $t = 3.1$ $P < 0.01$	+8 ± 6.9	+18 ± 7.3 $t = 2.4$ $P < 0.01$

* Per cent differences from the activity of the control in air and in the absence of added copper and cobalt ions (see above).

Effect of EDTA—The observation that the cupric ion so strongly accentuates oxygen toxicity and that other ions also influence the phenomenon made it likely that chelating agents would modify oxygen toxicity. The effect of the chelating agent EDTA was accordingly studied. This substance combines strongly with the calcium ion, less strongly with magnesium, and also removes cupric ions from solution. When EDTA was added to a rat heart homogenate activated by KCl and with glucose as

substrate, it was found that at low concentrations (6×10^{-5} M or lower) EDTA had no effect or caused only a slight depression of the initial rate of reaction. If the concentration was increased above 10^{-4} M, however, the initial rate was definitely depressed. At a concentration of 10^{-3} M, EDTA caused complete inhibition. The inhibition appeared to be caused by the removal of magnesium ions from the reaction medium since the addition of an equivalent or greater amount of $MgCl_2$ restored activity completely. When an adequate concentration of KCl is present, glucose oxidation will proceed rapidly without added $MgCl_2$, but it is apparent that the magnesium ions present in the tissue itself are necessary for activity.

Although EDTA in small concentrations had little or no effect on the initial rate of reaction, it did influence the activity of the enzyme preparations after incubation for 1 hour or more at 37°. The general action of EDTA at a concentration of 6×10^{-5} M was that of stabilizing the system; *i.e.*, of delaying the gradual fall in the rate of reaction that began at different times with every enzyme preparation studied. This stabilization of the system with EDTA occurred both in air and in oxygen but was significantly greater in oxygen than in air. In oxygen the time of the fall in activity (compared to the control in air) was, therefore, delayed and the difference in activity between the sample in air and oxygen was less pronounced. The observation that EDTA prolongs the activity of the system both in air and in oxygen makes it reasonable to assume that the fall in activity of the system in air, as in oxygen, is at least partly a result of the toxic action of oxygen; *i.e.*, that oxygen in this cell-free enzyme system is toxic at the tension present in air. If this be true, the enzyme system should be more stable when incubated under tensions of oxygen lower than 0.2 atmosphere than it is in air or oxygen. The oxygen uptake of the heart muscle enzyme system was therefore determined in the presence of glucose with 4 per cent oxygen (96 per cent nitrogen), air, or 100 per cent oxygen in the gas phase. At each tension of oxygen the effect of the addition of 6×10^{-5} M EDTA was also determined (Experiment 1, Table IV). In two subsequent experiments (Experiments 2 and 3, Table IV) the lowest oxygen concentration was 7.4 rather than 4 per cent. It was realized that at a concentration of 4 per cent oxygen almost all of the oxygen present was used up at the end of the experiment and that, under such conditions, diffusion of oxygen into the solution might become a limiting factor in the measurement of oxygen uptake. With 7.4 per cent oxygen in the gas phase, the concentration of oxygen at the end of an experiment will still be about half of the initial concentration, even with the most active enzyme preparation studied.

For each experiment we have recorded the initial rate of reaction in micromoles of oxygen per hour calculated from the rate during the 30 to 50

minute interval of the experiment. We chose this interval for calculation of the initial rate of reaction, since at 30 minutes the control without glucose

TABLE IV

Glucose Oxidation by Rat Heart Homogenate at Different Concentrations of Oxygen

Reaction system, Experiment 1: 0.5 ml. of homogenate (about 40 mg. of tissue) in 0.040 M sodium phosphate, 0.095 M NaCl, pH 7.2. Final concentrations of components: 0.012 M sodium phosphate, 0.029 M NaCl, 0.045 M KCl, 0.090 M sorbitol, 1.8 mg. per ml. of DPN, 0.0084 M glucose. Total volume, 1.65 ml. EDTA, when present, 6×10^{-5} M. Experiments 2 and 3: 0.5 ml. of homogenate (31 mg. of tissue) in 0.040 M sodium phosphate, 0.095 M KCl, pH 7.2. Final concentrations of components: 0.017 M sodium phosphate, 0.074 M KCl, 0.065 M sorbitol, 2.6 mg. per ml. of DPN, 0.012 M glucose. Total volume, 1.15 ml. EDTA, when present, 9×10^{-5} M. Oxygen uptake determined in Warburg vessels at 37°; CO_2 absorbed by alkali. Gas phase, 4 per cent O_2-96 per cent N_2, 7.4 per cent O_2-92.6 per cent N_2, air, or 100 per cent O_2, as indicated below. For each experiment the initial rate of oxygen uptake (calculated from the 30 to 50 minute rate) is recorded in micromoles per hour. Average rates during subsequent 40 minute intervals are given in per cent of the initial rate. Each figure is the mean of duplicate determinations.

Experiment No.	No EDTA	With EDTA	No EDTA	With EDTA	No EDTA	With EDTA
	4 per cent oxygen		Air		100 per cent oxygen	
1. Initial rate, *μmoles per hr.*	10.4	8.9	11.4	8.9	10.5	9.0
" " %						
70–110 min.	93	104	84	99	66	78
110–150 "	54	85	36	93	15	74
150–190 "	18	80	11	83	9	65
	7.4 per cent oxygen					
2. Initial rate, *μmoles per hr.*	14.1	12.8	14.6	13.2	14.1	12.5
" " %						
70–110 min.	79	87	81	88	67	81
110–150 "	76	84	67	82	56	76
150–190 "	54	64	29	69	11	55
190–230 "	46	51	10	59	11	49
230–270 "	38	42	5	51	7	42
3. Initial rate, *μmoles per hr.*	13.4	11.4	13.5	11.6	14.1	12.5
" " %						
70–110 min.	81	83	81	89	74	84
110–150 "	78	82	58	77	30	77
150–190 "	58	73	22	76	9	62
190–230 "	51	59	5	64	4	55
230–270 "	51	55	4	58	3	43

added had almost ceased to take up oxygen, and significant oxygen poisoning did not occur earlier than 50 minutes from the start of the experiment.

It is seen that the initial rate of reaction was almost the same at the different tensions of oxygen, indicating that the respiratory enzymes are saturated with oxygen even at 4 per cent concentrations. EDTA, at a concentration of 6×10^{-5} M in Experiment 1 and 9×10^{-5} M in Experiments 2 and 3, depressed the initial oxygen uptake slightly. The mean oxygen uptake values during subsequent 40 minute periods of incubation were calculated and expressed in per cent of the initial rate. There was in all cases a progressive fall in activity with time. This fall started earlier and was more pronounced in oxygen than in air. It was also found that the oxygen uptake was maintained better in 4 or 7.4 per cent oxygen than in air. EDTA protected the enzyme system against inactivation at all concentrations of oxygen. This protective effect of EDTA was particularly pronounced with 100 per cent oxygen in the gas phase. With EDTA present, the enzyme system has a remarkable stability. Even after 250 minutes of incubation at 37°, activity was still at a level of 50 per cent of the initial rate (Experiments 2 and 3).

DISCUSSION

In the experiments reported here the toxic action of oxygen on a cell-free multienzyme system is well demonstrated. The inhibitory action of oxygen is manifested by 100 per cent oxygen and also at the concentration of oxygen present in air. However, there is considerable evidence that most enzymes, when present in the intact cells of tissue slices or in the animal, are less susceptible to oxygen poisoning than they are when present in cell-free preparations such as the heart homogenate used in these experiments. This may be an indication that protective mechanisms are present in the cell that counteract the oxidizing potential of molecular oxygen. It has been observed that susceptibility to poisoning by oxygen varies widely from species to species (4, 20) and with the physiological state of the animal (21). It is possible that such differences in resistance to oxygen toxicity may parallel changes in the protective mechanisms of the cell. As an extreme case, one may consider the strictly anaerobic bacterium as an organism without any protective mechanism against oxygen poisoning.

That copper ions greatly accentuate oxygen poisoning and that the chelating agent EDTA exerts a protective action strongly support the view that the toxic action of oxygen on tissue metabolism is caused by a metal-catalyzed oxidation of one or more easily oxidizable tissue constituents. There is much evidence that the tissue constituents involved may be enzymes or coenzymes containing sulfhydryl groups: (1) The so called SH— enzymes are especially vulnerable to inactivation by oxygen. (2) The SH— coenzymes, glutathione and coenzyme A, have been shown by Barron (22) to be oxidized in water solution by high pressures of oxygen. (3)

Copper ions, found in our experiments to accentuate oxygen toxicity, are known to catalyze the oxidation of SH— groups (17).

The importance that the *in vitro* studies of oxygen toxicity may have for our understanding of the phenomenon of oxygen poisoning in the intact animal remains to be decided. The difficulties involved in conciliating the results from studies of tissue preparations with observations made on intact animals have been discussed by Stadie and Haugaard (11) and by Dickens (5). It should be pointed out, however, that recent studies with intact animals by Gerschman *et al.* (23) support the hypothesis that oxygen poisoning is caused by an oxidation of essential SH— groups. These authors found that mice were protected against death from high oxygen pressure by previous injection of the sulfhydryl compounds glutathione, cysteine, β-mercaptoethylamine, or BAL. Of importance in this connection are also the studies of Lambertsen *et al.* (24), who estimated that the oxygen tension in the brain cells of man exposed to toxic pressures of oxygen (3 to 4 atmospheres) was far below the tension of oxygen in the inspired gas. These considerations would tend to indicate that the tensions of oxygen that are toxic to cells *in vivo* are of the same order of magnitude as the oxygen tensions found to be toxic to tissue metabolism *in vitro*.

Much remains to be done before we understand the mechanism of oxygen poisoning. However, the evidence that has accumulated in favor of the hypothesis that oxidation of essential SH— groups is involved is impressive.

The authors wish to thank Miss Imelda Rusnock for valuable technical assistance.

SUMMARY

The toxic effect of oxygen on the enzyme systems in heart muscle oxidizing glucose and pyruvate has been studied. It was found that oxygen at a pressure of 1 atmosphere (compared to air) produced a gradual inhibition of enzyme activity. Cupric ions in trace amounts greatly accentuated this toxic action of oxygen. The chelating agent, ethylenediaminetetraacetic acid, protected against inactivation of the enzyme systems by oxygen. The relation of these findings to the phenomenon of oxygen poisoning has been discussed.

BIBLIOGRAPHY

1. Elliott, K. A. C., and Libet, B., *J. Biol. Chem.*, **143,** 227 (1942).
2. Noyons, A. K. M., and Van Goor, H., *Arch. nêrl. physiol.*, **25,** 553 (1940).
3. Mann, P. J. G., and Quastell, J. H., *Biochem. J.*, **40,** 139 (1946).
4. Stadie, W. C., Riggs, B. C., and Haugaard, N., *Am. J. Med. Sc.*, **207,** 84 (1944).
5. Dickens, F., in Elliott, K. A. C., Neurochemistry, Springfield, 631 (1955).
6. Dickens, F., *Biochem. J.*, **40,** 145 (1946).

7. Dickens, F., *Biochem. J.*, **40,** 171 (1946).
8. Stadie, W. C., Riggs, B. C., and Haugaard, N., *J. Biol. Chem.*, **160,** 191 (1945).
9. Stadie, W. C., and Haugaard, N., *J. Biol. Chem.*, **161,** 153 (1945).
10. Haugaard, N., *Nat. Res. Council Nat. Acad. Sc., Pub. 377,* 8 (1955).
11. Stadie, W. C., and Haugaard, N., *J. Biol. Chem.*, **164,** 257 (1946).
12. Barron, E. S. G., Dickman, S. R., Muntz, J. A., and Singer, T. P., *J. Gen. Physiol.*, **32,** 537 (1949).
13. Gerschman, R., in Colowick, S., Glutathione, New York, 288 (1954).
14. Wenner, C. E., Dunn, D. F., and Weinhouse, S., *J. Biol. Chem.*, **205,** 409 (1953).
15. Haugaard, N., and Itskovitz, H., *Arch. Biochem. and Biophys.*, **65,** 229 (1956).
16. Miller, B. F., and Van Slyke, D. D., *J. Biol. Chem.*, **114,** 583 (1936).
17. Wieland, T., in Colowick, S., Glutathione, New York, 51 (1954).
18. Hess, A. F., and Unger, L. J., *Proc. Soc. Exp. Biol. and Med.*, **19,** 119 (1921).
19. Standard values in blood, American Institute of Biological Sciences, National Research Council, 119 (1951).
20. Bean, J. W., *Physiol. Rev.*, **25,** 1 (1945).
21. Bean, J. W., *Nat. Res. Council Nat. Acad. Sc., Pub. 377,* 13 (1955).
22. Barron, E. S. G., *Arch. Biochem. and Biophys.*, **59,** 502 (1955).
23. Gerschman, R., Gilbert, D. L., Nye, S. W., Dyer, P., and Fenn, W. D., *Science,* **119,** 623 (1954).
24. Lambertsen, C. J., Kough, R. H., Cooper, D. Y., Emmel, G. L., Loeschcke, H. H., and Schmidt, C. F., *J. Appl. Physiol.*, **5,** 471 (1953).