REACTION OF THE CHICK TO ONE ATMOSPHERE OF OXYGEN

Harold S. Weiss, Ronald A. Wright and Edwin P. Hiatt

Environmental Physiology Laboratory
Department of Physiology
The Ohio State Univ. Coll. of Medicine
Columbus, Ohio 43210

Continuous exposure to 100% oxygen at atmospheric pressure (OAP) is toxic to most homeotherms, frequently causing death in as little as 2-4 days (see review by Bean, 1945). Congestion, atelectasis and other signs and symptoms of pulmonary damage predominate in this form of hyperoxia, and it has been suggested that alveolar surfactant may be a site of action of the increased oxygen (O_2) tension (Hackney et al.,1963; Collier, 1963; Jamieson & vanden Brenk, 1964). Since the avian lung is considered to be a semi-rigid structure (McLoed et al., 1964) and since there is some question about the quantity of surfactant present (Miller & Bondurant,1961; Klaus et al., 1962) it might be suspected that the bird would differ from the mammal in the nature of its reaction to OAP. The few comments in the literature on the exposure of birds to hyperoxia at pressures of either 1 atm. or higher (Bert, 1878; Smith, 1889; Thompson, 1889; Binet et al., 1939) make no such distinction, but these were limited studies. In this paper we present our findings on the response of the White Leghorn (W.L.) chick to OAP.

PROCEDURE

All studies were carried out in sealed flexible plastic chambers, approximately 4' x 2' x 2', similar in design to germ free isolators. The concentration of O_2 in the oxygen or experimental isolator was designed to be close to 100%, and in the control or air isolator as close to room air levels as possible. Isolators were kept inflated at a positive pressure of 1/2 to 1 in. water, either by counterweighted 100 liter spirometers filled with O_2, or by direct connection to cylinders of compressed O_2 through pressure regulators of the type used with breathing masks.

Carbon dioxide (CO_2) was absorbed from these closed chambers by pumping isolator gas through an externally located cylinder of soda lime and back into the isolator. Excess moisture was removed by passing the same stream of gas through a glass condenser cooled by a refrigerated anti-freeze solution. Heat was supplied as necessary by thermostatically controlled, externally placed infrared lamps. A fan within the isolator kept the gas continually stirred. Environmental variables were measured 1-3 times a day: O_2 on a paramagnetic analyzer (Beckman E-2); CO_2 on an infrared analyzer (Beckman LB-1); relative humidity (RH) on a hair type hygrometer; and temperature (T) by thermistors or conventional thermometers.

By means of gas locks and arm length dry-box gloves, a number of procedures were carried out within the chambers without loss or contamination of the isolator atmospheres. These included daily removal of fecal and urinary wastes to prevent accumulation of toxic decomposition products; frequent determination of body weights (BW) to the nearest gram; daily replenishment and measurement of intake of feed (to the nearest g) and water (to the nearest ml); obtaining of blood samples, either by venipuncture, heart stab, or [redacted]

posed under local anesthesia and determination of respiratory rate (RR) from 2 successive 1- minute- visual-counts on the bird lying quietly on its side with legs tied.

Arterial blood samples were capped in the syringe in which taken and placed immediately in ice water. Part of the blood was utilized for conventional erythrocyte counts (RBC) hematocrits (Hct.) and hemoglobin (Hb) measurements. The remainder was utilized to determine PO_2, PCO_2 and pH on Instrumentation Laboratory equipment.

At intervals some chicks were killed by cervical dislocation either while in the isolators or immediately after removal. The viscera were examined grossly and lungs preserved in 10% formalin. Subsequently, the lung tissue was prepared for histological examination by conventional H & E staining of 6μ sections.

A total of 36 W. L. chicks ranging in age from 2-7 weeks were used in the 3 trials on which this report is based. Selection was at random except for balancing the sexes. Each trial also included separate cages containing a group of adult white mice in each isolator. Numbers, ages, durations and measurements made on the chicks are listed in Table 1. Attention is called to trial II, in which atmospheres were reversed after 4 weeks (Part A), and observations continued for an additional 11 days (Part B). Also during the last 8 days of trial III feed intake of half the control birds was restricted to simulate the intake of those in 100% O_2.

RESULTS

Appearance, mortality and post mortems

No chicks died in any of the trials due to hyperoxia or confinement in the isolators (One chick was killed accidentally during a heart stab). How-

ever, in each of the trials, all the mice which had been included in the O_2 isolator were dead before the trial was over, with half of them dead within 5 days.

None of the mice in the air isolator died. As far as could be determined by simple visual observation, chicks in O_2 looked and behaved similarly to those in air, appearing active, alert and well preened. The O_2 mice, on the other hand, were obviously dyspneic, lethargic and ungroomed after the 2nd or 3rd day.

Autopsy of chicks killed after two weeks in 100% O_2 did not disclose anything noteworthy. Grossly, their lungs looked no different than controls. By contrast, lungs of mice dying in O_2 showed the typical severe pulmonary congestion, hypermia and edema. The pathologists report of the histological examination of the lungs of the chicks read as follows: "The lungs from the controls appeared normal. Those of the experimental chickens had the following lesions: the lining cells of the respiratory capillaries (alveoli) were swollen. Their cytoplasm was only faintly stained (hydropic degeneration) and their nuclei were vesicular and enlarged. A small amount of fibrin was seen in some air passages. There was no striking hyperemia, hyaline membrane formation, or septal fibrosis as reported in other animal species and man exposed to pure oxygen breathing. The lesions were mild and in our opinion reversible."

Growth, feed consumption and water intake

Fig. 1 shows the BW changes of the chicks in Trial II, including the effect of reversal of the atmospheres after 28 days, and a short period in the animal room following completion of the experiment. A decrease in growth rate is evident within 1-3 days after exposure to OAP and is particularly marked following the reversal of the gases. After one week of exposure to 100% O_2, when the chicks were 2-3 weeks old, their growth was depressed to about 3/4 of the controls.

By the fourth week, when they were about 4 1/2 weeks old, they were growing at about 1/2 the rate of the controls. After reversal, the birds now on O_2 for the first time, instead of the showing the lesser depression seen on the first O_2 exposure, grew only at about 1/4 the control rate. The trial was ended after 11 days because the chicks had outgrown the isolator cage facilities. At this point they were 7 weeks old and weighed about 550 g.

Equally as impressive as the depression in growth following O_2 exposure is the dramatic improvement in growth following return to breathing air. This is perhaps best seen in the last period of trial II, when both groups were removed from the isolators. Within a day or so their rate of growth appears to be the same as that of littermates kept continuously in the animal room, although the isolator birds show the smaller size resulting from their having undergone a period of depressed growth.

A similar pattern of depressed growth was observed in trial III. After one week in O_2, the regression coefficients for weight gain were 6.4 ± 1.3 g/day in O_2 and 13.5 ± 0.9 g/day in air, approximately a two fold difference. During the 2nd week the coefficients were 2.7 ± 0.8 and 9.6 ± 1.4 respectively, close to a four fold difference. At this time, the restricted-fed portion of the air group were gaining at the rate of 2.7 ± 1.0 g/day, essentially identical to the O_2 group. Chicks were between 5 and 7 weeks old and averaged about 500 g in BW in this trial.

Feed intake as a function of BW is plotted for trial II in Fig. 2. Almost immediately after exposure to O_2, the feed/BW ratio dropped sharply. This was true when the atmospheres were reversed, and was also completely duplicated in trial III. During the first week or two, the decrease in feed/BW ratio averaged about 25%; that is, the O_2 birds ate about 3/4 as much feed per unit body weight as the controls. It was on an estimate of this value made at the time that feed was restricted for part of the controls, but the actual amount consumed by them

turned out to be relatively less, 68% of the intake of the ad-lib controls.

In trial II, the difference in feed/BW ratio disappeared after four weeks of continuous exposure to O_2, as if a new balance had been struck between growth and intake. However, no confirmation of this was obtained, possibly because both the reversal phase of trial II and all of trial III lasted under 3 weeks, during which time the ratio remained significantly depressed for the O_2 birds.

Respiratory and blood changes.

Table 2 lists the results of measurements made on RR, hematology and blood gases. In most cases the values shown represent a pooling of data obtained in trials II and III. The table also shows the probable effect of decreased feed intake per se on these variables, as derived from comparison of the restricted-fed to the ad-lib fed portions of the controls of trial III.

In two tests in trial II (before and after reversal) and two in trial III, the O_2 birds exhibited a consistent statistically significant slower RR, averaging about 31% less than the controls. There was no indication that this slower rate was due to reduced feed intake. The measurements made 11 days after reversal of atmospheres in trial II showed that upon return to air breathing, RR returned to normal.

The hematological pattern as determined from a venous sample in trial II and an arterial sample in trial III shows approximately a 10% depression in RBC, Hb and Hct, the changes in the latter two being statistically significant. Restricted feeding produced on the contrary a significant increase in these variables.

Blood Po_2 and Pco_2 were measured only on the arterial samples obtained in trial II. Po_2 for the O_2 birds was highly significantly elevated to over 300 mm Hg. but Pco_2 was unaffected. Blood pH was determined on both heart stab blood (Trial II) and arterial blood (Trial III) without indication of any significant

difference due to 100% O_2. Restricted feeding apparently decreased Po_2 in the controls and possibly elevated pH, opposite to the trends shown in the O_2 birds.

Environmental conditions

Temperatures averaged within 3° F and RH values within 2% of each other in the O_2 and air isolators over the course of the 3 trials. (Overall averages were 76° F and 82% RH). CO_2 levels averaged 0.3% with the difference between systems never larger than 0.2%. The concentration of O_2 in the oxygen chamber was consistently 98% or better, with an overall average of 98.7%.

The only difficulty encountered in maintaining the desired environmental conditions within the isolators was in holding the O_2 concentration of the air system at 21%. Isolators leaked to varying degrees, and since they were kept inflated from a 100% O_2 source, more O_2 entered than was required to replace that used in metabolism. This tended to raise the O_2 concentration, which was desirable in the O_2 system, but unwanted in the controls. For example, in trial III, the air isolator averaged 33% O_2. Trial II was almost leak free, however, and the air isolator averaged a reasonable 22% O_2. Trial I was intermediate with an average control O_2 level of 25%.

DISCUSSION

This study shows clearly that the growing W.L. chick, at least up to the age of 7 weeks, is highly resistent to the lethal effects of 100% O_2 at one atm. pressure. No mortality, no obvious morbidity and no gross post mortem lesions were observed in continuous exposures lasting as long as 4 weeks. However, the hyperoxia is not completely without effect, for the chicks in O_2 grew at a slower rate, consumed less feed, breathed less rapidly, had fewer RBC's and their lungs showed some histological changes.

It would appear unlikely that extraneous environmental factors intervened

to give the chicks their apparent immunity. Although the O_2 exposure took place in a "sealed" type of system in which all environmental interactions may not be clearly delineated, control chicks were treated identically. T, RH and CO_2 were well controlled in the isolators and experimental O_2 concentrations were close to the desired 100% level. The one environmental defect, the increased O_2 concentration in the air system in trials I and III would have acted to decrease the differences between treated and control groups. But perhaps the best indication that an effective hyperoxic atmosphere has been achieved was the fact that half of the mice exposed simultaneously with the chicks died as expected within 5 days with all the signs of pulmonary damage typical of oxygen toxicity.

As the arterial O_2 tensions in the 100% O_2 birds were elevated about as much as expected (Bergovsky et al., 1964), one may deduce that the protection is not due to some block in the transmission of O_2 to or through the lungs. Furthermore, a decrease in RBC and RR is commonly noted in OAP exposure (Bean, 1945) and would indicate an effective elevation of tissue O_2 concentrations. It must be then that the lungs per se of the chick are comparatively resistant to the atelectasis, hypermia and edema generally associated with high O_2 concentrations.

While this interpretation supports the hypothesis that pulmonary surfactant is a site of action of high O_2 concentrations (Hackney et al., 1963; Collier, 1968; Jamieson & Vanden Brenk, 1964), since surfactant may be low in avian lungs (Miller & Bondurant, 1961; Klaus et al., 1962), such a conclusion must be approached with caution. For example, others using different extraction methods not only did not find a deficiency of surfactant in bird lungs (Pattle et al., 1961 & 1963), but claim that the requirement for a surface-tension-lowering agent may be greater in the bird than in the mammal because of the smaller diameter of air capillaries

compared to alveoli (Fedde and Burger, 1963). In addition, some have questioned whether surfactant plays any role at all in oxygen toxicity, (Bondurant & Smith), 1962; Fujiwara et al., 1964). Regardless of the surfactant question, the fact remains that the semi-rigid avian lung with its continuous air capillaries and numerous air sacs is anatomically quite different from the mammalian respiratory system and this difference might be related to the resistance of the chick to OAP.

An equally plausable alternative to the anatomical argument might be to consider the resistance of the chick to be a function of age. The young rat for example is markedly tolerant of OAP whereas the adult shows all the typical respiratory pathology (Smith et al., 1932). The young rat exposed to OAP also exhibits anorexia and progressively severer depression in growth with age as does the chick. Were this the explanation for the resistance of the chick, it also might explain why the phenomenon was not detailed before, since apparently the earlier studies on birds were based primarily on adults, although the species used did not include the domestic fowl (Bert, 1878; Smith, 1889; Thompson, 1889; Binet et al., 1939). Actually Binet et al., (1939) do comment on the unusual resistance of one pigeon which they describe as being in the period of growth.

It must not be assumed however, that all immature animals are necessarily resistant to OAP, for apparently the weanling mouse (Smith, 1889) and the puppy (Paine et al., 1941) are about as susceptible as the adult mouse and dog. Unfortunately, our present equipment was inadequate to make the necessary tests on adult chickens.

As for the decrease in RR and RBC which were observed in the O_2 chicks, the inclusion of a small group of restricted-fed controls for comparison shows that these changes were real effects of hyperoxia and not due to anorexia.

Further, the fact that restricting the feed intake of controls to approximately that of the O_2 birds produced an almost identical depression in growth suggest that the slower growth rate in O_2 could be entirely a function of the anorexia. The fact that the feed/BW ratio drops abruptly is further evidence as indicated by Smith et al. (1960), that among the first effects of the hyperoxia is a decrease in feed intake and not some wastage of or inability to utilize ingested feed. Neither is it likely due to any alteration in fluid balance, since the water/feed ratio remained unchanged.

These results provide little data relative to whether the decreased feed intake is in turn secondary to fall in metabolism, or a direct effect on digestive function or perhaps even to depression of appetite, although it has been noted that rats dying in OAP had intestines filled with food (Smith et al., 1960). The unchanged Pco_2 and blood pH suggest that there was no interference with CO_2 transport or acid base balance. Whatever the reason for the decreased food intake, it is rapidly and completely reversible in the young chick upon termination of the hyperoxic exposure.

SUMMARY

The White Leghorn chick between the ages of 2 and 7 weeks is markedly resistant to the toxic effects of 1 atm. O_2. Continuous exposure for as long as 4 weeks caused neither deaths nor obvious morbidity nor any signs of pulmonary damage on gross autopsy. Nevertheless the hyperoxia had some adverse effects, primarily reducing the growth rate to 3/4 to 1/4 of normal, reducing feed intake per unit body weight to 3/4 of normal, slowing respiratory rate by 31%, decreasing erythrocytes, hemoglobin and hematocrit by 9-12%, and causing reversible histological changes in the lungs. Arterial O_2 tensions were elevated over

300 mm Hg, but arterial PCO_2 and blood pH were unaffected. No residual effects were noted upon return to air breathing. It is possible that the anatomical peculiarities of the avian lung play some role in the chicks resistance to hyperoxia, but is also possible that it is a function of age, similar to the tolerance shown by the young rat, but not the adult.

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Table 1. Experimental design used in the exposure of the chick to 100% O_2.

Trial No.	No. of Chicks per Chamber	Type of cage	Age at start of 100% O_2 (weeks)	Duration of 100% O_2 (days)	Measurements made on animals
I	4	common	5	14	Behavior, autopsy, Lung histology
II* A	6	common	1 1/2	28	BW, feed+water intake, RR
B	4†	common	5 1/2	11	venous & heart stab blood samples
III	8	individual	4	16	BW, feed & water intake, RR, arterial blood samples.

* In trial II, experimental and control atmospheres were reversed after 4 weeks.

† Two chicks killed and autopsied after 3 wks of first exposure (IIA)

Table 2. Effect of 100% O_2 on various physiological variables in the White Leghorn Chick. (Birds between 5 & 7 weeks of age, exposed to O_2 for 2-3 weeks at time of measurements).

Measurement	Air Isolator (Controls)	Oxygen Isolator Experimentals	Pooled Stand. Error	Effect of restricting feed of controls $\Delta \pm$ SEMD †
Respiratory Rate, (/min)	(20) 42	(20) 29	3.6*	3 ± 4.1
Red Cell Count (10^6 /mm ^{3})	(9) 2.5	(10) 2.2	0.14	--------
Hemoglobin (g/.100 ml.)	(10) 8.6	(10) 7.8	0.29*	1.9 ± 0.72*
Hematocrit (%)	(10) 27.3	(10) 24.8	0.75*	6.4 ± 2.14*
Arterial O_2 Tension (mm Hg)	(6)97.1	(7) 330.8	13.55*	-24. 6 ± 3.33*
Arterial CO_2 Tension, (mmHg)	(6)26.2	(7) 25.7	0.47	0. 1 ± 1.05
Blood pH	(4) 7.50	(6) 7.45	0.021	0.07 ± 0.131

* Significant at the 5% level, or better

† Δ = difference between restricted- ad lib fed,

SEMD = standard error of mean difference (with 6 degrees of freedom)

Figure 1.

Growth pattern in White Leghorn chicks maintained in 100% O_2 at 1 atm. pressure. Chicks 12 days old at start of O_2 exposure. Six chicks per treatment.

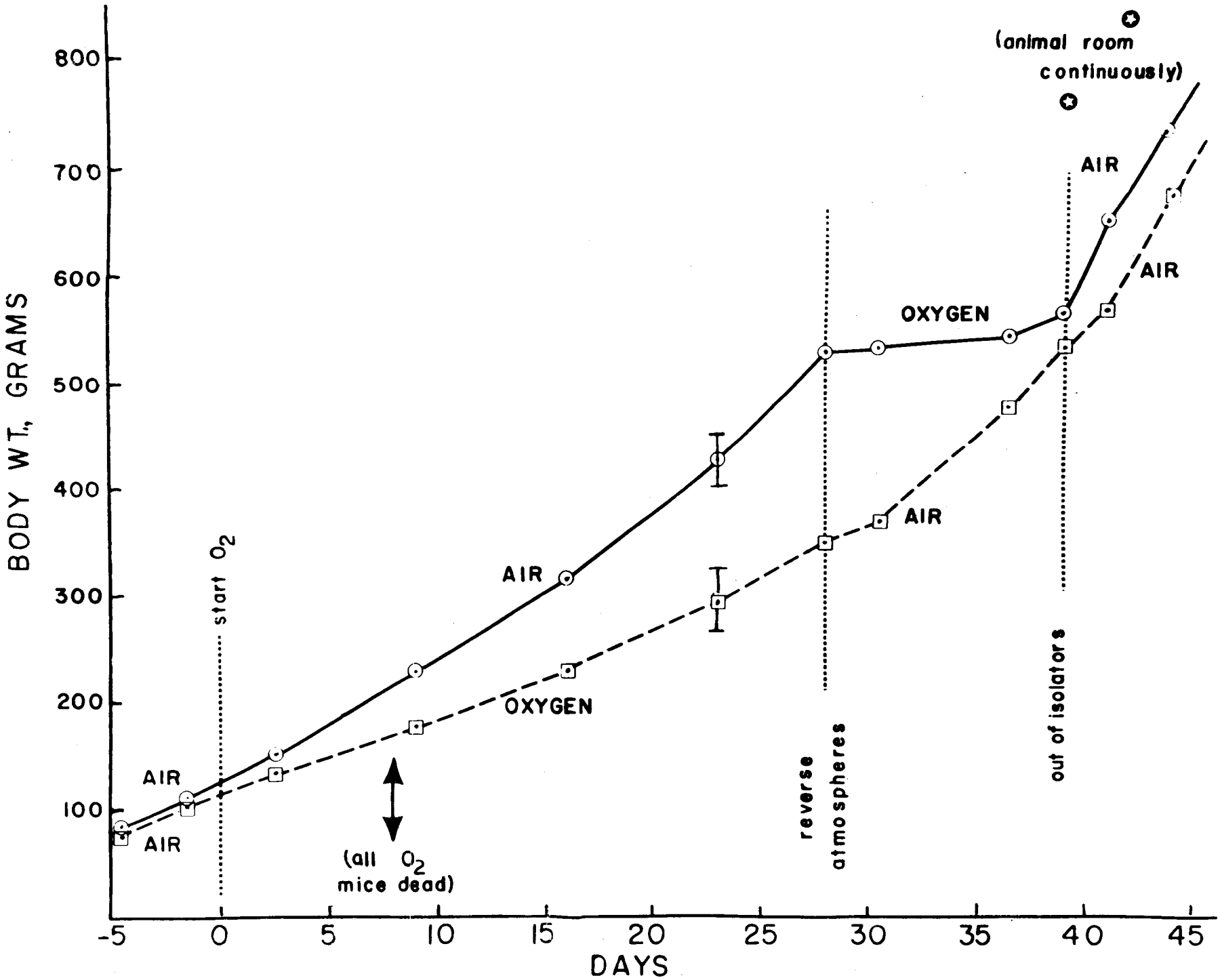
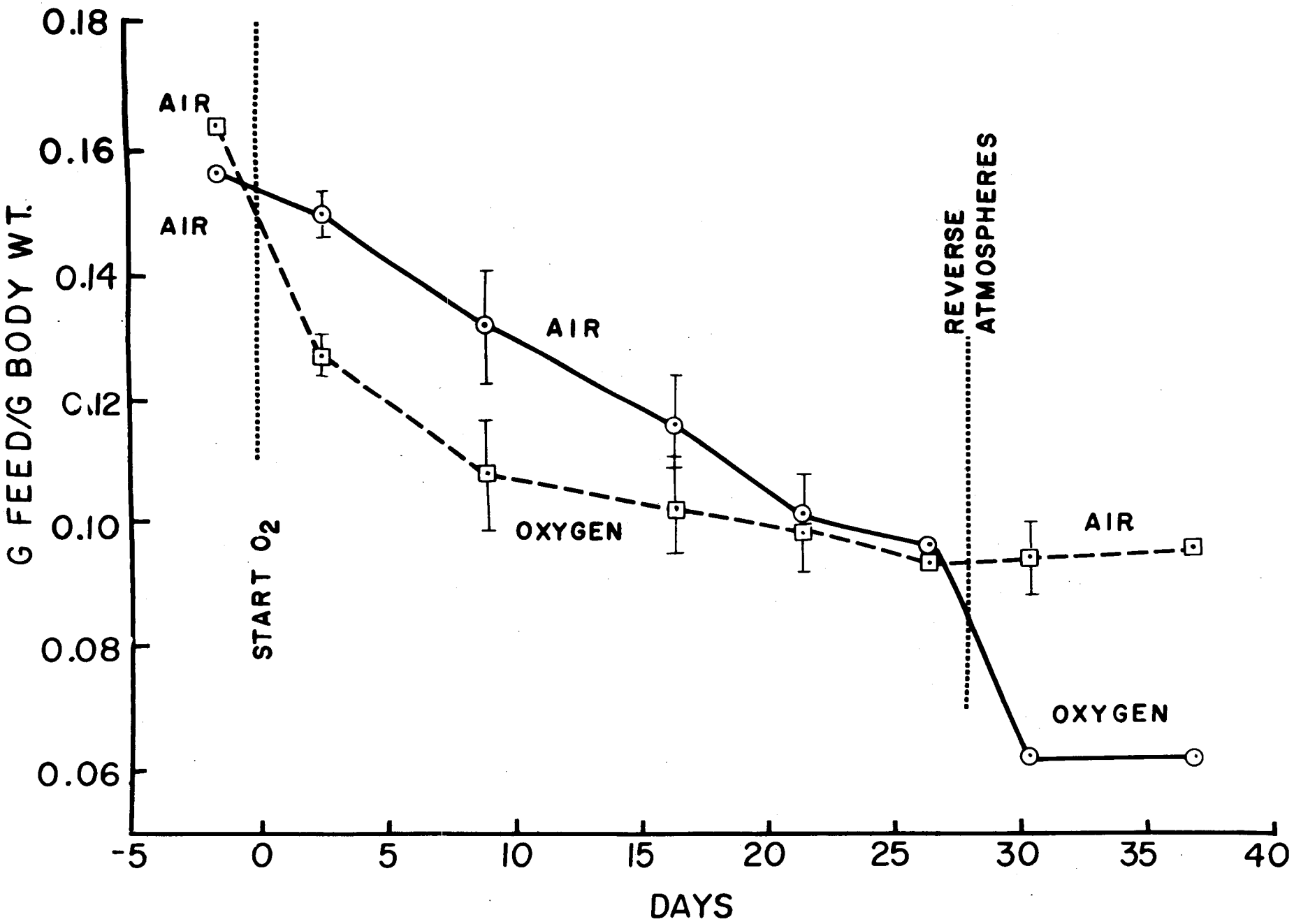

BODY WT, GRAMS
800
700
600
500
400
300
200
100
-5
0
5
10
15
20
25
30
35
40
45
DAYS
(animal room continuously)
AIR
AIR
OXYGEN
AIR
AIR
start O_2
AIR
OXYGEN
AIR
(all O_2 mice dead)
reverse atmospheres
out of isolators

Figure 2.

Pattern of feed intake as a function of body weight in White Leghorn chicks maintained in 100% O_2 at 1 atm. pressure. Chicks 12 days old at start of O_2 exposure. Six chicks per treatment.


0.18
0.16
0.14
0.12
0.10
0.08
0.06
G FEED/G BODY WT.
AIR
AIR
AIR
OXYGEN
AIR
OXYGEN
START O_2
REVERSE ATMOSPHERES
-5
0
5
10
15
20
25
30
35
40
DAYS

REFERENCES

1. Bean, J. W. Effects of oxygen at increased pressure. Physiol. Rev. 25:1-47, 1945.

2. Bergofsky, E. H., M. C. H. Wang, T. Yamaki, and J. H. Jacobson, II. J. Am. Med. Assoc. 189:841-844, 1964.

3. Bert, P. La Pression Barometrique. (1878). Translated by M. A. and F. A. Hitchcock, Columbus,Ohio,College Book Co.,1943 p 552-580.

4. Binet, L., M. Bochet, and A. Bryskier. Les atmospheres suroxygences. J. Physiol. Pathol. Gen., 37:524-535, 1939.

5. Bondurant, S. and C. Smith. Effect of oxygen intoxication on the surface characteristics of lung extracts. Physiologist. 5:111, 1962.

6. Collier, C. B. Pulmonary surface activity in O_2 poisoning. Fed. Proc. 22:339, 1963.

7. Fedde, M. R. and R. E. Burger. Death and pulmonary alterations following bilateral cervical vagotomy in the fowl. Poultry Sci. 42:1236 -1246, 1963.

8. Fujiwara, T., F. H. Adams and K. Seto. Lipids and surface tension of extracts of normal and oxygen-treated guinea pig lungs. J. Pediat. 65:45-52 1964.

9. Hackney, J. D., C. R. Collier, D. Conrad and J. Coggin. Pulmonary surface phenomena in oxygen poisoning. Clin. Res. 11:91, 1963.

10. Jamieson, D. & H. A. S. vanden Brenk. Pulmonary damage due to high pressure O_2 in rats. Changes in the surface active lung lining. Aust. J. Exper. Biol. & Med. Sci. 42: 483-490, 1964.

11. Klaus, M., O. Y. Reiss, W. H. Tooley, C. Piel, and J. A. Clements. Alveolar epithelial cell mitochondria as a source of the surface-active lung lining. Science 137:750-751, 1962.

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12. McLeod, W. M., D. M. Trotter, and J. W. Lumb. Avian Anatomy. Minneapolis, Minn. Burgess Publishing Co., 1964. pp 47-56

13. Miller, D. A. and S. Bondurant. Surface characteristics of vertebrate lung extracts. J. Appl. Physiol. 16:1075-1077, 1961.

14. Paine, J. R., D. Lynn, and A. Keys. Observations on the effects of the prolonged administration of high oxygen concentration to dogs. J. Thoracic Surg. 11:151-168, 1941.

15. Pattle, R. E. and F. Burgess. The lung lining film in some pathological conditions. J. Path. and Bact. 82:315-331, 1961.

16. Pattle, R. E. and D. A. W. Hopkinson. Lung lining in bird, reptile and amphibian. Nature. 200:894, 1963.

17. Smith, C. W., J. W. Bean, and R. Bauer. Thyroid influence in reactions to O_2 at atmospheric pressure. Am. J. Physiol. 199:883-888, 1960.

18. Smith, F. J. C., J. W. Heim, R. M. Thomson and C. K. Driker. Bodily changes and development of pulmonary resistance in rats living under compressed air conditions. J. Exp. Med. 56:63-78, 1932.

19. Smith, J. L. The pathological effects due to increase of oxygen tension in the air breathed. J. Physiol. 24:19-35, 1899.

20. Thompson, W. G. The therapeutic value of oxygen inhalation, with exhibition of animals under high pressure of oxygen. N. Y. Med. Record. 36:1-7, 1889.