

Oxygen Poisoning and X-irradiation: A Mechanism in Common¹

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A CONSIDERATION of various isolated reports in the literature has led us to the hypothesis that oxygen poisoning and radiation injury have at least one common basis of action, possibly through the formation of oxidizing free radicals. This article reviews the pertinent material that led to this hypothesis and also presents the supporting evidence obtained from (i) experiments on the protective action against oxygen poisoning by substances of varied chemical nature known to increase resistance to irradiation, and (ii) experiments on the survival in oxygen of mice irradiated and exposed to high oxygen tensions simultaneously or at different intervals.

Concerning free-radical formation, it is generally believed that the chemical actions of ionizing radiation on aqueous solutions are mainly indirect (1), involving the primary formation of the free radicals $H\cdot$ and $OH\cdot$ with subsequent formation of H_2O_2 , atomic oxygen, and $HO_2\cdot$ (2). In the presence of oxygen, increased amounts of the powerful and quantitatively important $OH\cdot$, as well as the less reactive but more persistent $HO_2\cdot$, would be expected.

Free-radical formation is also expected in normal oxidative metabolism. One mechanism by which molecular oxygen can be reduced is the compulsory univalent transfer of electrons described by Michaelis (3), according to which, in the presence of protons, one may expect the formation of $OH\cdot$, $HO_2\cdot$, and H_2O_2 . Daniels, *et al.* (4) have discussed the possible occurrence of an oxidizing free radical $RO_2\cdot$ during the reduction of oxygen, and several other authors (5-10) have indicated the occurrence of free radicals

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as such or bound with enzymes in normal metabolic reactions. As one of the reactants, it might be expected that increased concentrations of oxygen would increase the formation of oxidizing free radicals.

Indication of certain similarities between oxygen poisoning and x-irradiation results from the study of the many reports in the literature dealing with their effects. On the basis that increased metabolism might result in an increased production of free radicals, and vice versa, it is not surprising that variations in oxygen toxicity with metabolic activity have been noted. Thus, in oxygen poisoning, it has been observed that a decreased metabolism has a protective effect and an increased metabolism has a detrimental effect (11, 12). Several reports indicate that the same may be true for x-irradiation, but this matter has not been conclusively clarified (13-15).

The *in vitro* inactivation of some thiol enzymes has been demonstrated in oxygen poisoning (16, 17), and in irradiation (6, 18). On the other hand, a measurable *in vivo* decrease of SH groups right after irradiation (14) has not been observed. However, one must not necessarily rule out the possibility that the inactivation of SH groups may be responsible in part for the toxic effect of x-irradiation. Thus, Forssberg (19) has postulated that, even though a vital component in the cell may be almost completely protected against x-irradiation, the cell may still be highly sensitive in small but vital areas. This same type of reasoning could be applied to oxygen poisoning. Paul Bert and others (12, 20) have found that oxygen poisoning causes a decrease in metabolism. Some investigators, on the other hand, have not observed *in vivo* a decreased respiration after oxygen poisoning (21) or after x-irradiation (22, 23). However, it should be taken into consideration that oxygen uptake may not always be a reliable index of the energy that can be utilized by the cell. For example, if the phosphorylating mechanisms are altered (24), then, even though the oxygen uptake may not change or may even increase, the production of utilizable energy may be greatly decreased.

In ionizing radiation, there is evidence that the oxidizing free radicals may be responsible for denaturation of enzymes (18) as well as for the depolymerization and other chemical effects on nucleic acids with an associated *in vivo* (25) and *in vitro* aftereffect (4, 26-29). An *in vitro* aftereffect has also been reported for other substances (30, 31).

TABLE 1. The effect of protective agents* in mice submitted to 6 atm of oxygen.

	No. of animals	Mean survival time (min)	Difference (min)	"P" (%)
Glutathione (67 mg)	20	95.7 ± 6.01		
Saline control	20	42.8 ± 1.44	52.9 ± 6.18	0.0
β-mercaptoethylamine (3 mg)	19	76.9 ± 2.72		
Saline control	19	45.0 ± 1.39	31.9 ± 3.53	0.0
25% ethanol (0.7 ml)	14	59.6 ± 2.18		
Saline control	14	33.8 ± 2.07	25.8 ± 3.01	0.0
Propyl gallate (1.7 mg) †	20	86.7 ± 11.2		
Saline control	20	41.8 ± 1.64	44.9 ± 11.4	0.1
Nordihydroguaiaretic acid (2.5 mg)	20	56.1 ± 2.69		
Saline control	20	42.4 ± 1.69	13.7 ± 3.17	0.0
Cysteine (20 mg)	25	59.5 ± 2.80		
5% saline control	24	43.5 ± 1.65	16.0 ± 3.25	0.0
Oxytyramine (5 to 7 mg)	25	48.4 ± 2.71		
Saline control	25	39.9 ± 1.68	8.5 ± 3.19	0.8

* Doses are given per 20-g mouse I.P. Controls included solvents where necessary.

† Propyl gallate in a system containing citric acid and butylated hydroxyanisol.

It has been demonstrated, not only that anoxia decreases the acute lethal effects of ionizing radiations on rats and mice (32, 33) and on several other biological systems, but also that increased oxygen tensions enhance the effect of irradiation (34, 35). Both oxygen and x-rays produce identical aberrations of chromosomes of *Tradescantia* microspores (36) and strikingly similar histological changes in the testis of rats (37). Vitamin E (38-40), vitamin P (38, 41), and cobalt (16, 42, 43) have been shown to give some protection against oxygen poisoning and also against x-rays. Insulin, on the other hand, enhances sensitivity to irradiation (44) and to oxygen poisoning (45, 46).

By virtue of these considerations, we were led to two types of experiments: (i) to test, in the case of oxygen poisoning, the effectiveness of substances that have been demonstrated to protect against x-irradiation; and (ii) to test the combined effect of x-irradiation and oxygen poisoning on the survival of mice.

β-mercaptoethylamine (47), ethanol (48), glutathione (49, 50), cysteine (51, 52), and oxytyramine

(53) have been shown to protect mice against the lethal effect of x-irradiation. Table 1 demonstrates that these agents also increase the survival time of mice exposed to high oxygen tensions. β-mercaptoethylamine is not only of interest because it possesses an SH group, which might account for its protective action, but also because it is part of Coenzyme A. In this connection, Bacq and Herve (54) have found that Coenzyme A, in equimolecular concentrations, seems to be much more active than β-mercaptoethylamine as a protector against x-rays. In some preliminary experiments, we have also used Coenzyme A and have found that 3 mg of Pabst Coenzyme A per 20-g mouse, injected intraperitoneally, has some protective action against oxygen poisoning. Since Coenzyme A is a coenzyme for a number of enzymatic systems other than the pyruvic oxidase system, it would be well to keep in mind the possibility that x-rays and high oxygen pressure might affect any of these systems through a primary action on Coenzyme A.

The possible role of changes produced in the oxida-

TABLE 2. Effect of previous radiation on survival of mice in high oxygen pressures with varying intervals between radiation and oxygen exposure.*

Series	Interval	No. of expts.	Pressure (atm)	Sex	Mean survival time (min)			Std. error of dif. †	"P" (%)
					O ₂	rad. + O ₂	Dif.		
I	Simult.	3	5	m	71.3	56.9	14.4	5.3	0.7
Ia	5 hr	2	5	m	65.0	59.0	6.0	6.4	34.8
II	2 min	2	6	f	49.1	33.9	15.2	2.8	0.0
III	30 min	3	6	f	44.5	39.1	5.4	2.3	1.9
IV	2 hr	4	6	f	35.9	31.4	4.5	1.9	1.8
V	5 hr	3	6	f	35.9	37.2	-1.3	2.3	56.9
VI	18 hr	3	6	f	40.9	42.8	-1.9	2.3	40.7

* In each experiment, 20 mice were used, 10 irradiated and 10 controls (occasionally an observation on one mouse was missed).

† Notes on the statistical analysis of the data: There was no evidence of heterogeneity of variance from one group of animals to another of the same sex. Males varied substantially more than females. Within any series of experiments, there was no evidence of interaction between experiments and treatments. The standard errors for each sex are based on a pooled estimate of the within-group variance from all experiments with animals of that sex.

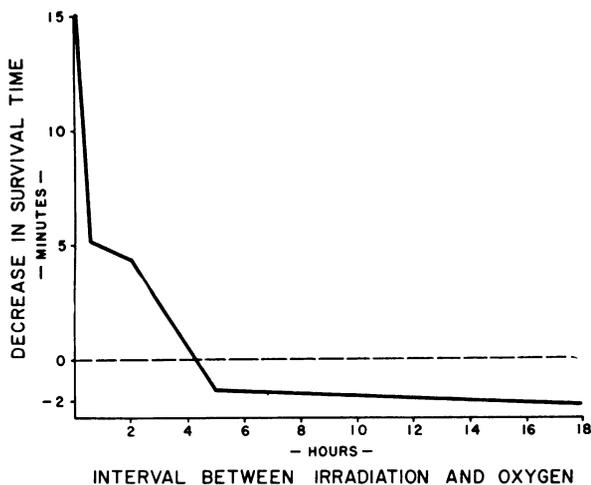


FIG. 1. The ordinates are differences in survival time (min) resulting from exposure to x-irradiation compared with that from exposure to oxygen alone. The abscissas are the intervals between exposure to radiation and to oxygen. The shortest interval is 2 min. The animals remained in the high oxygen until death, and the survival times were measured from the time 6 atm was attained until the time of death. The maximal shortening caused by prior irradiation is 31.0 percent. Data of series II-VI in Table 2.

tion of essential fatty acids by x-rays has been discussed by Mead (55) and by oxygen poisoning by Penrod (56). Of interest in connection with a preferential oxidation of the "antioxidants" propyl gallate and nordihydroguaiaretic acid, demonstrated by Tappel, *et al.* (57), is their protective effect against oxygen poisoning, as shown in Table 1. We have also obtained some evidence suggesting a possible protective effect against x-irradiation injury.

In the second type of study, mice were treated with x-rays and oxygen at the same time and in sequence with various intervening periods. In these experiments, the total time of irradiation was about 35 min, and the dose was approximately 8800 roentgens (r) (58). The survival times with irradiation in oxygen are given in Table 2. In series I, radiation was applied while the mice were in oxygen (59). In series II, oxygen was applied 2 min after the end of radiation and was continued until death occurred. In series III, IV, V, Ia, and VI, a delay of 30 min, 2 hr, 5 hr, 5 hr, and 18 hr, respectively, intervened between the end of radiation and the application of oxygen. Because the experiments with simultaneous irradiation and oxygen poisoning (series I) were done with male mice exposed to 5 atm, and the experiments with varying intervals between irradiation and oxygen poisoning were done with female mice exposed to 6 atm, series Ia was designed to facilitate comparison between these two sets of experiments. When male mice were exposed to 5 atm of oxygen 5 hr after the standard radiation exposure, no significant differences in survival time were noted between irradiated and control mice.

Figure 1 illustrates that irradiation produces an

effect that is synergistic with oxygen poisoning in decreasing the survival time of mice exposed to high oxygen pressure. When x-radiation and oxygen are applied simultaneously or within 2 min of each other, a marked decrease of survival time in oxygen is noted. The effect is still significant, although smaller, when oxygen treatment followed radiation by 30 min and 2 hr, and is completely absent or slightly negative after an intervening period of 5 hr.

From the experiments reported and the considerations presented, it would appear that irradiation and oxygen poisoning produce some of their lethal effects through at least one common mechanism, possibly that of the formation of oxidizing free radicals.

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58. The average survival time following this radiation alone was 4.1 ± 0.11 days in 22 mice.
59. One experiment was also tried in which 10 female mice were exposed simultaneously to radiation and oxygen, with another 10 mice exposed to oxygen only. Unfortunately, these mice could not be observed during the radiation because of danger to the observer; and when observations were resumed, 8 of the experimental and 7 of the control mice were already dead. Since no average survival time could be stated, these results are not listed in the table. Female mice are significantly less sensitive to oxygen than males. At a pressure of 5 atm, the female survival times were so widely dispersed that experimentation was difficult. For this reason, simultaneous exposures to radiation and oxygen could be tried only in males.

Plant Materials Used by Primitive Peoples to Affect Fertility

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RECENT publications (1-3) have stressed the need for finding inexpensive and harmless oral agents capable of controlling human fertility. Numerous agents are known which affect fertility, both in a positive and a negative sense but have side effects, some of which are undesirable. One method of finding useful materials is the screening of naturally occurring substances to discover those with the optimum characteristics that will guide chemists to the synthesis of the ideal drug.

As work goes forward on other approaches, it may be profitable to consider the information acquired by ancient and more remote peoples concerning the use of herbs and various plant materials for fertility control purposes. Such information, almost completely unevaluated, is available in fragmentary form about many plants found in all latitudes. It comes primarily from the collected folklore of primitive peoples, from the literature of so-called "popular medicine" of Western cultures, from 19th-century books on medical botany, and from old *materia medica*.

Information from such sources is to some extent held in question by modern investigators who often dismiss the accounts as superstitions or "old wives' tales." Confidence in such sources of information may be strengthened, however, by recognizing examples of valuable drugs recently isolated from plants used by primitive peoples for generations.

1. Many soils have been claimed to cure human ills; molds from them provide us today with valuable antibiotics (4).

2. The powerful antithrombic agent "Rutin" is found in *Ruta graveolens*.
3. Muscle relaxant Tubo-Curare is extracted from the Indian arrow poison of Guiana (5).
4. The heart-regulating agent, crataegolic acid, and other unidentified active substances are found in *Crataegus oxyacantha* (6).
5. The alkaloids of Indian *Rauwolfia serpentina* reduce blood pressure and act as a general sedative (7).
6. The glucoside Foliandrin from the oleander shrub provides a cardiac stimulant of great value to elderly patients (8).
7. The coronary stimulant "khellin" is obtained from *Amni visnaga* (9).
8. The antimalarial "Febrifugine" from Hydrangea (ancient Chinese Chang-Shan roots) has been isolated and synthesized very recently. (10).

Even greater confidence may be gained by referring to the recent scientific literature (2) on the desert herb, *Lithospermum ruderales*, used by Indians in the southwestern part of the United States for fertility control purposes.

The authors, working separately at first, have accumulated a list of more than 100 plants reported to contain substances that affect human reproduction. These have been classified into three categories:

Oc—so-called oral contraceptives thought to cause temporary sterility.

Oc₁—substances which in certain doses are believed to interfere with implantation or gestation but which might well prove to be in class *Oc* if used in smaller concentrations.

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