NSG-295-62

EMBRYO DEVELOPMENT AND CHICK GROWTH IN A HELIUM - OXYGEN ATMOSPHERE*

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

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

Mail Proofs to: Harold S. Weiss
Dept. of Physiology
The Ohio State University
College of Medicine
312 Hamilton Hall
1645 Neil Avenue
Columbus, Ohio 43210

UNPUBLISHED PRELIMINARY DATA

Running head: He - O_2 atmosphere on chick development

REPORTS CONTROL No. 1

* Footnote: Supported by Grant No. NsG-295-62 from the National Aeronautics and Space Agency.

ABSTRACT

Weiss, H.S., R. A. Wright and E. P. Hiatt. Embryo development and chick growth in a He-O_2 atmosphere. J. Appl. Physiol.

22195

Fertile chicken eggs were incubated in approximately 79% He -21% O_2 with up to 1-2% residual N_2 in a sealed, flexible, plastic isolator in which temp., relative humidity, O_2 and CO_2 were controlled. Live, healthy chicks were hatched in He-O_2, but only half as many as in a comparable air system (trial I, He-7 chicks/18 fertile eggs, air-16 chicks/18 fertile eggs; Trial II, He-8/35, air-20/35). The poorer He-O_2 hatch was due mainly to late embryonic death. Hatching time was similar, but the He chicks were 9% smaller. The He isolator required more electrical power to maintain incubation temp. but had lower inside surface temp. During development He embryos showed neither gross defects nor differences in dry wt. or in H_2 conc., but He eggs lost 27% more wt. During an additional 4 weeks in their respective atmospheres, chick growth and hematology were similar, but the He birds consumed up to 16% more feed. Higher heart and respiratory rates, lower T_B and a tendency to huddle in He suggested that the increased feed intake might be in response to a higher metabolism, stimulated by a more rapid loss of body heat because conduction of heat is 6 1/2 x greater in He than H_2. Increased conduction of heat in He may also be responsible for dehydration of eggs as well as direct effects on embryogenesis. Sudden removal of chicks into room air after 4 weeks in He-O_2 had no observable effect.

Author

INDEX TERMS

Inert gas on development
He on development
Embryo development in He
Artificial atmospheres
Absence of N_2 on development
Chick growth in He
Sealed systems

Current interest in "sealed environments" and artificial atmospheres has focused attention again on the role of gaseous nitrogen (N_2) in the breathing atmosphere. Using the avian embryo as a convenient assay organism, several workers (1, 3, 15) have indicated that departures from normal growth and development occur when helium (He) is substituted for N_2. Volskii (15) indicates that when eggs are incubated in such N_2 - low atmospheres, the embryos die by the ninth day and Allen (1) finds 93% of the embryos show some abnormality by the fourth day. Boriskin et al. (3), on the other hand, hatched normal chicks in an 80% He - 20% O_2 atmosphere, but only half as many as in a comparable air system. Although Allen (1) takes Boriskin et al's. (3) lower hatchability in the He atmosphere as support of his findings of only 7% normal embryos at four days, clearly, any size hatch is incompatible with Volskii's (15) observations that all embryos were dead by the ninth day.

Boriskin et al. (3) mention maintaining chicks in a He-O_2 atmosphere for several weeks, but gives no details. Neurospora are reported to grow more rapidly in He-O_2 , possibly because of elimination of the narcotizing influence of N_2 (12). Although early studies indicate normal growth of mice in He-O_2 (2, 6), recent work suggests that metabolic rates are elevated, possibly because of greater loss of body heat due to higher heat conduction in He compared to N_2 (7). Similarly, Boriskin et al. (3) suggest that their poorer hatch of chicks in He-O_2 was due to lowered intra-egg temperatures because of the more rapid conduction of heat in He. Volskii (13), however, raises (or perhaps better, resurrects) the intriguing question of whether failure in embryonic development may not be due to blockage of some essential N_2 fixation process.

As environmental systems which exclude gaseous N_2 are either in current use or are contemplated for long term space and underwater exploration and as therapeutic measures in surgery and medicine, it would seem important to pursue further the question of substitution of He for N_2. This report summarizes our results with the avian embryo through hatching and on chick growth thereafter, in a gas mixture of 79% He and 21% O_2 at one atmosphere total pressure.

PROCEDURE

Egg incubation and chick growth phases were carried out in clear flexible plastic containers, roughly 2 ft. x 3 ft. in size, very similar in design to the isolators used in germ free work. Slight positive pressure of 8 - 10 mm H_2O maintained by a weighted spirometer, kept the isolators inflated and insured that any leaks would be outboard. Changes in spirometer level also served as a measure of gas movement through the isolators, whether due to leakage or metabolism or both. During incubation the isolators were covered with a thin layer of reflective cloth to reduce heat loss.

Helium (He), oxygen (O_2), combinations of He and O_2, and room air were introduced into the isolators via the spirometers as required to maintain an atmosphere of approximately 79% He - 21% O_2 in the experimental and 79% N_2 -21% O_2 in the control system. Muffin fans of 100 CFM capacity kept the gas continually mixed. Transfer of materials in and out of the isolators was accomplished via an 18 in. diameter flushable gas lock and manipulations within the system via dry-box gloves, without contamination by room air. Electrical and gas connections were made through rubber stoppered nipples one inch in diameter.

Procedures were set up to keep isolator environment within the range considered normal for incubation of chicken eggs and growth of chicks (12, 14). Isolator gas was pumped through a closed external circuit containing indicating soda lime for carbon dioxide (CO_2) absorption and a water cooled condensor for removal of excess moisture. The volume of water condensed was measured to the nearest ml. Flow through the absorbing circuit was adjusted to keep CO_2, as measured on a Beckman LB-1 analyzer, below 0.5%.

Relative humidity(RH), as monitored on a hair-type hygrometer, was kept between 50-70% during incubation. Little use was required of the condensers during incubation, but before the growth phase was over, they were being operated at their maximum capacity.

Oxygen was monitored on a Beckman E-2 analyzer and He on a modified Cambridge analyzer. Temperatures (T) were monitored both by thermistors and conventional thermometers. Thermostats were used to keep gas temperature (gas T), close to 99.5°F during incubation and to allow it to decrease by 5° per week after hatching. In one trial, the inside top surface T of the isolator was monitored by a thermistor and electrical power input was followed on a kilowatt hour (KWhr.) meter. All variables were recorded at least daily and generally 3 times a day.

Up to 48 fertile eggs were incubated at one time in a wire tray pivoted in the center. The tray was turned through 90° three times a day for the first 18 days and kept horizontal thereafter. At intervals during incubation, eggs were weighed within the isolators to the nearest g, and groups of eggs were removed for various tests. Some of the removed eggs were opened for visual examination of the embryos in situ, following which the embryos were excised and held at 105°C for 24 hours in order to obtain dry weights. Other embryos were used for metabolic studies and tests of sensitivity to x-rays (17). Still other eggs were analyzed, shell and all, for total N_2 by macro-Kjeldahl, after first being digested in conc. HCl and thoroughly mixed. These particular eggs were weighed to 0.001 g before incubation, and the presence or absence of an embryo determined by candling before the N_2 analyses.

Time of hatching was determined by noting the number of chicks out of shell at the various observation times. After 22 days, all incubation materials, except for some of the chicks, were removed from the isolators and cages introduced in place of the hatching trays. Chick weights were determined at frequent intervals

to the nearest g. Chick starter mash and water were supplied ad libidum, and in one trial feed and water intake were measured daily. Feces were caught on aluminum foil and removed from the isolators approximately weekly. At intervals, heart rates (HR) were recorded on an ECG, respiration rates (RR) visually or by pneumograph connected to a pen writer, and body temperature (T_B) by rectal thermistor. Blood samples were taken from a wing vein or by heart stab for hematological studies (4).

Where possible, analysis of variance statistical techniques were applied to the data in order to detect treatment (He vs air), period (mainly week to week) and interaction (treatment vs period) effects. In general, however, only average treatment values are presented in the tables and reference to period and interaction made only where they were statistically significant and pertinent to the analysis.

RESULTS

Incubation phase

Hatchability of eggs in He-O_2 compared to air was studied in two trials with results as shown in Fig. 1. Normal appearing chicks hatched out in both systems, but significantly fewer from the eggs incubated in the He-O_2 atmosphere. Despite considerably poorer hatchability of all eggs, including the air controls, in Trial II, the depressing effect of [redacted] He was much the same in Trials I and II (5% and 30% decrease respectively). The 89% hatchability of the air eggs in Trial I may be considered quite as good as is normally encountered in commercial incubation, indicating that there was nothing inherently deleterious in the isolator procedure.

Times of death was estimated for the embryos in Trial II only (Table 1) and indicated that the major effect of the He was expressed in a higher number of late deaths (15 days or later). Had all the late deaths hatched, for example, there

would have been 28 chicks in air and 24 in He. On breaking open unhatched eggs to determine time of death, greater dehydration was consistently evidenced in the He eggs by increased size of the air space.

Average hatching time was estimated from the recorded data of number of chicks out-of-shell at various times between the 18th and 22nd day. This is a somewhat crude estimate and no statistical treatment was attempted. The only effects suggested are a slightly shortened hatching time for He eggs in Trial II, and for all eggs in Trial II (table 1). Body weights of those chicks which did hatch were significantly less in He than in air. The degree of depression in size was almost identical in the two trials, close to 9%, although control chick weights were about 7% higher in Trial II than in Trial I. In making weight measurements, air chicks were selected at random to equal the number of He chicks available.

Data obtained on isolator conditions during incubation are summarized in Table 2. For gas T, the only significant treatment effect was the 0.2°F higher level in the He system during Trial II. Average gas T in Trial I was 0.5° F lower and in Trial II 0.3°F higher than the planned for level of 99.5. If, in view of the 89% hatch in the controls, we consider the gas T in Trial I to be optimum for incubation in these isolator systems, then in Trial II, the higher overall T as well as the higher T in He is generally consistent with the associated smaller hatches and shorter hatching times. However, as all these incubation temperatures appear to be well within the range in which normal hatches could be expected (14) it would seem that at most, only a very small part of the observed differences in hatchability can be ascribed to differences in gas T.

Inner surface T of the isolator was 0.7°F lower in the He system. Most of the difference between systems in surface T developed during the last half of incubation and was due [illegible] to a rise in surface T of the air isolator while

that of the He isolator remained close to 94.0°F (interaction significant). For the last week of incubation, for example, the difference in surface T was 1.4°. Power input tended to be higher for the He system throughout incubation, but statistical significance could only be shown by resorting to analysis of the daily differences between systems. The smaller standard errors (SE) associated with temparature measurements in Trial II apparently reflect the greater stability and sensitivity of an electronic compared to a mechanical thermostat used in Trial I.

All O_2 concentrations tended to be 1-2 percentage points below the planned for level of 21% with the air system slightly (0.6 to 1.0 percentage points) but nevertheless significantly lower than He in both trials (Table 2). Thus whatever inhibition on hatching the lower O_2 levels might have had, should have been most apparent in the controls. No differences were found in the CO_2 concentrations which were below 0.4% in all trials. Measured He levels were close to the 79% level planned for. Nitrogen concentrations, determined by difference (100% - $\%O_2$ - $\%CO_2$ - %He), were close to the expected 79% in the air system, and less that 1% in the He system (where, theoretically there should have been none). Relative humidity varied between 55 and 65%, generally higher in the He system. In both systems, moisture tended to condense out and be trapped in the gloves, tubes and nipples extending outward from isolators. Some of this condensate was lost when connections to the isolators had to be manipulated, but no quantitative measurements were made.

Eggs in He lost more weight than those in air throughout incubation, the difference amounting to 1.9 g or 27% by the 19th day (Fig. 2). Analysis of this weight loss according to the degree of embryonic development (Table 3) shows that the greatest difference between treatments existed in the late death group (45%) and least in those which hatched (17%). Treatment had no effect on embryo dry weights during the first 2/3 of incubation, the average value shown in Table 3 being a

composite of measurements made after 4, 12, and 16 days on eggs from several different trials. No effect of incubation or of treatment could be demonstrated on the total N_2 content of the eggs with embryos (table 3). These data are also a composite of separate trials. Additional N_2 values at 8 and 23 days of incubation and on infertile eggs and eggs with early dead embryos have been omitted since they showed neither significant differences nor trends.

Growth Phase

Seven chicks in trial I and 8 in Trial II were held over in each isolator for observation of growth and behavior. For the 3-4 weeks during which the systems were kept sealed, growth was essentially the same in He-O_2 as in air (Fig. 3). The initially smaller size of the He birds (Table 1) tended to be maintained throughout the period of observation and even after removal from the isolators. In trial II for example, the He birds were on the average some 21g (7 1/2%) lighter at the 28th day. In Trial I, however, the maintenance of a smaller size in He is somewhat obscured due to an overnight failure on the 13th day in the O_2 supply of the control isolator which resulted in the death of 3 birds and a temporary inhibition of growth in those remaining.

In Trial II, inboard leaks developed on the 17th day and 19th day in the absorption circuit of the He isolator, resulting in 4-5 hours of increased N_2 concentration (marked by arrows in Fig. 3), but no effect on subsequent growth was detected. These short periods of increased N_2 had essentially no effect on the average values for isolator conditions. Growth rates in the isolators were apparently normal, as is suggested by the similarity in weight of littermates kept continuously in the animal room (marked by X's in Trial II, Fig. 3). Abrupt removal of chicks into room air after their incubation and growth in He-O_2 had no discernable effects, either immediately or over an additional week that they were

kept under observation.

During their sojourn in the isolators, the He chicks showed more of a tendency to huddle, as if they felt cold, but otherwise behaved similarly to those in air. Near the end of the 4th week in the isolators (in Trial II), a few chicks in both He and in air exhibited a peculiar drowsiness syndrome which persisted but with decreasing intensity even after removal from the isolators. This syndrome may be related to fecal and/or urinary toxicants, possibly ammonia, for in other studies where excretory wastes were removed more frequently, the ammonia order was much reduced and no drowsiness symptoms appeared.

Although growth rate was apparently little affected by the composition of the atmosphere, feed intake became progressively higher for the He birds throughout their stay in the isolator (Fig. 4). By the 4th week the He birds were consuming 16 % more feed than those in air. This divergence in feed intake was shown to be statistically reliable by a highly significant interaction term in the analysis of variance of the data plotted in Fig. 4. Further analysis of feed intake, using daily differences between systems, showed the average difference increasing from 1.0 ± 0.73 at week 2 to 4.1 ± 1.20 g/bird/day by week 4. However, when a similar analysis was made on a unit body weight basis, the difference between groups turned out to be uniform over the 3 weeks of measurements at either 0.02 ± 0.004g feed/g BW/day or 0.34 ± 0.134g feed/g gain/day. Water intake tended to be higher for the He birds, but this appeared to be a function of their greater food consumption, for the difference reversed itself when expressed per unit of feed intake (Table 5).

Additional measurements made on the birds at various times during growth in the isolators are listed in Table 4. The item marked condensate refers to the amount of water removed daily by the humidity control equipment. One of the interesting features here is that although significantly more water was removed from the He system in the control of RH, most of the difference disappears when

when expressed per unit water intake. Thus, since comparable RHs were maintained in the isolators (Table 5) there is little liklihood that dehydration of the animals or their wastes occurred.

Hematological studies revealed only one difference, of dubious import, between groups. This was the tendency for the hemoglobin to fall in He while rising in the controls between the 15th and 23rd days, as pointed up by a statistically significant interaction term in the analysis of variance. Another feature of possible interest was the low WBC in both systems, perhaps 1/2 - 1/3 of normal (13). This may be a function of their confinement in the isolators, although the remaining hematological values appear to fall in the normal range. A tendency for lower T_B in the birds was observed in both trials, although the difference is statistically significant only in Trial I. Heart rates and respiratory rates were significantly higher in He at all measurements (Table 4).

As for temperature and gas composition within the isolators during growth, the only differences of statistical significance have to do with surface T and power inputs. Although the average difference in surface T between air and He was not in itself statistically significant, the interaction between treatment and period was. As indicated in Table 5, the interaction effect is due to a reversal in the normally lower surface T of the He system (see eg. Table 3) Similarly, the normally higher power input of the He system as seen during incubation and in the first 9 day period of growth (statistically significant if analyzed on basis of daily differences between systems) was reversed by the last 9 days. The concentration of O_2 remained relatively normal during growth, but CO_2 levels were slightly higher than planned for, particularly for the He system in Trial I. Nitrogen levels, estimated by difference, averaged under 2% in the He isolator. Although RH was higher than normally desired for animal rearing, it was essentially the same in the two systems.

Discussion

Embryo Development Phase

In this study, the major effect on the development of the avian embryo which followed the replacement of N_2 by He was seen in the hatching of fewer chicks, due primarily to higher mortality during the last week of incubation. There were no differences in gas T or in O_2, CO_2 and R H levels which could account for the smaller hatch. On the other hand, associated with the He system during incubation were factors such as increased egg weight loss, lower chick hatching weights, increased electrical power input, and lower surface T.

In two trials, the reduction in numbers of chicks hatched in an atmosphere of roughly 79% He - 21% O_2 as compared with those hatched in air was 56% and 60%. This result is almost identical to the 58% depression in hatch size found by Boriskin et al.(**3**), who used eggs from a different breed of chickens and a different mechanical system, but a similar gas mixture. Large as this reduction in hatch may be, however, it is hard to reconcile with Allen's finding of only 7% "normal appearing" embryos after 4 days of incubation in 79% He- 21% O_2, and certainly would seem incompatible with Volskii's (**15**) report that all embryos were dead by the 9th day.

Equally hard to reconcile with the concept of widespread early embryonic derangement or death are the findings in the present study that the poorer hatch was due primarily to late embryonic death, an observation also noted by Boriskin et al. (**3**). Furthermore, in many trials in addition to the two described here, we have been unable to detect in the early phases of incubation in He-O_2 any difference in appearance or in dry weight of the embryos, or in N_2 content of egg and embryo. So far, it is only in greater loss in weight of eggs and a change in O_2 uptake of 8 day embryo homogenates (**17**) that we find evidence of a possible He effect in the first half of embryogenesis.

No factors stand out clearly at this time which might explain the different results obtained by the various investigators who have studied embryonic develop-

ment in He-O_2 atmospheres. Small differences in residual gaseous N_2 in the incubator atmospheres presumably are not involved inasmuch as Allen's (1) 93% abnormal embryos were observed in the presence of more than 10% N_2 (80 mm Hg partial pressure). Possibly many of the embryos which Allen (1) classified as "abnormal" at the 4th day may be capable of development into the last week of incubation and even to hatching. As far as can be determined from the published reports, such fundamental incubation criteria as T and RH were reasonably normal in all studies. Nevertheless, because of subtle differences between He and N_2 in certain physical properties, it is possible that normally overlooked differences in the mechanics of the systems used to incubate the eggs may become important.

In our trials, the significantly higher weight loss of the He eggs during incubation (Table 3), would appear to have some bearing on the poorer hatchability of these eggs. This was evident not only in the overall treatment difference, but also in the fact that the difference was highest for the late deads and least for those which hatched. Although Robertson (8) fails to find such effect of egg weight loss on hatchability, most workers agree that an inverse relationship exists between the two variables (16). Hays and Spear (5), for example, found weight losses greater than 12% by the 17th day were associated with significantly lower hatchability. However, by extrapolation of Hays and Spears' 5) data to our results it would seem that no more than half of the depressed hatch in He is likely to be accounted for by the greater egg weight loss.

Explanation of this greater weight loss of eggs in He is somewhat difficult. Presuming that it is essentially water which was lost, calculations can be made which show first that since leak rates were the same, any difference in RH of gas entering and leaving the isolator was far too small to account for the difference in egg wt. loss. Similarly these same calculations show that the

gas flow through either system could not have carried away the moisture evaporated from the eggs. For example, of the total of 258 g lost by the air eggs and 373 g by the He eggs in the first 2 weeks of incubation, only 50 and 90 g respectively could have been carried out by the gas flowing through the isolators, leaving 208 and 283 g unaccounted for, respectively.

Apparently the moisture condensed in the gloves, tubes, etc. extending out from the isolators, some of which was lost when connections to the isolators were manipulated, makes up this "unaccounted for" water. There is little doubt in our minds that 100-200 g a week could easily have been removed this way. It also seems reasonable to assume that more such moisture would condense in the He system than in air because of the liklihood of more rapid loss of heat from the trapped He-O_2 mixtures. In support of this view, there is the observation that during the growth phase more moisture was continually condensed out of the He isolator by the same type of cooling unit that was operating in the air system (Table 6).

Boriskin et al's.(3) explanation for their poorer hatch in He-O_2 centers around the 6 1/2 fold greater heat conducting capacity of He (He-0.000339 and N_2 - 0.0000524 cal/sec/cm^2 per cm thickness, per degree C. -Handbook of Chem., 37th ed., p. 225) The theory is that since intra-egg T during the latter half of incubation is normally 1-4°F higher than incubation T (10), more rapid dissipation of heat in He would result in lower than normal T immediately surrounding the embryo. Presumably this lower intra-egg T then could cause, among other things, higher late embryonic mortality, fewer chicks, delayed hatching and smaller chicks. With the disturbing exception that we observed no delay in hatching time in He in either trial, our results generally fit this description.

There is additional evidence, too, of some difference between systems in heat flow, as indicated by the higher electrical input and lower surface T in He. Higher electrical inputs are certainly connected with the idea of more rapid

heat loss. At least it can be shown that it is not due simply to He having about a 5 fold higher specific heat than N_2, since this is more than compensated for by He having a 7 fold lower molecular weight. Nor is it due to the heat required to vaporize the moisture from the He eggs, since this would require only about 0.01 KWhrs/day, in comparison to the observed 0.3 KWhrs/day. Finally a calculation of the possible heat contribution to the He isolators from embryo metabolism shows that during the last week of incubation this may have amounted to perhaps 0.06 KWhrs/day (9,11), insufficient to have greatly affected the observed difference in power input.

One factor which has not been fully evaluated, however, is the possibility of greater loss of heat from the He system via radiation, due to an accidental unfavorable location of the isolator in relation to walls, ceilings, windows, etc. Increased radiation loss might also enter into the lower surface T of the He isolator, for it is difficult to explain this observation as being due solely to more rapid conduction of heat within the gas. Alternatively, the boundary layer between He and the plastic surface may have physical characteristics that are different from those between N_2 and plastic.

Growth Phase

As long as BW was the only yardstick employed, the He-hatched chicks appeared to grow almost as well for the first 4 weeks of their life in the He-O_2 atmosphere as did the air controls. However, that the He chicks did differ somewhat from the controls was evident primarily in their higher feed intake, and also in a more rapid HR and RR and a lower T_B. No consistent differences were observed in gas T, O_2, CO_2 and RH between the two isolators which could account for this difference in efficiency of growth.

The most plausable explanation for the higher feed intake of the He birds centers around their more rapid loss of body heat due to the 6 1/2 fold greater heat conducting capacity of He. Metabolism presumably rises to compensate for this

heat loss, and since BW is maintained, feed intake must increase. Similar ideas have been advanced by Leon and Cook (7) with respect to metabolic changes observed in mice and rats held in He-O_2. In this study, such observations as higher RR, higher HR, increased tendency to huddle, and low T_B can also be considered compatible with either higher metabolism or a response to feeling cold.

The complete disappearance of the difference in power input between systems as the birds aged is also consistent with the idea that the He birds were contributing more metabolic heat to their isolator. An interesting calculation which can be made here shows that the caloric value of the extra feed eaten by the He chicks during the 4th week of growth accounts for 84% of the decrease in electrical power input below what should have been recorded for the He isolator had the same relative difference between systems persisted that was seen during incubation. The higher metabolic heat increment in the He isolator may also explain the slower fall in surface T during the growth phase (table 5).

Both the higher feed intake and higher metabolic rate would indirectly contribute more moisture to the He system. This moisture along with an increase in water intake apparently shows up in the larger volume of water condensed out of the isolator. It may perhaps be pointed out again that the same higher heat conductivity of He which presumably initiates the increased metabolism and food intake is probably also responsible for the greater efficiency of the condensor in the He-O_2 isolator in the control of RH.

Finally, it should be reemphasized that in both the incubation and growth phases, there remained perhaps 1- 2% residual N_2 in the He-O_2 isolator. Therefore, properly speaking, these results refer to a N_2 low rather than a N_2 free atmosphere. What effect this last 1-2% N_2 might have on development and growth remains to be determined.

ACKNOWLEDGEMENT

We gratefully acknowledge the time and effort of Dr. Charles O. Diesem in the hematological studies and the cooperation of Dr. Robert L. Clements and Mrs. Jack V. Cartwright in the nitrogen analysis. Special thanks are due Charles P. Wharton for continued technical support and Judith R. Alden for her help in the statistical analysis. The following individuals also assisted in various phases of the project: Dr. Robert Bartels, Edward K. Charnock, Gary E. Barnett, Paul M. Steinle and Frank S. McCullough.

Fig. 1. Percent normal chicks obtained from eggs incubated in air and wt 79% He- 21% O_2. Differences highly significant statistically in both trials.

HATCHABILITY

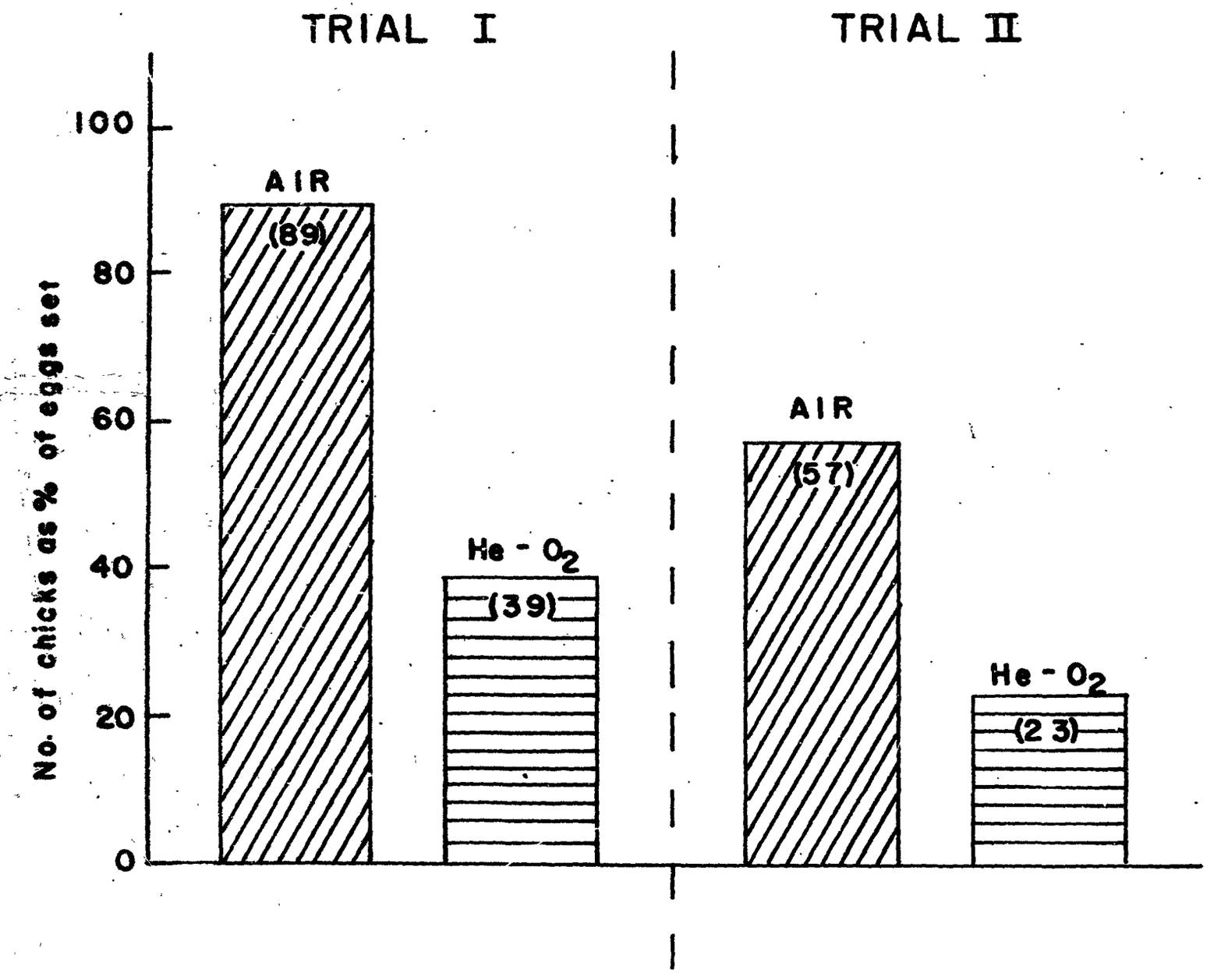

Fig. 2. Loss in weight of eggs during incubation in air and in 79% He - 21% O_2.

EGG WEIGHT LOSS

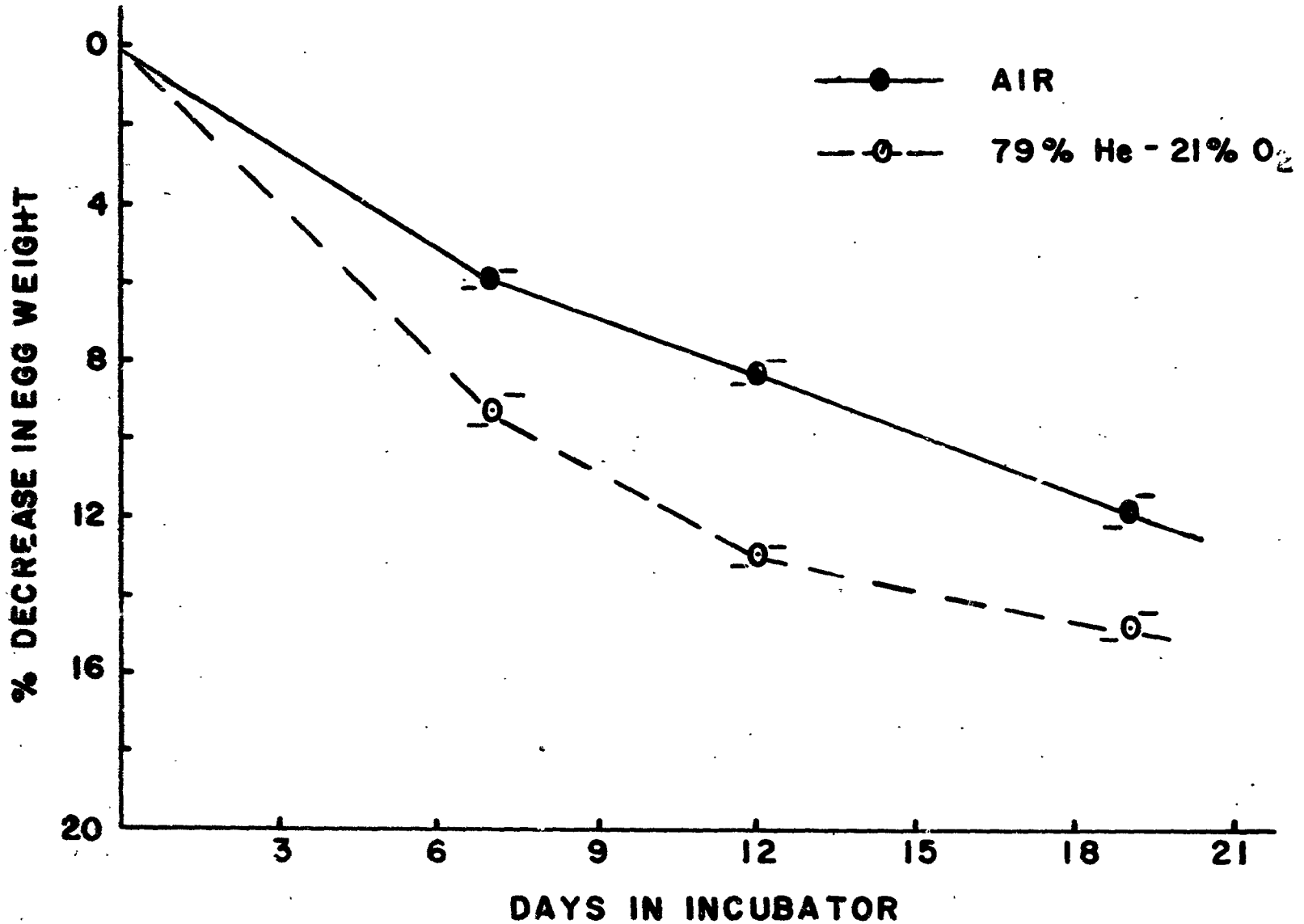

Fig. 3. Growth of chicks in air and in 79% He - 21% O_2. Death of 3 controls in trial I was due to overnight failure in O_2 supply. Points marked N_2 in trial II represent short intervals of increased N_2 concentration in the He isolator due to inboard leak in absorber-condenser circuit. See text for further details.

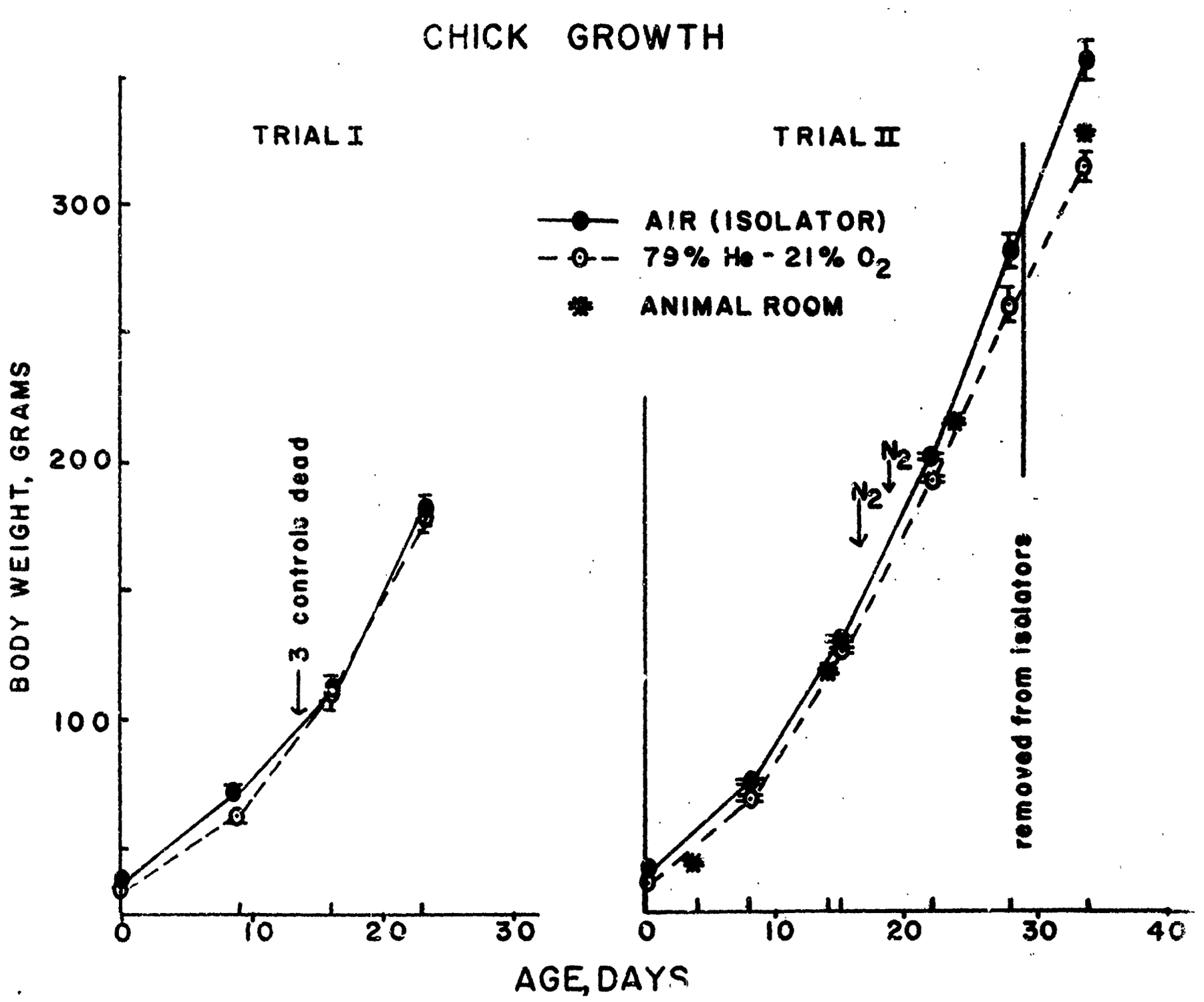
CHICK GROWTH
TRIAL I
TRIAL II
AIR (ISOLATOR)
79% He - 21% O2
ANIMAL ROOM
BODY WEIGHT, GRAMS
300
200
100
3 controls dead
N2
N2
removed from isolators
0
10
20
30
40
AGE, DAYS

Fig. 4 - Feed consumption of chicks in air and in 79% He - 21% O_2

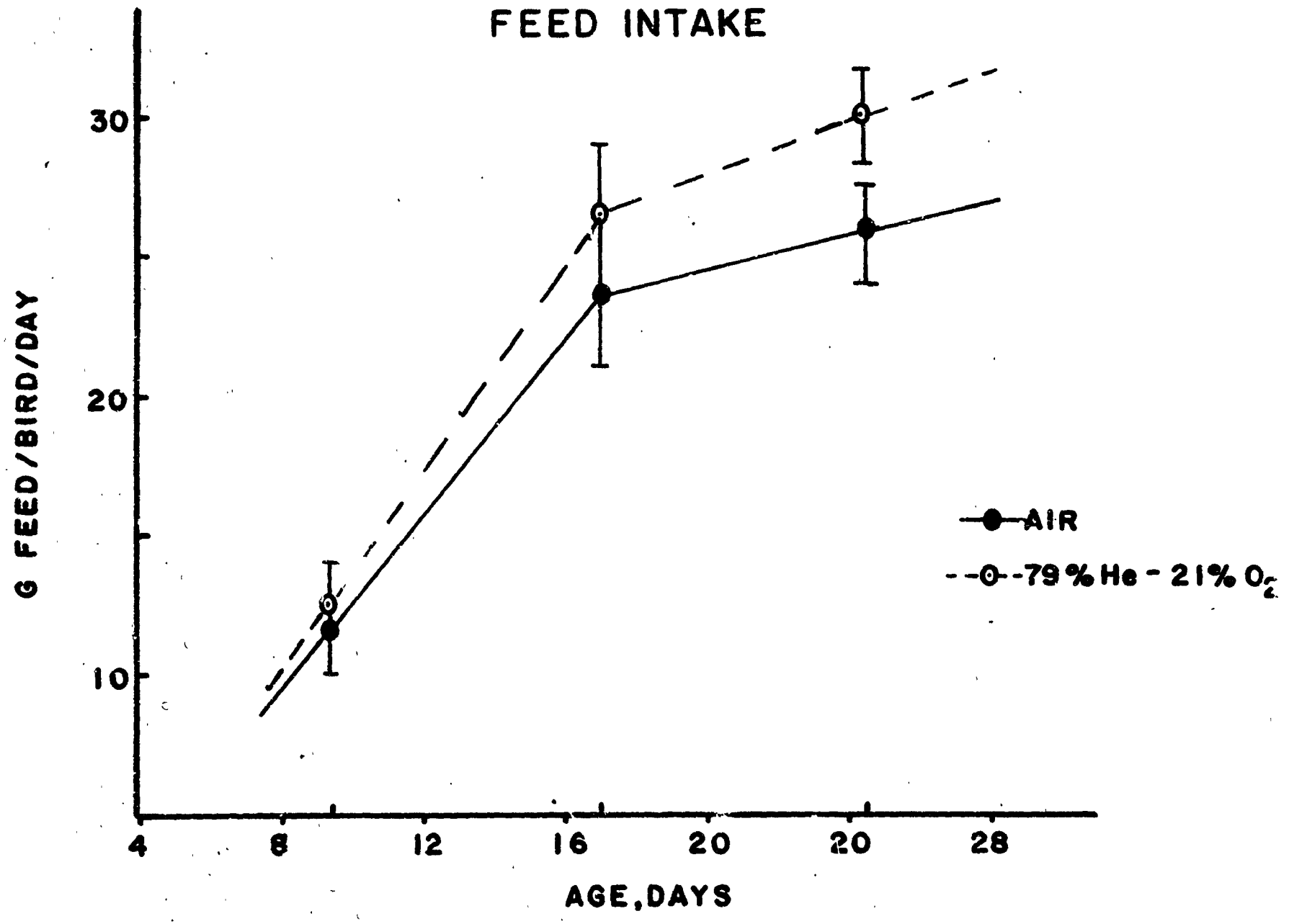
FEED INTAKE
G FEED/BIRD/DAY
30
20
10
4
8
12
16
20
20
28
AGE, DAYS
AIR
79% He - 21% O2

Table 1. Hatchability analysis on eggs incubated in 79% He-21% O_2.

Category	Trial	Air	He-O_2	Pooled SE(±) or Chi Sq.
Number of eggs set	I	18	18	-
	II	35	35	-
Initial egg wt., g	II	59.9	59.8	±0.29
Number of normal chicks	I	15	7	25.6**
	II	20	8	17.7**
No. dead ≤ 4 days	II	6	9	3.2
No. dead, 5-14 days	II	1	2	0.7
No. dead, ≥ 15 days	II	8	16	10.3**
Chick weight at hatching, (No)†, g	I	(7) 37.0	(7) 33.8	±0.8**
	II	(8) 39.5	(8) 36.1	±1.0*
Estimated hatching time, days	I	21.1	21.2	-
	II	20.5	19.7	-

*,** Significant at 5 and 1% level, respectively.

† Air chicks were selected at random to equal number of He-O_2 chicks available.

Table 2. Isolator conditions during incubation in 79% He-21% O_2.

Measurements	Trial	Air		He-O_2		Pooled
		(No)	av.	(No)	av.	SE
Gas temp. °F	I	(60)	99.1	(60)	98.9	0.27
	II	(64)	99.7	(64)	99.9	0.07**
Surface temp. °F	II	(63)	94.7	(63)	94.0	0.16**,†
Power input, KWhr/day	II	(21)	3.9	(42)	4.2	0.11‡
Oxygen, %	I	(22)	19.4	(22)	20.4	0.22**
	II	(63)	19.7	(63)	20.3	0.13**
Carbon dioxide, %	I	(26)	0.33	(26)	0.31	0.030
	II	(63)	0.20	(63)	0.20	0.019
Helium, %	I	-	-	(30)	78.4	0.25
	II	-	-	(63)	78.8	0.19
Nitrogen, % §	I	-	80.2	-	0.9	-
	II	-	80.1	-	0.7	-
Relative humidity, %	I	(59)	63.1	(59)	64.8	0.69
	II	(63)	57.2	(63)	60.7	0.65**
Av. leak rate, #/day	II	-	150	-	150	-

*,** Significant at the 5 and 1% level, respectively.

† Period and interaction effects are significant. See text for discussion.

‡ Analysis of daily differences between air and he was significant at the 5% level.

§ Nitrogen determined by difference of averages (100%-% He-% O_2-% CO_2).

Table 3. Measurements on eggs and embryos during incubation in 79% He-21% O_2.

(Various Trials)

Measurements		Air (No)	Air av.	He-O_2 (No)	He-O_2 av.	Pooled SE
Egg weight loss at 19 days, g	All eggs	(35)	7.1	(35)	9.0	0.23**
	Infertiles or early deads	(7)	7.3	(11)	9.2	0.47*
	Late deads	(6)	7.7	(14)	11.2	0.65**
	Hatched	(22)	7.0	(10)	8.2	0.50
Embryo dry weight at 10 days†, g		(52)	0.336	(50)	0.318	0.0179
g N_2/100g initial egg wt.	At 0 days (preincubation)		(22) 1.700			0.0264
	Eggs with embryos, 16½ days	(21)	1.663	(18)	1.656	0.0501

*,** Significant at the 5 and 1% levels respectively.

† Composite of measurements made at 4, 12, 16 days.

Table 4. Various measurements made during growth of chicks in 79% He-21% O_2.

(all Trial II, except as noted)

Category			day(s) of measurement	Air (No)	Air av.	He-O_2 (No)	He-O_2 av.	Pooled SE
Water	ml/day		7th-28th	(21)	392	(21)	426	25.3
Intake	ml/g feed/day		7th-28th	(21)	2.5	(21)	2.3	0.12
Condensate	ml/day		7th-28th	(21)	344	(21)	411	6.1**
	ml/ml H_2O intake/day		7th-28th	(21)	0.9	(21)	1.0	0.07
Red blood cells ($x10^6/mm^3$)			15th & 23rd	(13)	2.73	(16)	2.53	0.128
Hemoglobin g/100ml.			15th	(8)	9.4	(16)	9.3	0.21†
			23rd	(6)	9.9	(8)	9.2	
White blood cells	Total ($x10^3/mm^3$)		15th & 23rd	(13)	5.48	(16)	5.78	0.135
	% Lymphocytes		15th & 23rd	(13)	75	(16)	73	2.9
	% Heterophiles		15th & 23rd	(13)	22	(16)	23	2.4
Hematocrit, %			15th	(4)	32.0	(8)	33.5	1.27
Body temperature, F		Trial I	25th	(4)	107.3	(4)	106.0	0.13**
		Trial II	7,13,20 & 28	(32)	106.9	(32)	106.6	0.11
Heart rate, per min,			7,14,22 & 27	(32)	448	(32)	496	3.00**
Respiratory rate per minute		Trial I	25th	(4)	61	(6)	75	2.6**
		Trial II	7,13,20 & 28	(32)	68	(32)	79	1.5**

*,** Significant at 5 and 1% level respectively.

† Significant interaction term in analysis of variance.

Table 5. Average isolator conditions during 3-4 weeks of growth of chicks in 79% He-21% O_2.

Measurements		Trial	Air (No)	Air av.	He-O_2 (No)	He-O_2 av.	Pooled SE
Gas temperature, °F		I	(48)	89.6	(48)	90.1	1.00
		II	(55)	91.7	(55)	92.0	0.21
Surface temperature, °F	First 9 days	II	(19)	91.6	(19)	90.1	0.36†
	Last 9 days	II	(19)	84.0	(19)	84.7	
Power input KWhrs/day	First 9 days	II	(9)	3.7	(9)	4.1	0.21‡
	Last 9 days	II	(9)	1.4	(9)	1.3	
Percent oxygen		I	(39)	20.7	(39)	19.7	0.82
		II	(57)	20.5	(57)	21.4	0.57
Percent carbon dioxide		I	(44)	0.45	(44)	0.53	0.041
		II	(57)	0.30	(57)	0.29	0.025
Percent helium		I	-	-	(34)	78.0	1.02
		II	-	-	(56)	77.2	0.45
Percent nitrogen §		I	-	78.8	-	1.8	-
		II	-	79.2	-	1.0	-
Percent relative humidity		I	(43)	80.0	(43)	77.2	4.51
		II	(56)	79.3	(56)	80.8	0.58

† Interaction term in analysis of variance significant at 5% level.

‡ Significant difference for first 9 days if analysis based on dialy differences.

§ Nitrogen determined from differences of averages (100%-% He-% O_2-% CO_2).

REFERENCES

1. ALLEN, S.C. A comparison of the effects of nitrogen lack and hyperoxia on the vascular development of the chick embryo. Aerospace Med. 34: 897-899, 1963.

2. BARACH, A.L. Use of Helium as a new therapeutic gas. Proc. Soc. Exp. Biol. & Med., 32:462-464, 1934

3. BORISKIN, V.V., P.V. OBLAPENKO, V.V. BOL'NIK, & B.M. SAVIN. - possibility of growth of an animal organism under conditions of replacement of atmospheric nitrogen by helium. Doklady Akademii Nauk SSSR (Translation), 143: 475-478, 1962.

4. DIEGEM, C.O., W.G. VENZKE & E.N. MOORE. The hemograms of healthy chickens. Am. J. Vet. Res. 19:719-724, 1958.

5. HAYS, F.A. and W.W. SPEAR. Losses in egg weight during incubation associated with hatchability. Poultry Sci. 30:106-107, 1951.

6. HERSHEY, J.W. Components of air in relation to animal life. Science 71:394-396, 1930.

7. LEON, H.A. and S.F. COOK. A mechanism by which helium increases metabolism in small mammals. Am. J. Physiol. 199:243-245, 1960.

8. ROBERTSON, E.S. Studies on the effect of humidity on the hatchability of hen's eggs. II. A comparison of hatchability, weight loss and embryonic growth in eggs incubated at 40 and 70% RH J. Agric. Sci. 57:195-198, 1961.

9. ROMIJN, C. and W. LOKHORST. Chemical heat regulation in the chick embryo. Poultry Sci., 34: 649-654, 1955.

10. ROMIJN, C. and W. LOKHORST. The caloric equilibrium of the chicken embryo. Poultry Sci. 35:829-834, 1956.

11. ROMIJN, C. and W. LOKHORST. Foetal heat production in the fowl. J. Physiology 150:239-249, 1960.

12. SCHREINER, H.R., R.C. GREGORIE and J.A. LAWRIE. New biological effect of the gases of the helium group. Science 136: 652-653, 1962.

13. STURKIE, P.D. Avian Physiology, New York, Comstock Publishing Associates, 1954.

14. TAYLOR, L.W. Fertility and Hatchability of Chicken and Turkey Eggs. New York, John Wiley & Sons, Inc., 1949.

15. VOLSKI, M.I. The assimilation of nitrogen by animal organisms as exemplified by chicken embryos and honeybee pupae. Doklady Akademii Nauk SSSR (Translation), 128:895-897, 1960.

16. WINTER, R.R. and E.M. FUNK. Poultry Science and Practice, New York, J.B. Lippincott Co., 1956.

17. WRIGHT, R.A., M.A. LESSLER and H.S. WEISS. Metabolism and x-ray sensitivity of chick embryos incubated in a helium-oxygen atmosphere. Aerospace Med. 35:284 (abs.) 1964.