

Oxygen Poisoning in *Drosophila*

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ABSTRACT Fruit flies live longer at the partial pressure of oxygen found in air than at either larger or smaller partial pressures. Flies exposed to 1 atm of oxygen for 8 hr every day do not recover completely in the remaining 16 hr. In general, intermittent exposures to 1 atm of oxygen are better tolerated than continuous exposure to the same average oxygen concentration per day, but exposures to higher pressures of 2–5 atm of oxygen for as little as a half hour every two days markedly shorten the life-span. Older flies consume more oxygen per minute and are more sensitive to oxygen poisoning than young flies, and the rate of dying in 6 atm of O₂, or the reciprocal of the survival time, is a linear function of the age. The oxygen pressure-time curve can be well expressed by the general empirical equation $(P_{O_2})^2 \times \text{time} = 120$ where P is in atmosphere and survival time in hours. The progress of oxygen poisoning appears to be linear with time rather than exponential.

The most important previous study of oxygen poisoning in adult fruit flies is by Williams and Beecher (1944). Clark et al. (1954, 1958, 1960, 1961) have studied particularly the sensitivity of insect pupae which, in special cases at least, are irreversibly damaged by as little as 1 min exposure to 30 psi of oxygen. Goldsmith and Schneiderman (1956) have also observed the poisoning of pupae of *Mormoniella* by 1.6–10 atm of oxygen. In some respects our findings merely confirm those of Williams and Beecher, but the life-span at a wide range of different oxygen tensions and the effects of intermittent exposures have not been previously investigated. It does not seem to have been demonstrated previously, for any species, that the oxygen tension in air at sea level is, in fact, optimal for survival. Our studies of the rates of recovery from oxygen poisoning and the effects of high pressures of inert gases at different pressures of oxygen will be presented in later papers.

METHODS

The flies used in these experiments came from one culture of *Drosophila melanogaster*, the Swedish R wild type, obtained by Mr. R. C. Baxter of the Department of Radiation Biology, in 1956, from Cold Spring Harbor Laboratory. The flies were raised in

300-ml jars covered with nylon gauze over food made of 470 ml H₂O, 3.4 g agar, 50 ml molasses, 7 g brewer's yeast, 50 ml cornmeal, and 2.4 g methyl-*p*-hydroxybenzoate (dissolved in 70% alcohol). This is cooked until thickened and allowed to set solid in the culture vessels. The flies were kept in a constant temperature incubator at 24°C. Each day the newly hatched flies were removed to small vials containing food and set aside for experiments so that the age of the flies was usually accurately known.

For experiments under pressures above 1 atm, about 20–30 flies were placed in a Lucite chamber made of a cylinder 9 cm long and 5 cm outside diameter. The inside diameter was 1 cm. The brass cover was sealed by an O ring as shown in Fig. 1 of Thomas, Baxter, and Fenn, 1966. The inside of the chamber was polished after drilling to make it optically clear so that dead flies could be easily recognized. These chambers were attached to suitable gas tanks by pressure tubing and reduction valves and were immersed in a water bath during the experiment. For long experiments, the chamber was flushed out at intervals to remove CO₂, or a small capsule of soda lime was included to absorb it. In some cases it was also necessary to flush out the chamber to renew the oxygen.

For some of the experiments where frequent readings were not necessary, the flies were kept in vials 7.7 cm long and 1.9 cm in diameter, closed with nylon gauze, and the vials were inserted into an iron chamber which could withstand the desired pressures. In such cases soda lime was included in the chamber to control the CO₂. In this iron chamber many vials could be exposed at once.

For experiments at 1 atm pressure the vials were enclosed in jars of 300–500 ml capacity, closed with a two-hole rubber stopper so that the chambers could be flushed out everyday with the desired gas mixture. These jars were then kept in the incubator at 24°C and were inspected daily as needed. For long experiments the flies were changed to new vials with fresh food at least every 2 days.

RESULTS

1. *Life-Span at Different Oxygen Tensions*

In these experiments flies 1–4 (usually 1–3) days old were divided into two groups, control and experimental. Control flies were in air at 1 atm while the experimental flies were in different percentages of oxygen in nitrogen at 1 atm. Each group numbered about 30 flies in vials with about 8 mm of food at one end and the open end closed with nylon gauze.

In series A males and females were contained in the same vials but were scored separately, and the vials were kept upright in the jars. Under these conditions flies died more rapidly because they tended to get stuck in the food on the bottom of the vial. In series B the vials were kept on their sides with the food at one end. Under these conditions the absolute survival time was greater, but the effect of oxygen on the survival time in percentage of the control was about the same. In series B there were fewer experiments, and for the most part, only males were used.

In series C the technique was essentially the same, with the vials on their sides, and both males and females were included in the same vial. In most cases (but not all) the two sexes were scored separately. Series C was used to supplement some of the gaps in the data of series A and B. In all three series the observed survival times of all the flies were averaged, and the standard deviations of the means were calculated. The control flies numbered 537 males and 505 females in 35 vials in series A, 500 males in 14 vials in series B, and 484 flies of both sexes in 17 vials in series C. The average control life-span was 30 days.¹

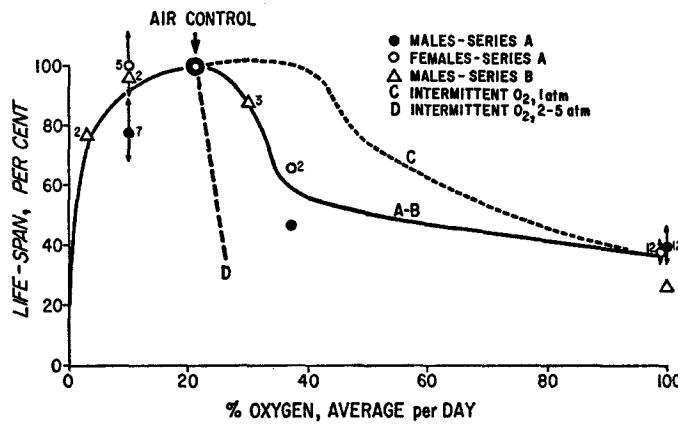


FIGURE 1. Survival time of flies in per cent of air control in different concentrations of oxygen at 1 atm pressure, solid line. Numbers on curve indicate number of experiments averaged. Vertical double arrows indicate standard deviations of the mean. Broken line C represents the data for intermittent exposures from Fig. 3. Broken line D represents intermittent exposures to high pressures from Fig. 4.

The data from series A, B, and C are all collected in Fig. 1 where the life-span in per cent of the control life-span is plotted against the concentration of oxygen in percentages of 1 atm. Most of the data come from series A and B but some figures from series C are included in the averages. The figures beside the various points represent the number of vial pairs (experimental and control) averaged together for that point. In some cases there were enough vials to calculate a standard deviation of the mean, and this is indicated by double arrows. The solid line represents the general trend of these points. The curve shows that the life-span is greatest in air and falls off at

¹ The numbers of these control vials exceeded the number of control vials listed in Fig. 1 because the air controls of the experiments in Fig. 3 were included. In general, females lived 1.23 ± 0.12 times longer than males when exposed to air only. There was a very wide variation in the life-spans of individual flies, some dying in the first week and the longest life-span being 100 days. This may indicate that some environmental factors, such as the wetness of the food, were not optimally controlled.

both higher and lower oxygen pressures. There is little or no decrease in life-span in going from air to 10% oxygen, but in 3% oxygen, there is a definite decrease presumably due to some hypoxia. The rather precipitous decrease in life-span above 30% is supported by rather few points and may be in reality less abrupt. In 1 atm of pure oxygen the life-span was only about 40% of that in air.

The broken lines in Fig. 1 represent the results of some intermittent exposures to oxygen and will be referred to later. We have also made a few observations of the survival times of flies kept in a pressure chamber at normal oxygen tension (0.21 atm) but without any nitrogen. The figures are too few to be quantitatively significant, but we could find no evidence that the presence of nitrogen made any difference. The experiments were a little difficult because frequent return to normal air was necessary to provide

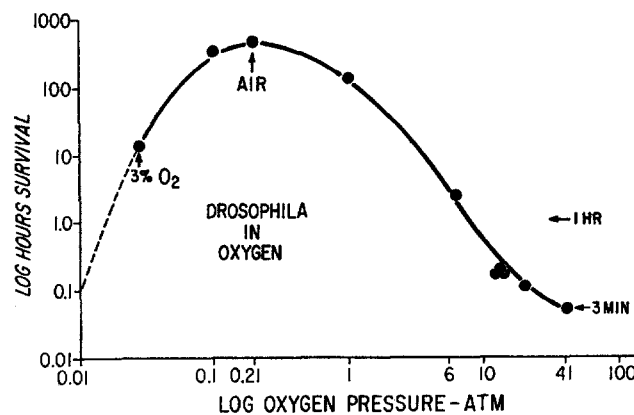


FIGURE 2. Survival time in hours as ordinates and oxygen pressures as abscissae, both plotted logarithmically.

fresh food, etc. In other respects the control flies were kept under exactly similar conditions in a closed, dark space at 24°C, and they seemed to live as long as their controls in air.

Observations have also been made of the survival times in acute exposures to oxygen pressures up to 41 atm where the flies collapse in about 3 min. These results are shown in Fig. 2. This is a double log plot, but the curve shows again that the maximum survival time is in the oxygen concentration found in air. Any increase or decrease in oxygen tension diminishes the survival time to some degree.

2. Intermittent Exposures to 1 atm of Oxygen

These experiments were designed to provide information concerning the rates of recovery from oxygen poisoning. To this end the flies were exposed, for a varying number of hours per day (mostly including weekends), to 1

atm of oxygen. The rest of the time they were kept in CO₂-free air. Except for the composition of the gas in which the flies were kept, both experimental and control flies were treated exactly alike. As before, for each experimental vial there was a control vial kept in air and containing approximately the same number of males and females of the same age and taken from the same culture at the same time; the experimental life-spans were expressed in per cent of the life-spans of the controls. All flies were transferred to new vials with fresh food every Monday, Wednesday, and Friday and were scored at frequent intervals, usually every day.

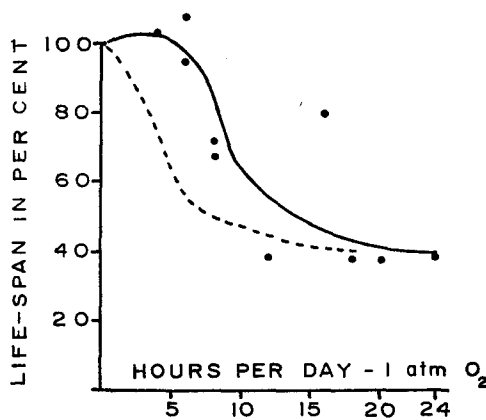


FIGURE 3. Life-span of *Drosophila* in per cent of the air control as ordinates and number of hours each day in 100% oxygen at 1 atm as abscissae. The remainder of the time the flies were in air. The points refer to the solid line. The broken line represents the solid curve of Fig. 1 for continuous exposures plotted on the same scale.

The results of these experiments are shown in Fig. 3 where the life-spans are plotted against the number of hours of 100% oxygen provided per day. The points scatter very widely and can be fitted best by an S-shaped curve, but it is hard to be sure that they deviate significantly from a straight line between 100% on air and 40% at 24 hr per day. There is some indication that 4 or 6 hr of oxygen per day is beneficial, but this is statistically insignificant, and certainly at 8 hr per day the life-span is definitely less than in air. This would mean that a recovery period of 16 hr in air was not sufficient to eliminate all the damage done by 8 hr in 1 atm of oxygen.

Some evidence of recovery is also obtained by a comparison of the data of Figs. 1 and 3. From the number of hours of 100% oxygen per day (H) it is possible to calculate the average oxygen concentration during the day by the formula

$$\frac{100 H + 20.93(24 - H)}{24}$$

Thus, the abscissae of Fig. 3 can be transferred to the abscissae of Fig. 1 and vice versa. The upper broken line in Fig. 1 represents the solid curve of Fig. 3 plotted in this way, and conversely, the broken line in Fig. 3 represents the curve of Fig. 1. In both cases, the life-spans were longer when the same number of oxygen hours was given intermittently than when given continuously. This seems to indicate that some recovery takes place when flies are transferred from 100% oxygen to air.

3. Intermittent Exposure to 2-5 atm of Oxygen

In some of the experiments, the two experimental vials of flies were exposed for 30 min every Monday, Wednesday, and Friday to different pressures of

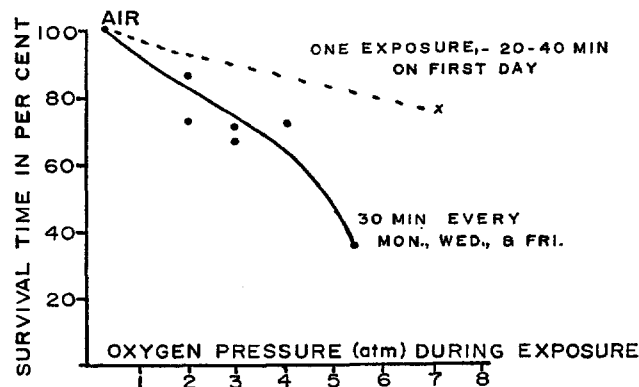


FIGURE 4. Survival times in per cent of air control in flies exposed for 30 min every Monday, Wednesday, and Friday to oxygen at pressures (in atmospheres) indicated on abscissae. Broken line indicates the average survival of flies in three vials, each exposed once for 20, 30, and 40 min respectively, to oxygen at 7.3 atm. These three values were averaged and expressed in per cent of a single control value.

oxygen varying from 2 to 5.5 atm. In this way they were given nearly 2 days to recover from the poisoning effect to which they had been subjected. Except in rare cases, this brief exposure to a high oxygen pressure was not immediately lethal. It seemed possible, however, that this procedure might shorten the eventual life-span by affecting some processes not involved with lower O_2 pressures.

The results of these experiments are plotted in Fig. 4 where life-span (in per cent of the control) is plotted against the pressure of the oxygen to which the flies were exposed for a half hour, three times a week. This treatment markedly diminished the life-span, the maximum change using 5.5 atmospheres of O_2 being a reduction from 27 to 10 days. The upper broken curve in Fig. 4 represents the result of an experiment in which three vials of flies (1-2 days old) were exposed for 20, 30, and 40 min, respectively, to

7 atm of oxygen pressure. Thereafter, they were left continuously in air and were compared with a control vial of flies not so treated. Even this single treatment to a high oxygen pressure caused an average decrease in life-span from 100% to 76%. This is only a single experiment and therefore not conclusive, but suggests that there is always some irreversible change produced by an exposure of this sort to a sufficiently high concentration of oxygen.²

In these experiments it is again possible to calculate the average concentration of oxygen per day resulting from such brief exposures every other day. Thus, the curve of Fig. 4 can also be plotted on Fig. 1 and gives the lower

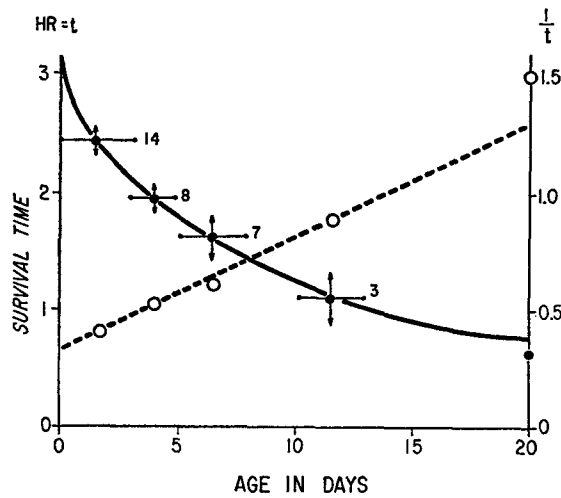


FIGURE 5. Survival times in hours, t , of flies of different ages when exposed to oxygen at 6 atm until death (solid line). Double vertical arrows indicate standard deviations of the mean. Numbers indicate the number averaged. Horizontal bars indicate the range of ages of the points averaged. The broken line is the reciprocal of the survival times, $1/t$, as indicated on the scale at the right. A single point is off the line for the 20-day flies. The solid line is calculated from the equation for the broken line.

broken curve, D . This shows that the effect on the life-span is very great even though the average increase in oxygen concentration is very small. With high oxygen (2 atm or above), intermittent exposures are worse than the same amount of oxygen hours given continuously, probably because they produce more irreversible damage.

4. Physiological Changes with Age

OXYGEN SENSITIVITY It is known from the work of Williams and Beecher (1944) that flies become more sensitive to oxygen with increasing age. This is well confirmed by our data as shown in Fig. 5. This curve represents all

A confirmation of this result by further experiments will appear in another paper.

the survival times which we have observed with flies of different ages, exposed to 90 psi (6 atm) of oxygen until death. The available points have been averaged in groups and the standard deviations of the mean calculated. As noted by Williams and Beecher, a fair straight line is obtained if the reciprocals of the survival times are plotted against age, the equation of the curve being

$$1/t = 0.32 + 0.49 \times \text{age in days.}$$

The curve in Fig. 4 is drawn to represent the equation and follows the experimental points well enough. A single experiment with 20-day old flies gives a point slightly off the line, but not significantly so. The equation derived from the graph of Williams and Beecher was

$$1/t = 1.32 + 0.147 \times \text{age}$$

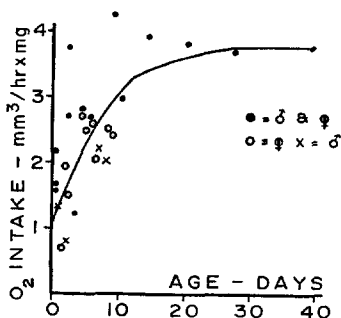


FIGURE 6. The effect of age of fly on the rate of oxygen consumption per milligram of fly. All measurements were made at 24°C in a differential volumeter with 6-12 flies enclosed in a capsule perforated for ventilation. Closed circles from data using both sexes; other data from experiments using males and females separately as indicated.

where t = hours. The constants have different values partly because they used 150 psi of oxygen and a temperature of 20°C instead of 90 psi and 24°C.

In four experiments a comparison was made between flies 1-2 days old and flies 2 weeks old, when both were exposed to 13.6 atm (200 psi) of O_2 for 25 min. All of the older flies were dead the next day, but only 46% of the younger flies. The immediate effect was also more severe in the older group.

METABOLIC RATE It was also of interest to measure the rate of oxygen consumption of the flies at different ages, and a progressive increase with age was found as indicated in Fig. 6. For these measurements the flies were enclosed in gelatin capsules, as used in the pharmacy for pills. These capsules were perforated with holes for ventilation. The capsule was weighed with and without the flies and was inserted into the respirometer for the measurement of oxygen consumption. The respirometers were differential volumeters (Fenn 1927). The points show a wide scatter, but the progressive increase with age seems well established. The later measurements on males and females separately seem, on the whole, a little lower than the earlier ones on mixed sexes,

but the reason for the difference is not clear since both workers used the same respirometer with the same calibration. It is possible also that the females had a slightly higher metabolic rate than the males. The correlation between metabolic rate and age is somewhat improved if the rate of oxygen consumption is expressed, as in Fig. 6, per milligram of body weight. There was no good correlation between metabolic rate and weight of the fly if age was disregarded. In the data the body weight tended to decline with age, and in these data the metabolic rate calculated per fly did not correlate with age unless the figures were corrected for the weight of the fly.

In some experiments the flies were measured both in air and in 1 atm of oxygen. Sometimes air was used first and sometimes 100% oxygen. The available data are found in Table I, and it is evident that there was no signifi-

TABLE I
EFFECT OF OXYGEN TENSION ON OXYGEN CONSUMPTION

Air	100% O ₂	Air/O ₂
<i>mm³ O₂/fy/hr</i>	<i>mm³ O₂/fy/hr</i>	<i>mm³ O₂/fy/hr</i>
2.81	2.32	1.21
1.71	1.90	0.90
0.82	0.70	1.17
2.52	2.50	1.01
2.31	2.21	1.05
3.45	2.72	1.27
Average 2.27	2.06	1.10±0.06

The same group of 6-12 flies were measured first in air, then in O₂, and then again in air. The average rate in air was then compared to the rate in O₂. In other experiments O₂ was used first. The difference is not significant.

cant difference due to oxygen tension, the average ratio of the rate in air to the rate in oxygen being 1.10 ± 0.06 . If anything, the oxygen was inhibitory rather than the reverse. We also made a few measurements on survivors from exposures to high oxygen pressures, but found no indications that significant changes could be established by more extensive data.

5. *Time Course of Oxygen Poisoning*

In 1 atm of oxygen flies live approximately 7 days. Most of the flies die between the 6th and 8th day. Previous to the 5th day they all appear normal. These experiments were designed to find out whether at 3.5 days the flies were half poisoned in some way not evident to the eye. For this purpose, the flies, at an initial age of 1-5 days, were exposed to 4.7 or 6.1 atm of oxygen pressure (absolute) before treatment with 1 atm of oxygen, and after different numbers of days of such treatment. At such high pressures it was believed

that poisoning was relatively so rapid that the time course would be approximately linear and the necessary lethal exposure time, T , would be a measure of the amount of further "poisoning" still necessary to obtain a lethal result. The average values of T for the controls were 1.7 ± 0.14 hr at 4.7 atm in 13 tests and 0.83 ± 0.1 hr at 6.1 atm in 21 tests. For every batch of about 20 flies (usually 10 males and 10 females) thus exposed to oxygen there was a control batch from the same culture exposed for an equal length of time to air at 1 atm and then treated with 6 atm of oxygen pressure until all col-

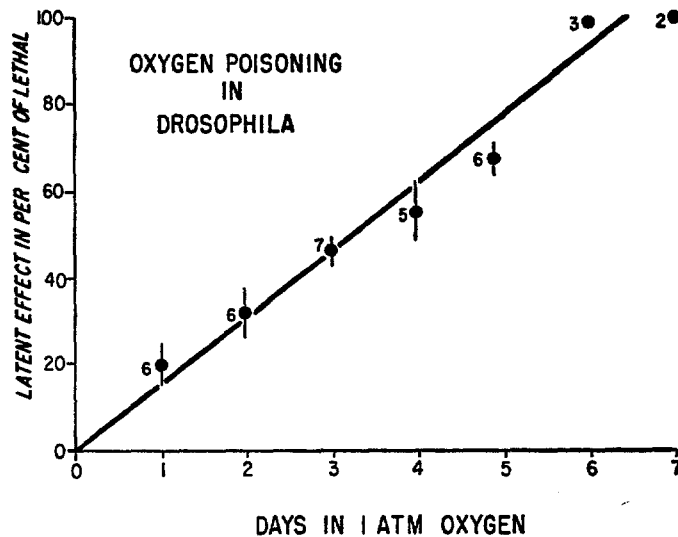


FIGURE 7. The time course of oxygen poisoning. Two vials of flies are exposed for different periods (abscissae), one to air and the other to 100% oxygen at 1 atm, and are then tested with 55 or 75 psi of oxygen and the average collapse times T_0 and T_1 are measured. These times are a measure of the amount of oxygen poisoning still required for collapse. The amount of oxygen poisoning already accomplished is then given (ordinates) as $100(1 - T_0/T_1)$. Bars represent standard deviations of the means, and the figures show the number of separate experiments averaged together for each.

lapsed. In both cases the time required for 50% collapse, or the mean collapse time, was calculated. The time required for the oxygen-treated flies was expressed in per cent of the control value. This result subtracted from 100% gives the amount of poisoning already accomplished and is plotted in Fig. 7. The standard deviation of the mean is indicated by bars, and the number of individual experiments averaged together for each point is indicated on the figure. The resulting points hardly deviate significantly from a straight line, both ends of which are pretty well fixed at 0 and 100% respectively. It is concluded that after 3 days in 1 atm of oxygen, the flies are $\frac{3}{4}$ poisoned, even though there is no apparent change in their behavior as judged by simple observation. The time course of oxygen poisoning then

appears to be linear rather than exponential. This is a significant finding because it appears to indicate that during the actual exposure to oxygen there is no significant rate of recovery which might be presumed to increase progressively in rate in proportion to the accumulation of some lethal metabolic product or condition. It can be said that the sensitivity to oxygen increases linearly during an exposure, although it may return to normal more or less rapidly after the termination of the exposure.

6. *Mathematical Analysis of Oxygen Toxicity Data*

Curves of oxygen pressure against survival time always show an increasing survival time as the pressure of oxygen decreases until a plateau is reached at the pressure of oxygen in air. Such curves have usually been expressed by

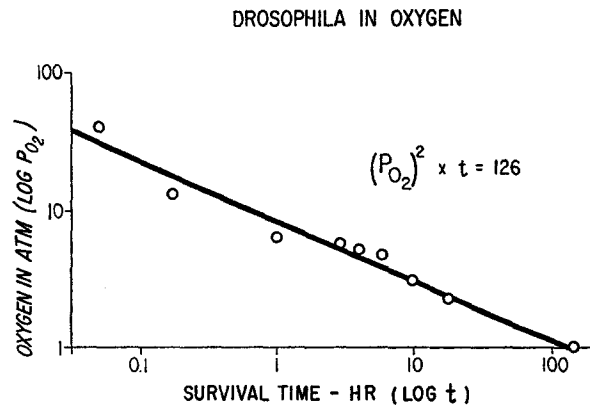


FIGURE 8. Logarithmic plot of survival time against oxygen pressure. The straight line represents the equation given.

an equation of the type $P^n \times \text{time} = \text{constant}$. Our data for *Drosophila* on a double log plot are shown in Fig. 8. They follow a straight line reasonably well, the equation being

$$P^2 \times t = 126$$

where the pressure of oxygen, P , is in atmospheres, and the survival time, t , is in hours. Expressed in the same way the data of Williams and Beecher (1944) follow the equation

$$P^{1.81} \times t = 120.$$

For all but the two highest pressures in our data the equation found is

$$(P_{O_2} - 0.21)^{2.2} \times t = 102.$$

The data for that curve are plotted directly (not as logarithms) in Fig. 9

where the experimental points are seen to follow the theoretical curve as closely as could be expected. In this connection, attention should also be called to the fact that Fig. 1 is a double log plot of even more extensive data. There, it is evident that the points representing pressures from 1 atm and above can be represented reasonably well by a straight line. The survival time in air is not included in either Figs. 8 or 9, and it does not fall on the same straight line. This presumably means that in air the flies do not die from oxygen poisoning but from other reasons which may be classed as merely "old age."

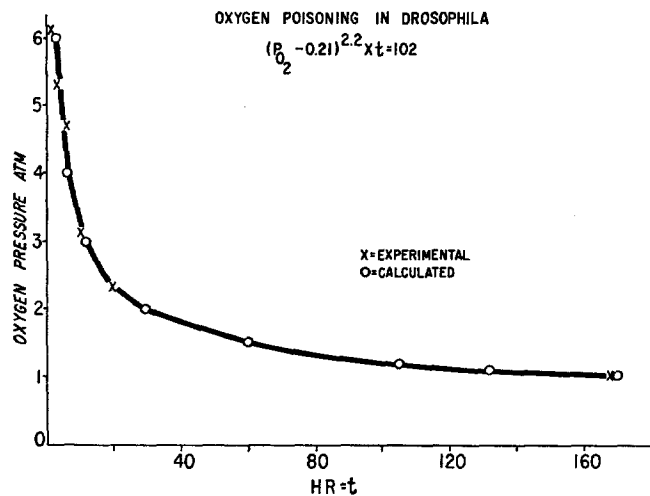


FIGURE 9. Survival time as abscissae against oxygen pressure as ordinates. The curve is drawn through the open circles which were calculated from the equation given. The crosses represent the experimental points.

Although this simple equation appears to fit the facts and is, therefore, of some practical value, it is a purely empirical equation and tells us nothing about the mechanism of oxygen poisoning. It seemed likely, at the beginning of this study, that the data should conform to some equation which included a term for the rate of recovery from oxygen poisoning, because we know that recovery does occur when a high oxygen concentration is replaced by air and probably at an exponential rate. For this reason we have tried the equation proposed by Blair (1932) for electrical stimulation of nerve. Thus:

$$\frac{dx}{dt} = KV - kx.$$

A similar equation was indeed proposed by Hederer and André (1935) but was not applied quantitatively. According to this equation, some toxic product or condition builds up at a rate proportional to the voltage V and

decays at a rate proportional to its own concentration. To test this equation Blair transformed it to

$$\log \frac{V}{V - R} = kt$$

where V is voltage, and R is the value of the rheobase. In applying this equation to oxygen, V is the pressure, P , and R would be the plateau reached at low pressures and long times. Taking $R = 0.21$ atm (or sometimes 1 atm), we have tested this equation on various oxygen pressure-time curves by plotting

$$\log \frac{P}{P - 0.21}$$

against t . Using the data of Gerschman, Gilbert, and Frost (1958) for *Paramecia*, with a limited range of pressures, we obtain a fair straight line through the origin with $k = 0.0059$, but no good fit could be obtained with our own data or those of Williams and Beecher (1944) for *Drosophila*, the data of Welch et al. for man, or the data of Hederer and André (1940) for rabbits. All of these data gave more or less satisfactory agreement with the empirical $P^n t = \text{constant}$ with values of n varying from 1.43 to 2.2. Our inability to fit the data with an equation including a recovery factor corroborates in some degree the conclusion from Fig. 7, that there is no simultaneous recovery process during exposure to high oxygen pressures.

DISCUSSION

The experiments described were carried out to provide a background for experiments to be presented later on rates of recovery from oxygen poisoning and on interactions between inert gases and various oxygen pressures. These data confirm the toxicity of oxygen in general, but provide comprehensive data over a wider range of pressures concerning the effects of oxygen on the life span of *Drosophila*. Perhaps the most important finding is that flies live longer in air than in any other concentration of oxygen. This seems reasonable enough, but it has never been demonstrated to our knowledge for any other species. Some have thought that animals would live longer at an oxygen tension less than that in air. It is also a common belief that any concentration of oxygen less than, perhaps 50%, is harmless to man. It is now possible to say that, in *Drosophila* at least, any deviation of oxygen tension, either above or below that of air, has some tendency to shorten the life span. It seems wise to apply this result tentatively to man until other evidence to the contrary becomes available.

The data show also that intermittent exposures to 1 atm of oxygen are

better tolerated than continuous exposures. However, intermittent treatment with 2–5 atm of oxygen for as little as 30 min every 2 days causes a marked reduction in life-span, which is far greater than the decrease caused by the same average oxygen pressure applied continuously. This seems to indicate that the effects of high pressures are less reversible than lower pressures applied for correspondingly longer times.

Even at 1 atm, oxygen produces some irreversible effect which diminished the life-span. So far, we have found no evidence of accommodation to abnormally high oxygen pressures. There is, however, ample evidence of increased sensitivity to oxygen as a result of previous treatment with sublethal doses of oxygen from which recovery is not complete.

SUMMARY

1. Measurements of survival time of fruit flies in different partial pressures of oxygen show that the maximum survival occurs in normal air and is less at either larger or smaller partial pressures of oxygen.
2. For the same average pressure of oxygen per day survival is better if the exposure to pressures above that in air is applied discontinuously rather than continuously. With intermittent exposures to pressures of 2–5 atm, however, the survival time is much diminished compared to the same average oxygen pressure continuously applied.
3. Flies exposed to 1 atm of oxygen for 8 hr everyday do not recover completely in the remaining 16 hr of air, and their life-span is shortened.
4. Older flies are more sensitive to oxygen poisoning than young flies, and the rate of dying, or the reciprocal of the survival time, is a linear function of age. Older flies have also a higher rate of oxygen consumption.
5. The progress of oxygen poisoning appears to be linear rather than exponential with time, even though no symptoms of poisoning may appear until shortly before death.
6. The lethal dose of oxygen appears to be proportional to the exposure time and to the square of the pressure of oxygen in excess of that in air.

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