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OXYGEN CONSUMPTION AND GROWTH OF NORTHERN BOBWHITE EMBRYOS UNDER NORMOXIC AND HYPEROXIC CONDITIONS[1]

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Abstract. The oxygen limitation hypothesis states that during the latter stages of incubation O_2 availability limits growth and metabolism of embryos of precocial birds. The hypothesis has received support when tested on chicken embryos (*Gallus gallus*); we have attempted to extend the hypothesis to another precocial species, the Northern Bobwhite (*Colinus virginianus*).

Quail embryos rapidly increased in mass until late in the incubation period when the growth rate declined. This pattern is typical of embryos of other similar-sized precocial birds. Data for the metabolic rate of quail embryos incubated in air (normoxia, 21% O_2) paralleled data for embryo mass with the preIP $\dot{V}O_2$ averaging 4.73 ± 0.81 ml/hr.

To test whether elevated levels of O_2 affected the growth rate of quail embryos, we switched the gas that eggs were receiving from normoxic to hyperoxic (60% O_2) on day 11. Wet mass of embryos from hyperoxic eggs did not differ from that of control embryos on days 12, 14, 16, 18, and 20 of incubation.

The metabolic rate of embryos incubated under hyperoxic conditions for 24 hr (days 14–15) or for 6 days (days 14–20) did not vary from controls. Moreover, the metabolic rate of normoxic eggs (days 17 and 18) did not increase when we exposed the air cell to ambient air. Thus we were unable to support the oxygen limitation hypothesis when tested on Northern Bobwhite embryos.

Key words: Oxygen-limitation hypothesis; hyperoxic; normoxic; embryo mechanism; Northern Bobwhite; quail.

INTRODUCTION

Embryos of altricial species increase in mass almost exponentially throughout incubation. In contrast, embryos of precocial species grow rapidly early in the incubation period, but as the embryo matures, growth slows, resulting in a relatively constant embryo mass during the last 25% of incubation (Vleck et al. 1979). Presumably, as a consequence of slowed growth, metabolic rate plateaus, or in some species, even declines until the time when the embryo penetrates the inner shell membrane (internal pipping; Vleck et al. 1979).

Working with white leghorn chicken eggs (*Gallus gallus*), Temple and Metcalfe (1970) found that embryos from eggs incubated chronically in 60% O_2 (hyperoxic) were significantly heavier than embryos from eggs incubated in room air (normoxic). This finding was later interpreted to indicate that precocial embryos outgrow the total O_2 diffusing capacity of their shell and chorioallantoic apparatus during the latter stages of incubation (Metcalfe et al. 1984, Stock and Metcalfe 1984). In response to limited O_2, embryonic growth is slowed and metabolism plateaus. The oxygen limitation hypothesis is consistent with the finding that oxygen consumption ($\dot{V}O_2$) of chicken eggs increased with a 2-hr exposure to pure O_2 during the last half of incubation (Hoiby et al. 1983). The hypothesis has received considerable support when applied to chicken embryos (Metcalfe et al. 1981, McCutcheon et al. 1982, Metcalfe et al. 1984), but it remains uncertain whether the hypothesis is applicable to precocial birds in general.

We tested the hypothesis that during the latter stages of incubation, O_2 availability limits growth and metabolism of the precocial Northern Bobwhite (*Colinus virginianus*), a species that lays a 10-g egg with a 23- to 24-day incubation period. Our specific objectives were: (1) to compare the growth of embryos incubated under normoxic and hyperoxic conditions; (2) to test if a short-term (24 hr) or a chronic (5 days) exposure to hyperoxia influenced embryo metabolism; and

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(3) to compare O_2 uptake of intact eggs during late incubation with eggs that had the air cell opened to ambient air.

MATERIALS AND METHODS

EGGS

We purchased freshly laid Northern Bobwhite eggs from Oakridge Game Farm, Gravette, Arkansas. We numbered each egg in pencil and recorded its length and breadth.

To test for random assignment of eggs to either normoxic or hyperoxic groups, we compared the mean egg volume (Hoyt 1979) for each treatment group in both growth and metabolic experiments. In every case, means were not significantly different.

GROWTH EXPERIMENTS

We placed eggs in several large airtight Tupperware containers (30 cm in diameter) within a forced draft incubator which maintained air temperature at 37.5 ± 0.5 (SD) °C. For the first 10 days (day of set = 0), we metered compressed air (400 to 500 ml/min; 20.90% O_2) through a dust and oil trap, a column of distilled water to humidify the air, a radiator within the incubator to warm the air, and then into the plastic containers. The O_2 concentrations of upstream and downstream air from the egg containers never differed by more than 1%. Temperatures within the containers were identical to those in the incubator. Relative humidity was periodically checked within the egg containers with a dewpoint hygrometer (EG and G, model 911) and varied between 60% and 65%. Beginning on day 11, half of the eggs received a gas mixture containing 60.0% O_2 (balance N_2), and the other half continued receiving compressed air. On days 12, 14, 16, 18, and 20, we removed a sample of eggs from the normoxic and from the hyperoxic groups and opened them. Embryos were separated from extra-embryonic membranes and external yolk, laid on tissue paper to remove excess H_2O, and then weighed to the nearest milligram. In all experiments, we turned eggs by hand three or four times daily, wearing vinyl gloves to prevent dirt and skin oils from clogging shell pores.

METABOLISM EXPERIMENTS

Eggs received compressed air, as previously described, for the first 13 days of incubation. Beginning on day 14, we metered a mixture of gas containing 60% O_2 through the egg containers of the hyperoxic treatment group; the control group continued receiving compressed air. Metabolic rates of both groups of eggs were subsequently measured on day 15 (short-term, 24-hr exposure) and on days 16 to 20 (chronic exposure). Our metabolic chambers consisted of either 200- or 500-ml Plexiglas syringes fitted with a three-way stopcock on one end and a movable plunger sealed with two O-rings on the other (Vleck et al. 1979). Our syringes were relatively impermeable to oxygen, considering that after we filled them with 60% O_2, we could not detect any change in oxygen concentration after 1 hr. This finding is similar to Vleck et al. (1979), who used an identical system. While in the syringes, eggs were protected from breakage and contamination by resting them in small (50 mm in diameter) plastic film containers, cut to allow free air circulation.

To quantify O_2 uptake of embryos under normoxic conditions, we placed an egg inside a syringe with the plunger removed, and allowed the system to equilibrate inside the incubator. Then we replaced the plunger and removed a 50-ml sample of air from the syringe for initial O_2 content determination by partially depressing the plunger. After 60 to 120 min, depending on the age of the embryo, we took a final gas sample from the syringe. For eggs incubated in hyperoxia, we warmed each syringe inside the incubator for 15 min, placed an egg inside, filled the syringe with gas containing 60% O_2, replaced the plunger, and then removed an initial sample of gas from the syringe. After 60 to 90 min, we removed a final sample of gas from the syringe and measured O_2 content. In these experiments, only data from eggs which subsequently hatched are included in our analyses.

In a final experiment, two groups of eggs were incubated in air until day 17 or 18. On each of these 2 days, we exposed experimental embryos to ambient air by cutting a hole 2 mm in diameter in the shell above the air cell. We then placed the eggs in our syringes for 1 hr to measure their metabolic rate. At the same time, we placed intact control eggs in separate syringes and measured their metabolic rate. All gas samples were analyzed for O_2 concentration using an Applied Electrochemistry S3-A oxygen analyzer. Each gas sample was passed through a tube (10 mm in diameter) containing a layer of drierite, ascarite, and then drierite, prior to entry into the oxygen analyzer.

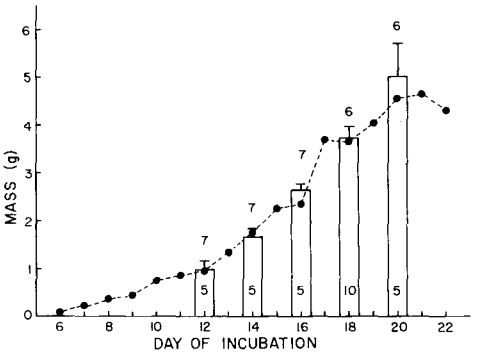

FIGURE 1. Growth of embryonic Northern Bobwhites during incubation. Darkened circles represent the mean wet mass of embryos incubated in air (n = 52). Bars represent the mean wet mass of embryos grown in hyperoxia (60% O_2). Lines above bars represent 95% confidence intervals. Numbers within bars are sample sizes for hyperoxic eggs, numbers above bars are sample sizes for normoxic eggs on that day.

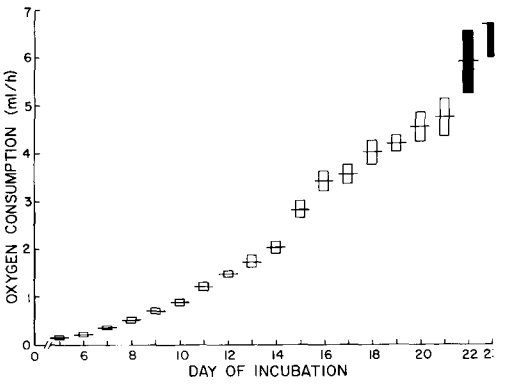

FIGURE 2. Oxygen consumption (ml/hr per egg) of embryonic Northern Bobwhites during incubation. Means are shown as horizontal lines. Bars indicate 95% confidence intervals about the mean. Shaded bars represent eggs that were externally pipped.

$\dot{V}O_2$ (ml/min) was calculated using the formula

$$\dot{V}O_2 = \frac{V - V_{I_{H_2O}} - V_{I_{CO_2}}(F_I - F_E)}{(1 - F_E)(t)}$$

where V = volume of gas (STPD) in the metabolic chamber at the start of the experiment (adjusted for egg and container volume), $V_{I_{H_2O}}$ = initial volume of water vapor in the syringe, $V_{I_{CO_2}}$ = initial volume of CO_2 estimated as 0.03%, F_I and F_E are the initial and end fractional concentrations of O_2 after both water vapor and CO_2 are removed from the sample, and t is time in minutes.

MEASUREMENT OF AIR-CELL GASES

We measured the partial pressure of oxygen (P_{O_2}) and of carbon dioxide (P_{CO_2}) within the air cell of hyperoxic (chronically exposed) and normoxic eggs following the technique of Rahn et al. (1974). On days 16, 18, and 20 of incubation, we candled a sample of both groups of eggs, outlined the air cell with pencil, then with the egg under acidified water (pH = 6.0), broke two small holes in the shell above the air cell with a hypodermic needle. We next aspirated the gas contents of the air cell with a 1-ml glass syringe fitted with a glass capillary tube and transferred the sample to a Scholander 0.5-ml gas analyzer to determine the concentrations of O_2 and CO_2 (Scholander 1947).

RESULTS AND DISCUSSION

GROWTH UNDER NORMOXIC CONDITIONS

Quail embryos (n = 52) incubated under normoxic conditions rapidly increased in mass until the last few days prior to hatch (Fig. 1). By day 17, or by about 75% of the way through incubation, the growth rate had markedly declined. This pattern, typical of small precocial species, is similar to data for the Coturnix Quail (*Coturnix coturnix*; Vleck et al. 1979), a species that lays an egg of similar size.

GROWTH UNDER HYPEROXIC CONDITIONS

Embryos incubated in 60% O_2 continuously from day 11 through 20 showed no accelerated growth when compared to normoxic embryos (Fig. 1; ANCOVA; $t_{slopes} = 1.45$, $P > 0.10$; $t_{elevation} = 0.51$, $P > 0.5$). Our data do not support the notion that exposure to elevated O_2 concentrations accelerates the growth of bobwhite embryos.

METABOLISM OF NORMOXIC EGGS

Consistent with our growth data, $\dot{V}O_2$ for eggs incubated in normal air increased rapidly during the first portion of the incubation period, but the rate of increase slowed markedly as embryos matured (Fig. 2). This pattern conforms to data from Coturnix Quail embryos (Vleck et al. 1979). For days 5 to 15, $\dot{V}O_2$ was described by the equation: ml O_2/hr = $e^{0.27(d)-2.96}$ where d equals the day of incubation (n = 142 measurements on 71 eggs, $r^2 = 0.91$, $P < 0.001$).

For comparative purposes, investigators have

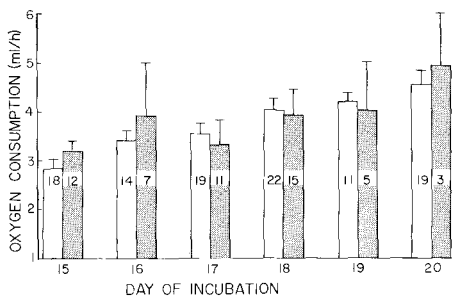

FIGURE 3. Oxygen consumption (ml/hr per egg) of embryonic Northern Bobwhites. Open bars represent eggs that were incubated in air; stippled bars indicate eggs incubated in hyperoxia (60% O_2) beginning on day 14. Lines above bars represent 95% confidence intervals about the mean. Numbers inside bars indicate sample size.

reported $\dot{V}O_2$ just prior to internal pipping (preIP), the time at which the beak of the embryo penetrates the air cell (Hoyt and Rahn 1980). Most normoxic eggs hatched after 23 days of incubation, but some hatched on the 24th day. We did not detect any evidence of internal pipping. Using day 21 as preIP (90% of the way through incubation), $\dot{V}O_2$ at this time is 4.73 ml/hr or 113.5 ml/day (n = 18). The preIP $\dot{V}O_2$ for Coturnix Quail eggs, which also weigh about 10 g, but require only 18 days to hatch, equalled 7.67 ml/hr in the study of Vleck et al. (1979) and 6.88 ml/hr according to Hoyt and Rahn (1980).

A close correlation exists between preIP $\dot{V}O_2$, initial egg mass, and incubation period, for eggs ranging over three orders of magnitude in size (Hoyt and Rahn 1980): preIP ml O_2/day = $139(M^{0.85}/I^{0.65})$ where M = the initial egg mass in grams, and I = length of incubation in days. This equation predicts a preIP $\dot{V}O_2$ of 129.3 ml/day for a 10.1-g quail egg, a value which is 13.9% higher than we have found.

As $\dot{V}O_2$ increases, the air-cell oxygen tension (P_{AO_2}) declines (Wangensteen and Rahn 1970). Late in incubation (day 20), P_{AO_2} averaged 79.3 ± 10.4 torr (n = 2). This value is lower than the average value of 101 torr (n = 34 species) reported by Hoyt and Rahn (1980), and lower than the two values reported for *C. coturnix*, 92 torr and 88 torr (Vleck et al. 1979, Hoyt and Rahn 1980).

METABOLISM OF HYPEROXIC EGGS

A short-term exposure of 24 hr (days 14–15) to 60% O_2 produced no significant effect on the $\dot{V}O_2$ of embryonic quail (Fig. 3). On day 15, $\dot{V}O_2$ of normoxic eggs averaged 2.83 ± 0.37 ml/hr (n = 17), and for hyperoxic eggs averaged 3.21 ± 0.94 ml/hr (n = 12). These values were not significantly different (t = 0.5, $P > 0.4$). In contrast, for white leghorn chicken eggs (day = 17; egg mass = 63.3 g; incubation period = 21 days), $\dot{V}O_2$ was elevated from 21.6 ml/hr when incubated in air, to 26.4 ml/hr when placed in pure O_2 for a period of 2 hr (Hoiby et al. 1983). In the same study, for bantam eggs (mass = 46 g), the acute (2 hr) effect of pure O_2 on metabolism could not be detected until the 15th day of incubation. However, during the last 4 days before hatching, $\dot{V}O_2$ of bantam eggs placed in pure O_2 for 2 hr was 23% higher than eggs tested in normoxic air.

TABLE 1. Oxygen tension (P_{AO_2}), and carbon dioxide tension (P_{ACO_2}) in air cells of Northern Bobwhite eggs. Mean values ± 1 SD.

Age (days)[a]	(n)	P_{AO_2} (torr)	P_{ACO_2} (torr)
Normoxic			
16	(3)	79.1 ± 12.0	48.8 ± 7.8
18	(3)	74.8 ± 9.0	58.4 ± 3.2
20	(2)	79.3 ± 10.4	54.4 ± 9.7
Hyperoxic			
16	(3)	259.9 ± 37.2	52.3 ± 12.6
18	(2)	195.7 ± 42.4	79.4 ± 15.0
20	(3)	177.2 ± 63.8	67.6 ± 22.1

[a] Day of set = 0.

We could not demonstrate a long-term effect of hyperoxia on metabolism of quail eggs (Fig. 3). Oxygen consumption by normoxic eggs (n = 103 measurements on 71 eggs) did not differ from that of hyperoxic eggs (n = 53 observations on 45 eggs) when we compared them for days 15 to 20 (ANCOVA; t_{slope} = 0.29, $P > 0.6$; $t_{elevation}$ = 0.87, $P > 0.2$). The metabolic rate of eggs from the two groups did not differ on day 20 (t = 1.29, $P > 0.7$). Temple and Metcalfe (1970) reported that continuous exposure of hen eggs to 60% O_2 stimulated growth of the embryo, suggesting an elevated metabolic rate. We have found no evidence that hyperoxia influences the metabolic rate of Northern Bobwhite embryos.

Air-cell gas from hyperoxic eggs contained a higher O_2 concentration than did that from normoxic eggs (Table 1). Pooled values for each treatment differed statistically (t = 6.49, $P <$ 0.001, n = 8 for each treatment). Air-cell P_{CO_2} was not significantly different between the two

groups ($t = 2.0$, $P > 0.05$). Though our sample sizes are small, these results confirm that embryos exposed to hyperoxic conditions experienced higher levels of O_2 than did embryos exposed to air. Further, if increased O_2 availability stimulated the metabolic rate of eggs from the hyperoxic group, then one would expect higher levels of CO_2 in their air cells; this was not the case.

METABOLISM OF EGGS WITH AIR CELLS OPENED

On day 17, $\dot{V}O_2$ for 10 eggs incubated in air averaged 3.84 ± 0.45 ml/hr, while $\dot{V}O_2$ for 10 eggs with their air cells exposed to ambient air averaged 3.85 ± 0.38 ml/hr. The following day, $\dot{V}O_2$ of intact eggs equalled 4.1 ± 0.4 ml/hr ($n = 9$), and for eggs with their air cells opened, averaged 3.92 ± 0.38 ml/hr ($n = 10$). In neither case were means significantly different ($t = 0.02$, $P > 0.98$; $t = 1.62$, $P > 0.12$, respectively). In contrast, during the last half of the incubation period, the O_2 uptake by chicken eggs incubated in air increased as much as 13% when a hole was made in the shell above the air cell (Hoiby et al. 1983).

HATCHLING DRY MASS

Dry mass of hatchlings from the normoxic group averaged 2.08 ± 0.27 g ($n = 31$), while those from the hyperoxic group (chronic exposure) weighed 2.09 ± 0.24 g ($n = 45$). That these values were not significantly different ($t = 0.31$, $P > 0.6$) indicates that net growth was unaffected by prolonged exposure to 60% O_2 during the latter part of incubation.

CONCLUSIONS

In precocial embryos, the asymptote in $\dot{V}O_2$ coincides with the time during which embryonic growth slows (Vleck et al. 1979). Research on chicken embryos has led Metcalfe and his coworkers to postulate that oxygen availability limits both growth and metabolic rate at this time. Results to date support this idea when experiments were performed on the relatively large chicken egg or on bantam hen eggs, but when we tested the hypothesis on Northern Bobwhite eggs, which weigh only 10 g, we could find no effect of increased O_2 availability on metabolism or growth. Apparently the hypothesis cannot be extended to precocial birds in general.

O_2 limitation may be in some way related to the size of the developing embryo, or more specifically, to the surface area of the chorioallantoic apparatus relative to the metabolic rate of the embryo. Consistent with this speculation is the finding by Hoiby et al. (1983) that the effect of a 2-hr exposure to pure O_2 was not evident in the smaller bantam hen eggs (46 g) until much later in the incubation period than for white leghorn chicken eggs (63 g). The surface area (SA) and presumably the chorioallantoic membrane during late incubation of small (10 g) and large (60 g) eggs is 22.2 cm^2 and 72.7 cm^2, respectively (Paganelli et al. 1974). The preIP $\dot{V}O_2$ equals 113.5 ml/day (this study) and 537.6 ml/day for chicken eggs as given by an allometric equation (Hoyt and Rahn 1980), and thus the $\dot{V}O_2$/SA ratio is 5.1 (quail) and 7.4 (chicken), a 31.1% difference. This suggests that surface area may be a factor involved in the disparity between our results and those of Metcalfe and his coworkers and warrants further study.

Alternatively, we envision that the results on O_2 limitation found for chicken embryos may be an artifact produced in some way by the intense artificial selection pressures placed on this species. Clearly the hypothesis needs to be tested on a wide range of other species. The next step could be to test the oxygen limitation hypothesis on eggs of a relatively large precocial species other than the chicken.

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