

BODILY CHANGES AND DEVELOPMENT OF PULMONARY
RESISTANCE IN RATS LIVING UNDER
COMPRESSED AIR CONDITIONS*

BY F. J. C. SMITH, J. W. HEIM, R. M. THOMSON, AND C. K. DRINKER, M.D.
(From the Departments of Physiology and Industrial Hygiene, Harvard School of
Public Health, Boston)

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No previous work on the toxicity of increased oxygen tensions in the respired air has been conducted under conditions in which all other factors were constantly and perfectly controlled, and most of the investigations upon this subject have been based upon data obtained from observing a limited number of animals. The possession of a large chamber, susceptible to accurate and steady adjustment, has made it possible to carry out decisive experiments on large groups of animals. The first project to which this new apparatus has been devoted consisted of observations on the life history of a standard laboratory animal living for prolonged periods in an environment normal in all respects except for the increase in the oxygen tension of the air. It was our intention to determine whether or not any acclimatization could be produced against the toxic action upon the lungs of high oxygen tensions and, if so, what changes in the animal were responsible for this adaptation. The data we have accumulated deal with a single species, the standard albino rat, bred from Wistar Institute stock, exposed to compressed air having an oxygen tension of 635 mm. of mercury equivalent to 83.6 per cent oxygen at normal barometric pressure.

EXPERIMENTAL

A. The High Pressure Chamber.—A detailed description of the construction, operation, and experimental capabilities of the apparatus used in this research has been published by Thomson (1). The pressure chamber consists of a $\frac{1}{2}$ inch thick steel cylinder, 31 feet long and 8 feet in diameter. It is divided into three

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compartments, each of which may be used independently of the others. The center compartment is the smallest and is known as the lock. By means of it one may enter and leave the high pressure end compartments in which the animals are kept, without altering the conditions of their environment. Two electrically driven air compressors located in an adjoining room supply air at the desired pressure and volume flow to the pressure chambers. After leaving the compressors the air passes through an automatically controlled air-conditioning apparatus where it is scrubbed and brought to predetermined dry and wet bulb temperature readings. Continuous written records of the air pressure and dry and wet bulb temperatures were obtained automatically during the entire experiment.

B. Conditions of the Experiment. 1. *Barometric Pressure.*—Increased oxygen tension and increased barometric pressure were the two possible abnormal factors in these experiments. Many authors (2, 3,¹ 4-9) have presented concise evidence that the increased oxygen tension, and not the barometric pressure, was responsible for the symptoms of poisoning found under such conditions. The results of our experiments have confirmed these earlier observations, and therefore it may be assumed that the only factor varying from the normal in our experiments was the oxygen tension. The barometric pressure in the experimental chamber was maintained at 3040 mm. Hg for 30 days in the first experiment. In the second, this same pressure was held for 72 days except for approximately 1 hour each day, when it was reduced to 2280 mm. Hg while observers entered the animal chamber. This was done in order to lessen the time necessary for the decompression of attendants from 82 minutes to 32 minutes. The results of each experiment were identical, showing that this transient variation had no effect upon the animals. The oxygen tension, therefore, was approximately 635 mm. Hg or 83.6 per cent of an atmosphere, except for the temporary daily drop to 60 per cent during the second experiment.

2. *Temperature.*—The dry bulb temperature was kept at 28°C. plus or minus 1°C. This was found to be the temperature at which the animals were most active and apparently most contented. Benedict and MacLeod (10) have showed this to be the critical temperature for the albino rat, as the metabolism becomes basal at this reading.

3. *Humidity.*—The relative humidity ranged from 49 to 50 per cent during all the experiments.

4. *Volume Flow.*—Conditioned outdoor air was circulated through the animal chamber at the rate of 2660 liters per minute. The composition of the air was found to be practically identical to that of outdoor air on numerous gas analyses. This rate of ventilation was also sufficient to keep the odors of animal excretions from accumulating in the chamber.

5. *Duration of Exposure.*—The first group of animals was exposed continuously for 30 days. A second group was exposed for 72 days, and then after an interval of 40 days at normal pressure was reexposed for 10 more days.

¹ Bert (3), pp. 611-612.

6. *The Albino Rat.*—The albino rat is a standard laboratory animal whose life history is accurately known. Although sporadic cases of pulmonary infection are found, they occur in a relatively small percentage of animals. The life cycle is completed rapidly, and it is therefore possible to observe the reaction of the animal as it passes from adolescence to maturity.

7. *Number of Animals Employed.*—At the beginning of these experiments 147 animals of representative age groups and sexes were selected for observation. Twenty-one of these were killed and autopsied to give normal tissue sections to serve as controls for a pathological study. During the course of the experiments 97 rats were born and observed for varying periods of time under the prevailing experimental conditions, making a total of 244 animals studied.

8. *Care of Animals.*—All rats were cared for in the same manner. Two standard diets were employed, each being used alternately.² Daily feedings were maintained at all times except on Sundays and holidays. In addition to the standard diets lettuce and fresh whole milk were given daily. 20 to 30 cc. of cod liver oil were added per liter of milk twice a week. No more than eight rats were housed in a single cage, 2 feet square and 1 foot high. A certain number of cages was changed daily, so that all animals had clean quarters at least once a week.

² *Standard Rat Diets.*—

No. 1. Fairhall, L. T., *Am. J. Physiol.*, 1928, **84**, 382.

	<i>per cent</i>
Wheat flour	35.3
Corn meal	23.5
Rolled oats	23.6
Dextrin	11.7
Powdered liver	5.9
	100.0

Add 10 gm. of calcium phosphate to each kilo of food. Make into a dough, then roll and cook into biscuits.

No. 2. Greenman, M. J., and Duhring, F. L., *Breeding and care of the albino rat for research purposes*, Philadelphia, Wistar Institute, 1923, 51.

	<i>per cent</i>
Lima beans	19.0
Rice	27.0
Whole wheat	27.0
Rolled oats	27.0
	100.0

Add water and cook until well done. Allow excess moisture to evaporate, then pour on large trays. It may be kept for many days in a cold room.

A liberal amount of wood wool was supplied to absorb the moisture and provide nesting material. Especial care was taken in the handling of the animals. They were fondled daily and were absolutely gentle during all observations. Rats are exceptionally responsive to this kind of treatment, and their behavior served as an important index of the general well-being. If in good health all animals clung to the front of the cages when observers entered the chamber. They displayed intense interest in the feeding procedures and daily weighings. When ill all such interest disappeared, and the animals became markedly apathetic, failing to respond even when the cages were opened.

9. *Accumulation of Data.*—Weighings were made under pressure on a direct-reading, Toledo, springless balance. Two light aluminum boxes were constructed to hold about ten rats, so that all in one cage could be weighed at once, thus eliminating individual weighings. A small number of animals were weighed individually. Daily notes were made regarding the condition of the rats, and any dead or obviously dying animals were removed for autopsy. The details of the pathological findings are reported in a second publication (11).

In order to avoid caisson disease the stage decompression table compiled by Boycott, Damant, and Haldane (12) for the British Admiralty was followed. Neither observers nor animals showed the slightest evidence of nitrogen bubble formation.

The Effect of the First Exposure to Increased Oxygen Tension

A. *Acute Oxygen Poisoning.*—Many authors (2, 4, 5, 7, 8, 11, 13, 14–18) have showed that the damage from acute poisoning by oxygen in concentrations such as we have provided is centered in the lungs. Active hyperemia and pulmonary edema dominate the picture. The significant fact brought out by our observations is the comparative immunity of young rats to an oxygen concentration which invariably causes serious illness or death in adults. By young rats we refer to animals from birth to 3 months of age. After the 3rd month rats still display a degree of the resistance to high oxygen concentrations which is characteristic of early life, but this quality diminishes steadily and by the 6th month is gone. Faulkner and Binger (19) found that young turtles were less susceptible to oxygen poisoning than were adults, but the differences reported were not so definite as we have found in rats.

1. *Old Rats (over 3 Months of Age).*—The animals lived in the experimental chamber under normal atmospheric conditions for 5 days prior to compression. They were in excellent condition during this period, and their weight curves showed a normal upward trend

(Text-fig. 1). On the 6th day the air pressure was gradually raised to 3040 mm. Hg within an hour, and remained at this point for 30 days in the first experiment and 72 days in the second. The results of both experiments were identical, and for the sake of simplicity they will be considered together in the presentation of data.

All rats were in excellent health until the 3rd day of exposure. At this time several were found to be weak, apathetic, and dyspneic. They showed no interest in the feedings. The majority, however, were still in good health. The weight curves showed that since the beginning of exposure the normal growth rate had decreased in some of the groups of animals (Text-fig. 1, Curves 2, 3, and 4).

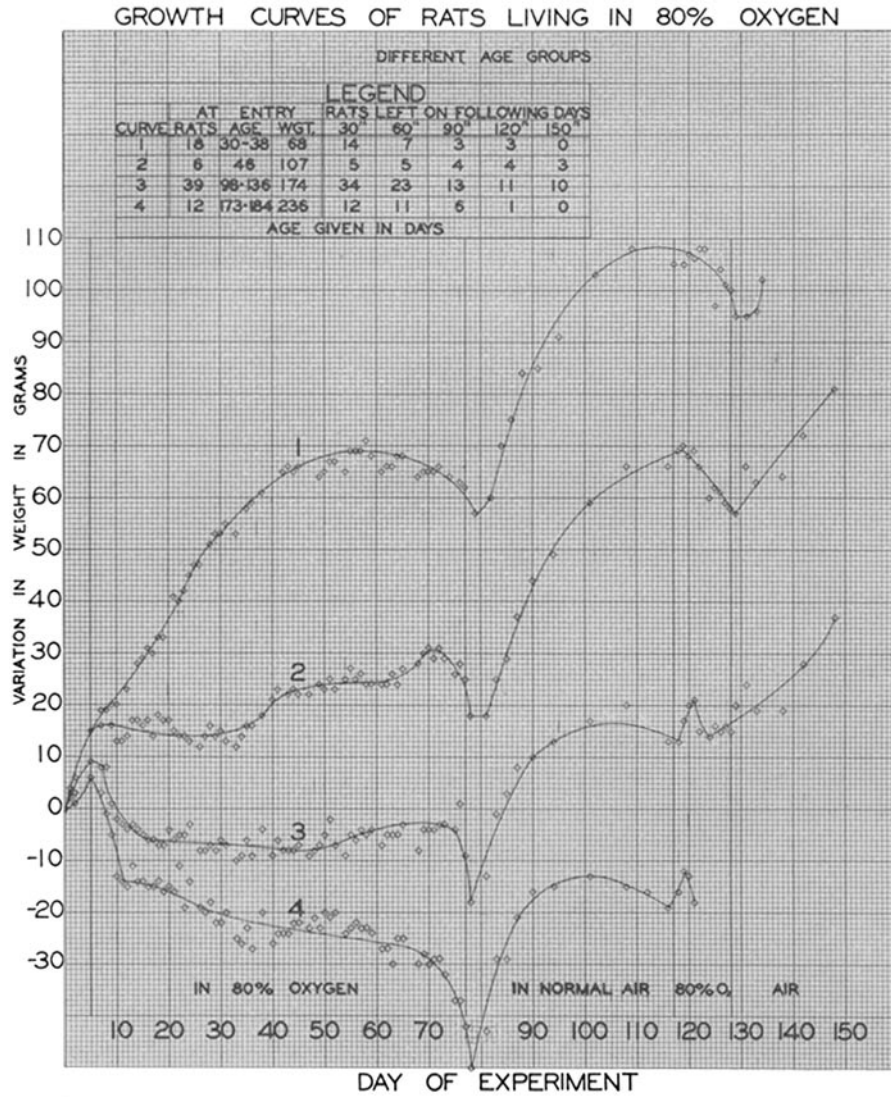
During the 4th day the symptoms of acute oxygen poisoning became marked in all, and the characteristic pathological findings were present (11). There was an average loss of 10 to 15 gm. in weight from the 3rd day. The rats were acutely ill, and showed extreme respiratory embarrassment and hyperpnea. Various degrees of cyanosis were present. There was practically no food consumption during the preceding 24 hours (Text-fig. 3, Curve 2), and the mortality rate was 13 per cent, all the deaths occurring on the 4th day (Table I).

On the 5th day most of the surviving rats were in good condition but had continued fasting during the preceding 24 hours (Text-fig. 3, Curve 2).

By the 6th day all rats were in excellent health objectively, and the pathological changes found on the 3rd and 4th days had almost disappeared. They were eating again, but the food consumption was only half the amount prior to exposure, and the original loss in weight persisted with slight fluctuations.

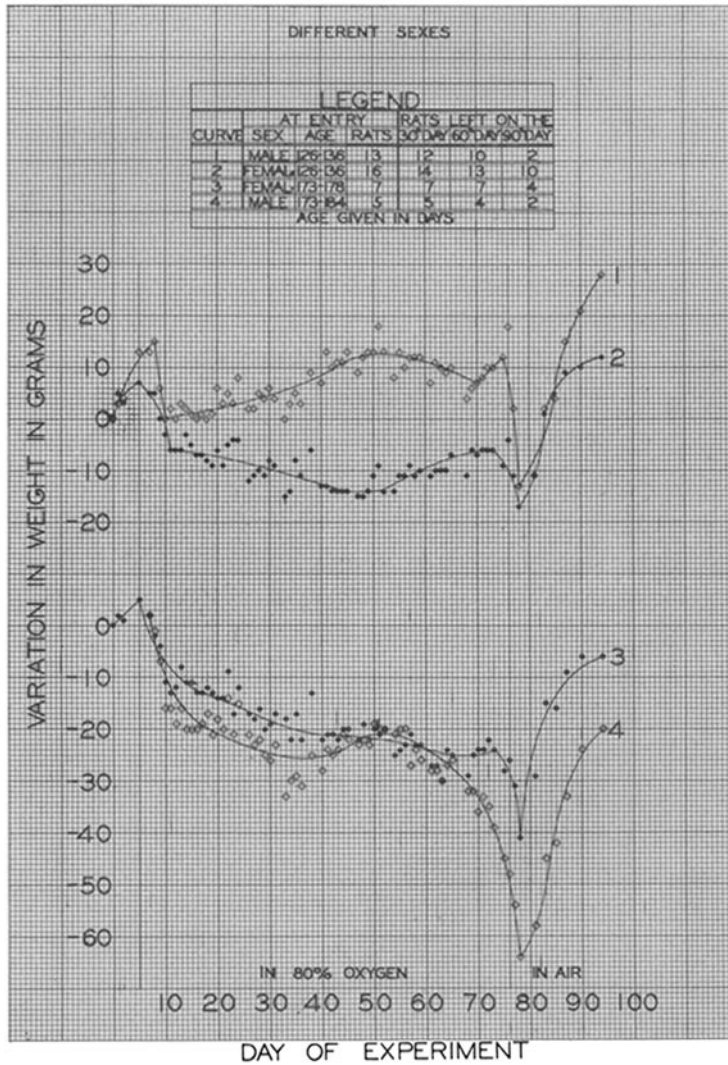
2. *Young Rats (under 3 Months of Age)*.—These animals did not show objective signs of acute oxygen poisoning at any time during exposure. They continued to be exceedingly active and playful, and their appetite was good. In contrast with old rats they showed no loss in weight, yet there was a definite depression of the normal growth rate.

Young adult rats from 100 to 150 days of age reacted in an intermediary manner to that described above. Symptoms of illness were observed during the acute stage of pulmonary edema on the 3rd and 4th days, but the animals remained in much better condition than



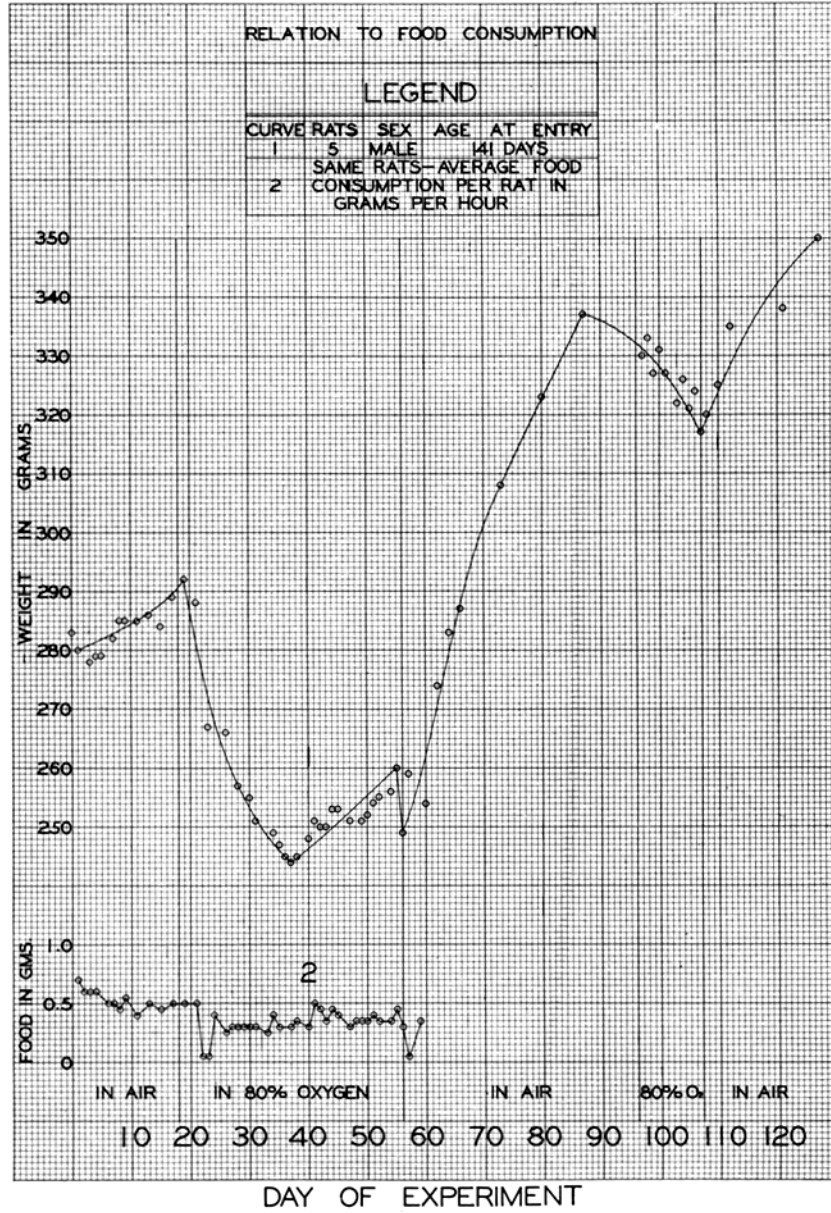
TEXT-FIG. 1. The relationship of age to the development of acute oxygen poisoning, showing the increased susceptibility of rats with advancing age.

GROWTH CURVES OF RATS LIVING IN 80% OXYGEN



TEXT-FIG. 2. The relationship of sex to the development of acute oxygen poisoning, showing that both sexes react in essentially the same manner.

GROWTH CURVE OF RATS LIVING IN 80% OXYGEN



TEXT-FIG. 3. The relation of growth curves to food consumption, showing a decrease in appetite.

older rats. There was less loss in weight and a much lower mortality rate (Text-fig. 1, Curve 3; Table I, Group 4, 4th day of exposure).

The difference in the effect of 83.6 per cent oxygen on old and young animals can be correlated with a difference in lung structure (11). This difference is essentially a far greater cellularity of the alveolar walls in young rats.

B. Prolonged Exposure.—During the 2nd week all rats continued in good condition, although the weight of the older ones remained at the low level established on the 4th day of exposure (Text-fig. 1, Curves 2, 3, and 4). The young rats were gaining in weight (Text-fig. 1, Curve 1). The food consumption continued to be about half the amount prior to exposure (Text-fig. 3, Curve 2).

Sporadic cases of illness appeared early in the 3rd week in old rats. The animals affected had a poor appetite, lost weight rapidly, became dyspneic, apathetic, inactive, weak, and eventually died of a bronchopneumonia. The incidence of this disease was practically the same as that of the sporadic respiratory infection found in supposedly normal rats, although in the latter it did not progress further than lobar atelectasis and purulent bronchitis (11). Cases of chronic bronchopneumonia were found during the remainder of exposure in roughly 22 per cent of the rats. Eleven died at various times without showing any evidence of bronchopneumonia; in all these there was considerable fibrosis in the lungs apparently referable to the acute experience suffered on the 3rd and 4th days. The final cause of death in these animals cannot be given.

With the exception of the animals dying as has been described, the rats remained in good health after the 1st week, but they were not entirely normal. Food consumption and weight were low, indicating that although nothing seriously wrong could be detected on daily inspection, some definite influence was operating steadily to keep them in a slightly subnormal state. Such animals, when killed and examined microscopically, always showed changes in the lungs—most characteristically an increased cellularity, so that the lungs resembled those of young animals (11).

On the 72nd day the exposure was discontinued. During decompression or within 24 hours afterwards, thirteen, or 28 per cent, of the rats surviving the 72 days exposure died (Table I). All showed marked degrees of lung damage, and in the majority it was due to bronchopneumonia. No paralyses were observed, and none of the

deaths could be attributed to caisson disease. The animals that lived through decompression were in good health and showed no bronchopneumonia at subsequent autopsies (11).

A temporary loss in weight followed removal to normal air. During the next few weeks a great increase in growth occurred, being most rapid the first few days after the conclusion of exposure (Text-figs. 1 to 3). The gain in weight averaged 40 to 50 gm.

Adaptation—Reaction during Second Exposure to Increased Oxygen Tension

All rats surviving the 72 day period in 83.6 per cent oxygen were kept in normal air for 40 days. They were then reexposed for 10 days to the previous conditions of high oxygen concentration. No acute change in health occurred during this time, although a slow loss in weight was seen. There were no signs of acute oxygen poisoning either clinically or pathologically (11). Five control rats, for which this was the first exposure, developed typical signs of oxygen poisoning beginning on the 3rd day. Unquestionably, during the first exposure the animals had become resistant to the toxic effect of this environment. Nine litters were born during the first exposure and all died. One litter was born in the second period of exposure, and six out of the eight young survived and developed normally. This was further evidence that some permanent adaptation had been produced. On removal to normal air all rats remained in excellent health, contrary to the outcome at the end of the first exposure when there was a mortality of 28 per cent. At the present writing, 214 days after the conclusion of this experiment, the survivors are in excellent condition.

Mortality Statistics

The mortality rates for males and females of different age groups have been compiled in Table I. Since a large number of rats were killed at different times during exposure, it was impossible to obtain accurate data regarding the mortality for the entire period. Nevertheless, an approximation has been reached by dividing the total number of deaths during exposure and decompression by the sum of the number of rats surviving the experiment plus the number dying during the experiment.

Table I shows that all deaths from acute oxygen poisoning occurred on the 4th day in 80 per cent oxygen. There were no deaths in rats

TABLE I
Mortality Statistics for Different Age Groups and Sexes after Varying Periods of Exposure to 83.6 Per Cent Oxygen Tension

Day of exposure.....		1st					2nd					3rd					4th				
Age group.....		1	3	4	5-6	Total	1	3	4	5-6	Total	1	3	4	5-6	Total	1	3	4	5-6	Total
No. of rats exposed	M	12	1	26	19	58	12	1	25	19	57	12	1	24	18	55	12	1	23	17	53
	F	32	4	26	15	77	32	4	25	15	76	32	4	24	15	75	32	4	23	15	74
	MF	44	5	52	34	135	44	5	50	34	133	44	5	48	33	130	44	5	46	32	127
No. of rats dying	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	4
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2
	MF	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	4	6
Per cent mortality	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	18	7
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	7	3
	MF	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	13	5

Day of exposure.....		5th					During decompression					During all experiment				
Age group.....		1	3	4	5-6	Total	1	3	4	5-6	Total	1	3	4	5-6	Total
No. of rats exposed	M	12	1	21	14	48	3	1	7	4	15	4	1	9	7	21
	F	31	4	21	14	70	7	2	12	10	31	10	2	15	12	39
	MF	43	5	42	28	118	10	3	19	14	46	14	3	21	19	57
No. of rats dying	M	0	0	0	0	0	1	1	3	2	7	2	1	6	5	14
	F	0	0	0	0	0	2	0	2	2	6	5	0	5	4	14
	MF	0	0	0	0	0	3	1	5	4	13	7	1	11	9	28
Per cent mortality	M	0	0	0	0	0	33	100	43	50	47	50	100	67	71	67
	F	0	0	0	0	0	29	0	17	20	19	50	0	33	33	36
	MF	0	0	0	0	0	30	33	26	29	28	50	33	52	47	50

M, male, F, female. MF, male and female. Age group, approximate age in months.

less than 120 days old, while in older ones the number of deaths was proportional to the advance in age. There was no significant difference in the mortality rate for males and females.

Effect of Pregnancy

Six pregnant rats in the late stage of gestation were exposed with the others at the beginning of the experiment. Three of these were 9 months of age, and all died of acute oxygen poisoning, one as early as the 2nd day. The other three pregnant rats were 130 days old, and two of them died. The third survived and gave birth to a normal

TABLE II

Mortality Rate and Average Weight of Litters Born during and after Exposure of Mothers to 83.6 Per Cent Oxygen Tension

No. of litters	Total No. of rats in litters	No. dying within 24 hrs. after birth	No. surviving	Mortality	Total weight of litters	Average weight per rat
Litters born in tank during first exposure of mothers						
				<i>per cent</i>	<i>gm.</i>	<i>gm.</i>
3	16	All	0	100	—	—
1	5	All	0	100	14.7	3.34
1	8	All	0	100	25.6	3.20
1	9	All	0	100	32.4	3.60
6	38	All	0	100	72.7	3.30
Litters born within 24 hrs. after decompression of mothers						
2	12	All	0	100	—	—
1	8	All	0	100	29.9	3.70
3	20	All	0	100	29.9	3.70
Litters born 6 to 39 days after decompression of mothers						
4	31	2	29	6.5	—	—
Litters born in tank during second exposure of mothers						
1	8	2	6	25	—	—

litter after she was removed from the high oxygen. Apparently rats advanced in pregnancy cannot survive acute oxygen poisoning as well as non-pregnant animals of the same age. The encroachment upon the thoracic cavity by the enlarging fetuses is probably the cause of this increased susceptibility.

Rats entering 80 per cent oxygen in the early stages of pregnancy,

as well as those becoming pregnant during exposure, were as resistant to acute oxygen poisoning as non-pregnant animals. The litters, however, were never carried to term under these conditions. Table II shows the average weights of litters born during the experiment. Each survived for a few hours, but according to the birth weight given for normal rats by the Wistar Institute these litters were almost 50 per cent underweight. Nine litters were born during the first exposure of the mothers, and all died within 24 hours after birth.

During the second exposure an acclimatized female gave birth to a litter on the 4th day, and six of the eight young survived. The mother remained in excellent health. This is a striking example of the difference in reaction of adapted animals, because during first exposure all the litters, and almost 100 per cent of the mothers advanced in pregnancy died. Furthermore, this litter was born on the 4th day of exposure when the symptoms of acute oxygen poisoning are most intense in non-adapted rats (Table II).

The fertility of rats was not altered by prolonged exposure. Females living with males throughout the exposure had frequent litters.

DISCUSSION

Previous investigators have failed to demonstrate any acclimatization or adaptation of animals to toxic oxygen tensions (16, 20). Although there may be several reasons for this, the most important is probably the fact that definite damage to the animal was not produced during the first exposure, so that there was no stimulation to the lungs or other defensive mechanisms of the body. Barach (20) kept rabbits in 60 per cent oxygen for a prolonged period in an attempt to produce acclimatization and then raised the oxygen tension to toxic heights, but oxygen poisoning developed in a typical manner. The exact border-line of toxic oxygen tensions has not been established, although the available evidence (2, 3,³ 4, 13, 20) suggests that it lies around 70 per cent of an atmosphere. Species variation to this toxicity may be expected, so that it can be assumed that 80 per cent oxygen is just at the border-line of dangerous toxicity for the albino rat. Whereas higher tensions might prove fatal during the acute

³ Bert (3), pp. 611 and 844.

stage of oxygen poisoning, most rats are able to combat successfully the acute pulmonary edema produced by 80 per cent oxygen with eventual complete removal of exudate. The lung damage or stimulation leads to distinct changes in morphology (11) which persist for at least 53 days after returning to normal air.

The significance of these alterations in the adaptation occurring in old rats and the difference in the reaction of young rats to acute oxygen poisoning is the subject of a second paper (11) to which we have frequently referred.

The gradual loss in weight, even though all signs of acute oxygen poisoning have disappeared, is conclusive evidence that the normal physiological processes are constantly altered during exposure. No satisfactory pathological changes were found in the first 30 days of exposure to account for this weight loss in animals remaining otherwise normal. After the 1st month of exposure the presence of chronic vascular lesions in the lungs (11) was confirmatory evidence of the toxicity of this environment, although no clinical changes in health could be detected. The effects produced by prolonged exposure to toxic oxygen tensions should be termed "chronic oxygen poisoning" in contradistinction to the pulmonary edema associated with acute oxygen poisoning. On removal to normal air the rapidity of growth indicated that the inhibitory effect of the previous environment quickly disappeared. The loss of weight during a second exposure in the absence of any clinical or pathological changes was again evidence of this alteration in the normal physiological processes. A certain number of determinations of basal metabolism were made upon animals under continuous exposure to high oxygen. While the subject should be explored further, it is safe to say that the metabolism was not increased, so the loss of weight may consequently be ascribed to loss of appetite, the final reason for which cannot be given.

The steady though subnormal gain in weight in young rats is probably in part due to greater resistance to oxygen poisoning and in part to a greater stimulus for growth in these than in adults, so that growth progresses in spite of the adverse environment.

SUMMARY AND CONCLUSIONS

1. 244 albino rats from standard Wistar Institute stock have been kept for periods up to 72 days under the following conditions.

(a) Barometric pressure: 3040 mm. Hg. This means a partial pressure of oxygen of 635 mm. Hg and is equivalent to an 83.6 per cent oxygen mixture at normal barometric pressure.

(b) Temperature: 28°C.

(c) Humidity: 50 per cent relative.

(d) Rate of ventilation: 2660 liters per minute for all animals.

(e) Food and daily care were provided which induced normal growth in rats in the usual laboratory quarters.

2. The harmful factor in this environment was the increased oxygen tension. In our experiments the acute effects were active hyperemia and edema of the lungs, just as have been described by many investigators.

3. Only a small percentage of rats die from acute oxygen poisoning at the pressure employed. The majority return to good health objectively and survive several months of exposure.

4. Rats under 1 month of age display no clinical signs of acute oxygen poisoning, while in older animals the severity of the reaction and the mortality is directly proportional to the advance in age.

5. The symptoms of acute oxygen poisoning appear on the 3rd day of exposure in adults and reach maximum intensity during the 4th day, all deaths occurring at this time.

6. A continued weight loss is found in old rats, while the young gain weight, but not with normal vigor.

7. Adaptation to this toxic oxygen tension occurs in the albino rat during the first exposure, so that on reexposure acute oxygen poisoning does not develop.

8. Respiratory infection occurred sporadically in roughly 20 per cent of the normal adult rats in our laboratory colony. About the same incidence was found in the experimental rats during exposure; in most of these, chronic bronchiectasis and bronchopneumonia followed and proved fatal, indicating a lowered resistance in exposed animals.

9. Most rats exposed to an 80 per cent oxygen tension late in pregnancy have premature litters and die of acute oxygen poisoning, but if exposed early in pregnancy the majority survive.

10. Litters born during the first exposure of the mother are approximately 50 per cent underweight and die during the first 24 hours after birth.

11. A female, rendered resistant by a first exposure, produced a healthy litter during second exposure.

12. The continuous failure of adults to gain in weight and the fact that young animals grow slowly, together with the slowly progressing pulmonary pathology (11), indicate that high oxygen tensions not only produce acute changes in the lungs but also some alteration in the normal physiological processes, which may be termed "chronic oxygen poisoning."

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