Interactions of Oxygen at High Pressure and Radiation in *Drosophila*

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ABSTRACT Oxygen at high pressure (OHP) and X-irradiation can interact in the fruit fly *Drosophila melanogaster* to potentiate toxic actions characteristic of one agent alone. 1000 kvp X-irradiation in doses of 30, 60, and 75 kr accelerated the acute immobilization of young male *Drosophila* by oxygen at 7.8 atm, up to rates twice that observed with such oxygen pressure alone. X-irradiation alone in these dosages did not acutely immobilize the *Drosophila*. X-irradiation *during* exposure to 7.8 atm pO_2 was more effective and consistent in producing this potentiation than was X-irradiation that preceded exposure to OHP. Acute OHP toxicity in young female *Drosophila* was not potentiated by 75 kr of X-irradiation. On the other hand, shortening of the life span of young male *Drosophila* by the above doses of X-irradiation was augmented significantly by a concurrent 40 min exposure to OHP (which alone did not significantly decrease life span). This shows, for the first time, that oxygen can affect not only the acute effects of radiation, but also the residual irreversible effects indicated by the life span shortening.

INTRODUCTION

The similarity of the toxic effects of oxygen at high pressure (OHP) to the deleterious processes produced by ionizing radiation has been observed by many workers in the past, but has never been so emphatically stressed as by Gerschman and her colleagues with their "free radical theory" for oxygen toxicity (1, 2). In all likelihood molecular oxygen does react in biological media through the formation of free radicals, both in normal physiological energy-yielding processes and in pathological destructive phenomena. Other than the observation that many agents which "protected" mice against the acute effects of X-irradiation also seemed to "protect" mice against oxygen at high pressure, the most relevant finding by Gerschman's group was that X-irradiation, immediately preceding or concurrent with exposure of mice to oxygen at high pressure, appeared to "potentiate" the acute lethal effects of oxygen (1, 2). The small but statistically valid augmentation of the lethal effects of oxygen could be interpreted as caused by an increased "supply" of

free radicals to initiate "chain reactions" with oxygen. Perhaps another explanation would be that the X-irradiation stimulated the release of adrenal corticosteroids (3) which are well known to potentiate the toxic effects of oxygen at high pressure on mammals (4, 5).

To confirm these important results we have used the fruit fly, *Drosophila melanogaster*. This animal was chosen because it is convenient to handle in large numbers, and because careful studies have already been made of its reactions to both oxygen (6) and to radiation (7). The results (*a*) confirm Gerschman's finding that oxygen at high pressure kills more quickly if accompanied by simultaneous radiation which by itself is not lethal for 2 wk, and (*b*) show that X-irradiation shortens the life span more if accompanied by simultaneous high pressure of oxygen, even though the same dose of oxygen by itself had little or no effect.

MATERIALS AND METHODS

Test Animals

For most experiments reported in this paper we have used 2 to 3 day old male *Drosophila melanogaster* of the Swedish R wild type strain originally obtained (by R. C. B.) from Cold Spring Harbor in 1956. In preliminary studies flies of various ages were employed in demonstrating again that sensitivity to oxygen as well as oxygen consumption increases with age. With the Fenn respirometer it was found that a 2 to 3 day old male fruit fly consumes O_2 at the rate of about 2 μl/hr (8), a rate which is quite similar to that observed by previous investigators (9), and which is unchanged subsequent to a 40 min exposure to oxygen at 7.8 atm absolute pressure. In a limited number of experiments, female *Drosophila* were also studied. Sexes were separated after light etherization at least 4 hr and usually 18 hr previous to exposure to radiation and/or oxygen at high pressure, because ether anesthetization of flies immediately preceding (within 1 to 2 hr) their exposure to oxygen at high pressure appeared to alter their sensitivity to OHP. Flies were maintained in an air-conditioned room at 25°C on a standard diet of brewer's yeast, molasses, and corn meal, incorporated in an agar gel containing the antimold agent methyl-*p*-hydroxybenzoate. Thirty to 100 flies were kept in shell vials (20 × 75 mm OD) each containing 2 to 3 cc of food, and stoppered by a piece of nylon stocking held in place by a rubber band. During experiments in which life span was measured, the live flies were usually changed daily, or every other day, to fresh vials while the number of deceased flies was counted and discarded.

Exposure to Oxygen at High Pressure

Flies were shaken from their vials through a small funnel into the narrow, cylindrical inner chamber of the lucite pressure vessel shown in Fig. 1. Up to 40 flies could be easily observed within the 4.4 cc of the inner chamber, which was approximately 0.96 cm in diameter and 6.2 cm in length. After a flush with O_2 for 15 to 30 sec, the O_2 pressure was steadily raised to 7.8 atm (abs) over approximately 30 sec. Such a

"slow" compression was utilized because very rapid or explosive compression accelerated the toxic effects of oxygen. Likewise, decompression was "slowly" performed in about 1 min because explosive decompression of flies exposed to air or O_2 at 7.8 atm for more than 30 min caused many flies previously mobile to be at least temporarily incapacitated. During the experiments the lucite chamber was immersed in a water bath usually maintained at 24 ± 0.3°C.

During the exposures to oxygen at high pressure, observers noted the behavior of the flies at intervals of 5 min or less, and recorded the number of flies which could or could not maintain an erect posture. For simplicity, this was recorded as the "number of flies that remained *up*." The CO_2 output of 40 such flies in the 4.4 cc chamber for 1 hr would not be expected to raise the pCO_2 more than 14 mm Hg. For longer exposures the CO_2 could be flushed out periodically without serious change in the pressure.

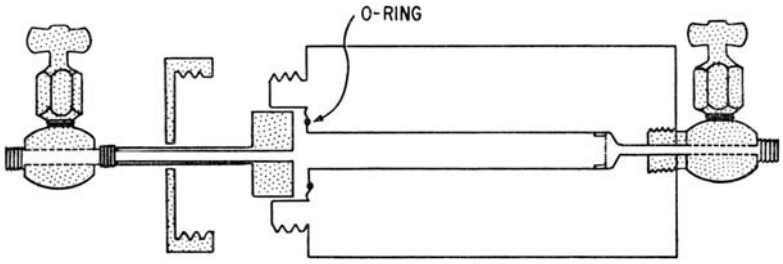

FIGURE 1. Longitudinal view of the cylindrical pressure chamber showing the body of lucite capped by a screw-on brass head piece. Dimensions are noted in the text.

Irradiation

For most exposures of *Drosophila* to X-irradiation, a 1000 kvp General Electric industrial X-ray unit was employed without added filtration; this machine produced an X-ray beam with a half-value layer of 3.8 mm of lead while operating at a current of 3.0 ma. Usually the pressure chamber containing the flies to be radiated was so placed that its longitudinal axis was 10 cm from the X-ray target, while a control chamber could be placed within a lead shield, to the side of and well behind the primary beam. Radiation exposures were estimated by a Victoreen Radacon Rate Meter using a thimble-type ionization chamber placed within a model lucite tube similar to the experimental chamber. While absolute exposure rates cannot be determined precisely with this instrumentation, the measured exposure rates within the model chamber were consistently between 6200 and 6400 roentgens per minute (R/min). Because of this purposely high dose rate (designed to give the flies an exposure of as high as 75 kr in "only" 12 min), and also because of a time lag (about 12 sec) in achieving full X-ray power, the commonly employed Victoreen R chambers could not provide satisfactory measurements, except in demonstrating that the shielded control flies received less than 1 R.

All radiation exposures with oxygen at high pressure were begun as soon as possible after 7.8 atm pO_2 was attained (usually 3 to 4 min) in the lucite pressure chamber,

using the 1000 kvp machine. However, in certain experiments the flies were exposed while inside a perforated gelatin capsule, inside an 18 cc food vial, or inside a specially modified 10 cc syringe. With the latter device, 40 to 100 *Drosophila* could be comfortably maintained within a disclike space, 2 mm thick, between the end of the plunger and a piece of nylon stocking fitted over the opened end of the barrel of the syringe. X-irradiation of flies within such chambers could be satisfactorily performed only at atmospheric pressure, with a subsequent delay in pressurization; but, on the other hand, less energetic X-rays could be employed for experiments testing effects of *previous* X-irradiation. For this purpose a Norelco dental X-ray unit, operated at 50 kvp and 2 ma with a beryllium window and a 0.06 mm aluminum filter, was utilized, producing an X-ray beam with a half-value layer of 0.064 mm of aluminum and dose rates of about 16,000 R/min at 3 cm from the X-ray target. A 250 R Victoreen ionization chamber was used to measure such radiation exposures.

RESULTS

Acute Effects

Quantitative data have usually been calculated in terms of the *mean* and the *median* duration of OHP exposure that was necessary to inhibit ability to assume and maintain erect stance. For brevity, such a duration is termed "up time." In employing these criteria in preliminary experiments using only hyperbaric oxygen, we observed characteristics of acute oxygen toxicity that were similar to those reported by Williams and Beecher (6): toxicity of oxygen varied as the oxygen pressure, the age of the flies, the temperature of the water bath, and the partial pressure of CO_2. Qualitatively, the initial response of flies exposed to oxygen at high pressure was a brief period of hyperactivity which was followed by a somewhat longer period of apparently normal activity. This intermediate stage of apparent normalcy gradually merged into a progressive state of lethargy and lack of flight, followed by the inability to maintain erect stance, then loss of spontaneous movements, and finally loss of reflex excitability and death. When flies were exposed to greater than 30 kr of X-irradiation during or before exposure to 7.8 atm pO_2, the most striking observation other than decreased up times was the almost immediate appearance of a very lethargic state, in which the flies could maintain their stance, but would remain almost motionless.

In Fig. 2 we have summarized the quantitative observations recorded during 7 successive experiments in which groups of 40 or less male flies were irradiated immediately after pressurization to 7.8 atm pO_2, for comparison with concurrent control OHP experiments (without radiation). Each point represents the mean value (averaged for the stated number of experiments) of the percentage of flies still standing after the indicated duration of exposure to hyperbaric oxygen. This kinetic presentation clearly demonstrates that 30, 60, and 75 kr during exposure to hyperbaric oxygen significantly accelerate

the incapacitation of flies by 7.8 atm pO_2. The *Drosophila* which were exposed to similar doses of X-irradiation in air or oxygen at atmospheric pressure within the same chamber were able to maintain stance, activity, and flight for many days after some brief (at most—10 min) but transient mild lethargy. However, 1000 kvp X-ray exposures greater than about 80 kr at atmospheric pressure caused immediate albeit temporary disability of a significant number of flies; therefore, we have not included such experiments in this report. Very

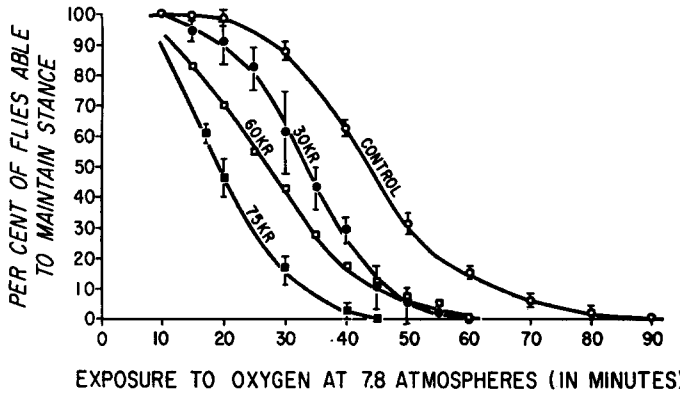

FIGURE 2. Time course of immobilization of *Drosophila melanogaster* by oxygen at high pressure, with and without simultaneous X-irradiation. In each experimental trial 35 to 40 male flies were exposed to oxygen at 7.8 atm. For the control (non- X-irradiated) flies, each point, represented by an open circle (○), indicates the mean of the findings from 8 separate trials; for flies simultaneously exposed to 75,000 R, each point, represented by a closed square (■), indicates the mean of the findings from 4 separate trials; for flies simultaneously exposed to 30,000 R, each point, represented by a closed circle (●), indicates the mean of the findings from 2 separate trials; for flies simultaneously exposed to 60,000 R, each point, represented by an open square (□), indicates a finding from a single trial. The vertical brackets enclose one estimated standard error of the mean, in either direction, for the number of trials stated above.

similar curves were found in another series of similar experiments using 100 (instead of 40) male flies exposed to 0, 30, 60, and 75 kr at 7.8 atm pO_2.

The data tabulated in Table I in terms of mean up times reemphasize the conclusion that simultaneous X-irradiation of young male *Drosophila* with appropriate doses increases the rate of onset of the toxic effects of hyperbaric oxygen (the use of the median up time yielded comparable results). It can be seen from experiment 8 that preoxygenation of flies with 100% oxygen may sensitize them to subsequent exposure to OHP, with or without concurrent exposure to X-irradiation. More notably, concomitant X-irradiation did not appear to accelerate significantly the acute effects of OHP upon female *Drosophila* as measured by up time in oxygen at 7.8 atm. However, rapid onset of lethargy was seen with X-irradiated females at OHP, with a severity

TABLE I

IMMOBILIZATION OF *DROSOPHILA* BY OXYGEN AT 7.8 ATM WITH AND WITHOUT CONCURRENT X-IRRADIATION

Experiment No.	Exposure	Sex	No. of flies studied	Mean up time	Ratio Experimental / Control
	kr			*min*	
1	0	Male	22	43.9	1.00
	75	Male	30	23.6	0.54
	75*	Male	30	28.6	0.65
2	0	Male	40	44.6	1.00
	75	Male	39	19.9	0.45
	75*	Male	40	25.2	0.58
3	0	Male	40	49.1	1.00
	75	Male	40	28.6	0.58
	0	Female	40	66.1	1.00
	75	Female	40	61.6	0.92
4	0	Female	39	47.9	1.00
	75	Female	40	38.9	0.81
5	0	Female	40	53.5	1.00
	75	Female	36	58.2	1.09
6	0	Male	40	48.6	1.00
	30	Male	40	36.2	0.73
	60	Male	40	29.4	0.59
7	0	Male	54	52.3	1.00
	30	Male	39	39.0	0.74
8‡	0	Male	45	37.1	1.00
	30	Male	36	24.7	0.67
9	0	Male	40	50.1	1.00
	20	Male	40	55.4	1.10
	10	Male	40	52.4	1.04

* X-irradiation was performed in three doses of 25 kr each, spaced about 4 min apart.

‡ Both groups of flies had been preoxygenated with 100% O_2 for ½ hr before pressurization.

similar to that observed with the males. There was no significant difference between the behavior of male and female *Drosophila* with oxygen alone at high pressure. The slight increases in up time observed when 10 or 20 kr were applied to male *Drosophila* at OHP are not considered significant without further data.

In some experiments the 12 min 75 kr exposures were divided into 3 parts, with 4 min intervals between, so that the flies could be counted during this critical period. This use of a divided dose resulted in a small increase in up

TABLE II

EFFECTS OF X-IRRADIATION BEFORE THE EXPOSURE OF MALE *DROSOPHILA** TO OXYGEN AT 7.8 ATM

Experiment No.	X-irradiation			Delay between X-irradiation and OHP	No. of mobile flies before OHP	Mean up time	Ratio Experimental / Control
	Peak kilo voltages	Exposure	Location of flies during X-irradiation‡				
		kr				*min*	
1	0	0	*a*	—	16	45.6	1.00
	1000	75§	*a*	2 min	40	22.3	0.49
	0	0	*b*	(25 hr)	37	46.2	1.00
	1000	75§	*a*	25 hr	31	28.8	0.62
2	0	0	*a*	—	40	57.2	1.00
	1000	75§	*a*	2 min	40	14.3	0.25
	1000	75§	*a*	20 min	40	21.0	0.38
	1000	75§	*a*	60 min	38	10.3	0.18
	0	0	*b*	(6 days)	36	50.6	1.00
	1000	75§	*a*	6 days	37	74.4	1.47
3	0	0	*a*	—	40	58.8	1.00
	1000	75	*a*	3 min	40	41.8	0.71
	1000	75	*a*	−18 min‖	40	34.2	0.58
	1000	75	*b*	3 min	40	51.9	0.88
4	0	0	*c*	0	40	50.7	1.00
	50	30	*c*	3 min	39	40.2	0.78
	50	60	*c*	6 min	40	41.0	0.82
5	0	0	*c*	0	32	33.2	1.00
	50	30	*c*	11 min	26	23.4	0.70
	0	0	*b*	0	39	36.1	1.09

* 2 to 3 day old flies were tested in all experiments except experiment 3 in which 1 to 2 day old *Drosophila* were studied, and experiment 5, in which 11 to 12 day old *Drosophila* were used.

‡ Location of flies during X-irradiation: (*a*) lucite pressure chamber; (*b*) food vial; (*c*) specially modified syringe.

§ In these experiments the flies were maintained in 100% O_2 at 1 atm during period of X-irradiation. In other experiments they were irradiated in air.

‖ In this trial, X-irradiation was performed with oxygen at 7.8 atm for comparison with accompanying trials.

time suggesting some degree of recovery in 4 min, but this difference was not statistically significant for the number of experiments involved.

When the *Drosophila* were irradiated before exposure to oxygen at high pressure (Table II), the results were less clear cut but definite, particularly

when the flies were X-irradiated in 100% oxygen at 1 atm inside the lucite pressure chamber by the 1000 kvp machine. Interestingly, up time was still decreased 1 day after X-irradiation (experiment 1), but was prolonged in one experiment after a 6 day interval between X-irradiation and exposure to hyperbaric oxygen (experiment 2). The unexpectedly large decreases in up times at 20 and 60 min after X-irradiation (experiment 2) could have been caused by insufficient replacement of oxygen by air during the waiting period. Such prolonged preoxygenation could have added to the effects of

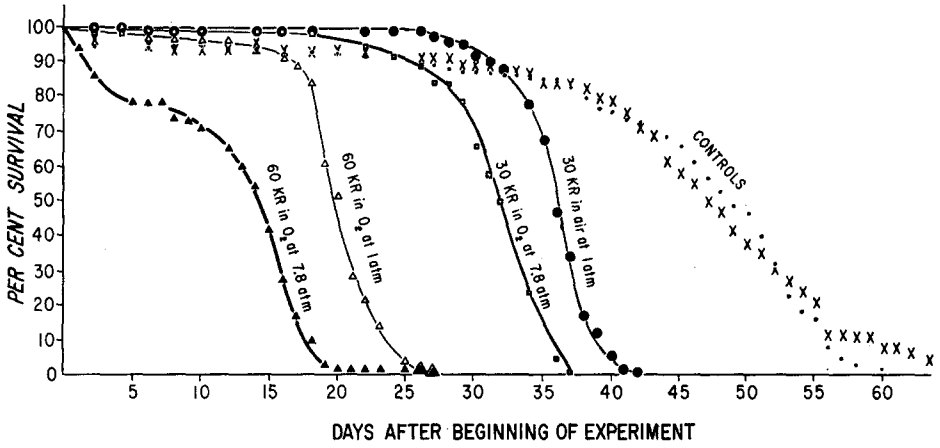

FIGURE 3. Shortening of life span of *Drosophila melanogaster* by 30,000 and 60,000 R of 1000 kvp X-irradiation, with and without concurrent exposure to oxygen at 7.8 atm. 100 male flies were studied in each experimental trial. Control data without X-irradiation were obtained from 40 min exposures in the lucite pressure chamber to air at 1.0 atm (represented by the *small* closed circles— •) or to oxygen at 7.8 atm (represented by the crosses—×). For X-irradiation of flies in the lucite pressure chamber, the data are represented by: (▲) for 60,000 R with 40 min in oxygen at 7.8 atm; (△) for 60,000 R with 40 min in oxygen at 1.0 atm; (□) for 30,000 R with 40 min in oxygen at 7.8 atm; (●— a *large* closed circle) for 30,000 R with 40 min in air at 1.0 atm.

preirradiation. Effects of a lesser magnitude were seen in experiment 3 when X-irradiation was performed in air instead of 100% O_2, inside or outside the pressure chamber.

Observations with the *soft* 50 kvp X-irradiations suggested that OHP toxicity in young male *Drosophila* was enhanced by such preirradiation (experiments 4 and 5). Unfortunately, the results with the lower exposures (30 and 60 kr) are not supported by a sufficient number of experiments for a conclusive opinion. Results with 75 kr of 50 kvp X-irradiation are not included, because this exposure immediately disabled many of the flies.

Effects on Life Span

The well known "oxygen effect" on the biological effects of radiation has usually involved a comparison between atmospheric oxygen and no oxygen.

Our experiments have involved rather a comparison between atmospheric oxygen and OHP at 7.8 atm. The survival curves of Fig. 3 show that exposures to oxygen at this pressure for 40 min have little or no effect on the total life span of the flies, but such exposures do enhance the life-shortening effect of simultaneous radiation (30 or 60 kr). Similarly, the curves in Fig. 4 show that an exposure to 75 kr of 1000 kvp X-irradiation is more effective in shortening the life span if given simultaneously with OHP. Even at 1 atm of oxygen the radiation is more effective than if given in air. At reduced oxygen tension

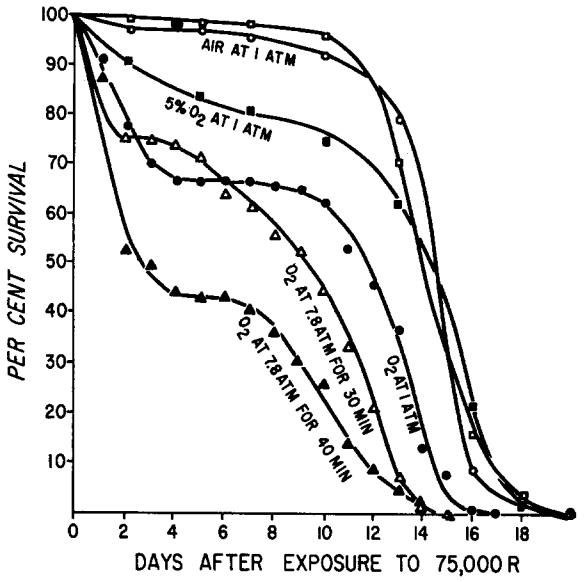

FIGURE 4. Shortening of life span in male *Drosophila* by 75,000 R of X-irradiation during exposure to various pressures of oxygen. X-irradiations were performed in: (■) 5% O_2 in N_2 at 1 atm in the pressure chamber for 20 min; (□) air at 1 atm in a glass vial; (○) air in the pressure chamber at 1 atm for 20 min; (●) O_2 at 1 atm in the pressure chamber for 20 min; (△) O_2 at 7.8 atm for 30 min; (▲) O_2 at 7.8 atm for 40 min.

(5%) there were some early deaths, perhaps due to some degree of anoxia or to the slight trauma involved in transferring the flies to the lucite chamber. The anoxia was not sufficient, however, to provide any protection from the radiation. In general, all these survival curves tend to be rectangular in shape, indicating that all the flies in any one group tend to die at approximately the same time. This is a sign of a "well kept homogeneous population" according to Casarett (10), and the displacement to the left by radiation, without diminishing the rectangularity, is a sign of "premature" aging (10).

The early deaths shown especially in Fig. 4, and at 60 kr in Fig. 3, make calculation of a median life span difficult. Since this effect seems to be due to some process other than aging, we have arbitrarily calculated the life span from the part of the curve beginning at the plateau. Thus, the initial number

of flies was taken as the number alive 2 days after the exposure to radiation. Qualitatively the conclusion is the same whichever way the curve is treated. The figures so obtained are shown in Table III. Here there is indication of

TABLE III

LIFE SPAN OF *DROSOPHILA* AFTER SIMULTANEOUS EXPOSURE TO X-IRRADIATION AND OXYGEN AT 7.8 ATM

Oxygen exposures		X-irradiation exposure	No. of experiments	Total No. of flies	No. of flies alive 2 days after exposure	Corrected* avg‡ median life span ± SEM
pO_2	Duration					
atm	*min*	*kr*				*days*
0.21	(Controls)	0	5	350	345	46.8±2.4
7.8	30	0	1	112	107	44.1
7.8	40	0	3	311	301	45.1±2.1
7.8	60	0	1	22	20	39.3
7.8	75	0	2	77	61	34.6±0.4
0.21	20	30	1	100	100	35.9
7.8	40	30	1	100	99	32.0
1.0	40	60	1	100	100	20.1
7.8	40	60	1	100	87	14.8
0.05	20	75	1	87	79	14.2
0.21	20	75	2	209	204	14.3±0.0
1.00	20	75	1	115	89	12.8
7.8	20	75	1	100	88	11.0
7.8	30	75	1	114	87	10.5
7.8	40	75	1	115	61	9.9
7.8§	40	75	1	40	39	13.2
7.8‖	50	75	1	40	27	11.6
7.8‖	60	75	3	120	106	11.4±1.4

* "Corrected" median life span signifies that the median was determined from the number of flies remaining alive 2 days after exposure.

‡ Averages of the median life spans were determined when more than one experiment was used with an estimated standard error of the mean (SEM) calculated for each average (mean) of the medians.

§ OHP preceded X-irradiation in O_2 in 1 atm.

‖ OHP followed X-irradiation in air at 1 atm.

some shortening of the life span due to oxygen alone for 60 to 75 min, but no effect at 40 min or less. When combined with irradiation, however, oxygen regularly enhances the toxic effects of radiation. In the last 3 lines of Table III it appears that oxygen enhances the effect of radiation even though it is given just before or just after the radiation. These effects, however, are small and the post-X-irradiation exposures to OHP (50 and 60 min respectively) were of sufficient duration to significantly shorten the life span. Note that

there also seem to be some irreversible effects of oxygen alone at sufficiently high dosages which eventually shorten the life span. It is consequently not certain just how simultaneous the exposures to radiation and OHP must be to produce this type of summation.

DISCUSSION

By these simple experiments we have found that X-irradiation can markedly potentiate the acute neurological effects of oxygen at high pressure. This phenomenon provides further and more definite evidence to suggest that oxygen exerts its toxic effects as well as its physiological benefits via free radical intermediates. Most likely this toxic potentiation is brought about by the transformation of the sluggish oxidant O_2 to very potent oxidants such as $HO_2^{\cdot}$, O_2^{-}, and organic peroxy radicals. Such powerful and relatively long lived oxidants would be far more effective than molecular oxygen in high concentration alone. Actually, O_2 does need activation to a higher energy (and oxidizing) state to exert any of its actions, for reasons well explained by Gilbert (11).

X-irradiation during exposure of *Drosophila* to hyperbaric oxygen did provide strong evidence in support of the data with mice obtained by Gerschman's group (1). We should mention that for a fly 75 kr is an intermediate dose; it produces temporary immobilization, but is not lethal for about 2 wk. The dose used by Gerschman et al. (1) in similar experiments on mice was considerably larger relative to the sensitivity of the animal to radiation; she used 8.8 kr which killed the mice in 4 days. However, radiation preceding exposure to oxygen at high pressure did not reveal such large and consistent accelerations of the acute neurological effects of OHP. Persistence of organic peroxy radicals probably is of relatively short duration in animals such as fruit flies as compared to long persistence of organic radicals in bacterial spores (12) or seeds (13). The long persistence of a significant number of free radicals in hydrated animal tissues is extremely improbable according to present concepts. However, scanty knowledge of such molecular events in living tissues precludes any definite conclusion on this point. Evidently, some effects did persist, however, and we cannot explain delayed potentiation of OHP toxicity in fruit flies as easily as we did with mice, in which release of corticosteroids by X-irradiation could enhance the effects of oxygen.

There was, further, a tendency for preirradiation in the pressure chamber to be more effective and consistent in potentiating subsequent O_2 effects than was irradiation in the modified syringe. In the case of X-irradiation in the pressure chamber, the flies were subjected to bombardment by electrons as well as by electromagnetic radiation, since the chamber acted like an ion chamber. Dose measurements with the thimble-type ion chamber within the pressure chamber measured only the electromagnetic radiation and neglected

the electron radiation. The latter could have contributed another 5% of the total dose, as suggested by incomplete measurements using glass-rod dosimetry. It is noteworthy that X-irradiation with the "soft" x-rays of the 50 kvp machine also potentiated subsequent effects of oxygen at high pressure. Consistent with most other reports, the acute lethal effects of soft X-irradiation indicated a higher biological efficiency than with the "harder" 1000 kvp radiation.

Why the female *Drosophila* did not show the impressive radiation potentiation of OHP toxicity as measured by up time is not at all clear, although it is generally accepted that female flies are usually more resistant to radiation than are males. Nevertheless, the females were rendered lethargic by combined OHP and X-irradiation to the same extent qualitatively as were the males.

The enhancement of the effects of X-irradiation by concurrent hyperoxygenation, as measured by shortening of the life span, is an interesting, though not so sharply defined a phenomenon, as is the previously discussed interaction. The principal reasons for difficulty with observations on this type of phenomenon were (*a*) tissue pO_2 was not known; (*b*) acute lethal effects interfered with accurate estimation of the life span when higher doses of X-irradiation were used; (*c*) the oxygen pressure used was perhaps too high for the dosages of X-irradiation employed. Nevertheless, it is obvious that oxygen has an effect on the life span shortening by radiation, and this effect occurred when oxygen was applied at a pressure greater than atmospheric. Both these features are interesting when considering the opinions of Gray (14) and Bacq and Alexander (3) that there is no "O_2 effect" on life span shortening induced by radiation. Of course, the magnitude of the OHP-induced potentiation of life span shortening by X-irradiation was relatively small (never by a factor greater than 1.4) as compared to many other studies of oxygen potentiation of X-irradiation effects on *other* parameters (factors up to 2.5). Likewise, such previous experiments contrasted effects of radiation with various levels of oxygen against effects *without* oxygen, which we could not do in these experiments. Therefore, our findings are not strictly comparable with classical measurements of the oxygen effect on radiation damage. Nevertheless, these experiments have demonstrated that high pressure oxygen can influence and potentiate the effects of X-irradiation on the life span beyond the effect of atmospheric oxygen. Also, one must not overlook the fact that repeated doses of oxygen at less than atmospheric pressure can also shorten the life span in *Drosophila* (8) just as did oxygen at 7.8 atm for 60 min or longer.

As an important side light, these experiments further support the findings of Baxter and Tuttle (7) that the median life span of *Drosophila melanogaster* is shortened roughly in proportion to the exposure to ionizing radiation

(gamma radiation from Co^{60}). Although Sacher (15) has reported an increase in survival of heavily irradiated *Drosophila* through periodic exposures to X-irradiation, his data from single massive exposures (50 to 100 KR) are in good agreement with our single dose experiments and those of Baxter and Tuttle (7). It should be pointed out that the life spans of Sacher's controls were in some cases abnormally short and quite variable. This might explain his conclusion that periodic exposure to X-irradiation served to lengthen survival.

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