AVIAN REPRODUCTION OVER AN ALTITUDINAL GRADIENT: INCUBATION PERIOD, HATCHLING MASS, AND EMBRYONIC OXYGEN CONSUMPTION

CYNTHIA CAREY,[1] EDWARD L. THOMPSON,[1] CAROL M. VLECK,[2,3]
AND FRANCES C. JAMES[4]

[1]Department of EPO Biology, University of Colorado, Boulder, Colorado 80309 USA,
[2]Department of Zoology, University of Washington, Seattle, Washington 98105 USA, and
[4]Department of Biological Science, Florida State University, Tallahassee, Florida 32306 USA

ABSTRACT.—Embryonic oxygen consumption, incubation period, and hatchling mass do not vary significantly among populations of Red-winged Blackbirds (Agelaius phoeniceus) breeding over a 2,900-m altitudinal gradient, despite a 28% decrease in oxygen tension from the lowest to the highest altitude. Calculations suggest that embryos develop normally at 2,900 m at air-cell O_2 tensions of 7.1 kPa. The regulation of loss of water vapor or CO_2 by a reduction of eggshell conductance appears to have been more important for populations breeding in montane environments than maximizing O_2 availability to the embryo. The superior tolerance to hypoxia demonstrated by embryos of wild birds, compared to that of embryos of domestic chickens, may relate to the modification of the diffusive resistance of O_2 inside the shell. Received 28 September 1981, accepted 24 April 1982.

THE ability of a species to invade a new habitat and to establish permanent residency there depends importantly upon successful reproduction. Avian species have exploited a remarkable diversity of habitats. This success is particularly striking in view of the potential vulnerability of avian embryos to environmental stresses. Because avian embryos develop externally from the body of the adult, they are less protected by the homeostatic systems of the adult than are embryos of viviparous vertebrates. Although the physiology of avian embryos has received considerable attention during the last decade, embryonic tolerances of and adaptations to harsh environments have received little study.

Avian embryos exchange gases with the environment by diffusion (Wangensteen et al. 1970/71). The diffusive flux of O_2 into the egg, and CO_2 and water vapor outwards, is described by a modification of the Fick equation (Wangensteen et al. 1970/71, Paganelli et al. 1975):

$$\dot{M} = (D/RT) \cdot (Ap/L) \cdot \Delta P \qquad (1)$$

where $\dot{M}$ = gas flux (cm^3 STPD·s^{-1}), D = diffusion coefficient (cm^2·s^{-1}), Ap = effective pore area (cm^2), L = length of diffusion path or shell thickness (cm), RT = gas constant and absolute temperature (cm^3 STPD·cm^{-3}·kPa^{-1}), and ΔP = partial pressure difference of gas across shell (kPa). The terms $(D/RT) \cdot (Ap/L)$ are often combined into the term "G" (cm^{-3}·s^{-1}·kPa^{-1}), representing the conductance of the eggshell to each gas (Ar et al. 1974).

The progressive decrease in barometric pressure, P_B, in montane habitats is an environmental gradient that poses distinct problems for diffusive respiratory systems. Equation 1 suggests two factors that should become progressively more important to avian development as P_B falls with increasing altitude. First, O_2 flux ($\dot{M}_{O_2}$) is influenced by the P_{O_2} difference across the eggshell (ΔP_{O_2}). Because ambient P_{O_2} falls as P_B decreases, the driving force for O_2 diffusion will decrease unless internal P_{O_2} is lowered commensurately. Therefore, an embryo may not obtain sufficient O_2 above certain altitudes to sustain normal metabolism and growth. Second, theoretical predictions and empirical results show that D is inversely proportional to P_B (Reid and Sherwood 1966, Paganelli et al. 1975). Therefore, gases exchanged between the embryo and the environment will diffuse more rapidly as P_B decreases with al-

titude, if all other factors in Eq. 1 are constant. The enhanced diffusion of O_2 into the egg may compensate in part for the detrimental effects of hypoxia on metabolism (Visschedijk et al. 1980), but augmented rates of diffusion of CO_2 and water vapor from the egg during incubation may prove harmful to normal embryonic growth (Rahn et al. 1977, Carey 1980). Therefore, successful breeding by montane populations of birds has undoubtedly required that the resistant properties of the eggshell to gaseous diffusion be designed to meet the demands for maximizing O_2 supply and minimizing excessive losses of CO_2 and water vapor at high elevations.

Tolerance to hypoxia has been studied thus far only in embryos of domestic fowl (*Gallus domesticus*). They are unable to maintain normal levels of metabolism or growth when subjected acutely or for the duration of incubation to moderate hypoxia (Visschedijk et al. 1980). Growth and metabolism of embryos obtained from a colony of chickens raised for more than 15 yr at 3,800 m were significantly reduced below control levels (Wangensteen et al. 1974). These results raise interesting questions concerning the abilities of embryos of wild birds breeding at high altitudes to obtain sufficient O_2 for normal metabolism and growth. The purpose of this study was to evaluate O_2 uptake directly by measuring embryonic metabolic rate and indirectly by studying incubation period, hatchling mass, and embryonic mortality in Red-winged Blackbirds (*Agelaius phoeniceus*) breeding over a 2,900-m altitudinal gradient.

METHODS

Incubation periods, hatchling masses, and embryonic mortality.—Nests were found at the following locations: Tallahassee, Leon County, Florida (30 m); Olema, Marin County, California (30 m); Lake Itasca, Clearwater County, Minnesota (460 m); Boulder, Boulder County, Colorado (1,600 m); Gunnison, Gunnison County, Colorado (2,400 m); and near Gothic, Gunnison County, Colorado (2,900 m). Nests were located during the laying period and were checked daily between 0800 and 1200 during the incubation period. Because the eggs in a clutch usually did not hatch on the same day, two measures of incubation period were used. First, the incubation period of the first egg was defined as the number of days between the date the last egg was laid (Day 0) and the date on which the first egg in each clutch hatched. Therefore, means for each population were

calculated using one value per nest. Second, the incubation period for all other eggs was defined as the number of days between Day 0 and the date of hatching of all eggs (other than the first one) in the clutch. Populational means using this latter method represent 2–4 values per nest, depending on clutch size.

Hatchlings were weighed on a portable Torbal or Ohaus balance, precise to 0.005 and 0.01 g, respectively, between 0800 and 1200 on the day they were first observed in the nest. Some variability in the data may be due to the fact that some chicks may have hatched the previous afternoon and received food before weighing. Spot checks of nests in Boulder, Gunnison, and Gothic indicated that about 15% of the eggs in each population hatched in the afternoon and that this proportion did not vary significantly among collecting locations.

Eggs that had not hatched within 3 days after hatching of the remainder of the clutch were cracked open. The contents were inspected to determine whether the failure to hatch was due to infertility or to death in the shell at advanced stages of development.

One-way analysis of variance was used in conjunction with Scheffe's and LSD procedures to test for significant differences in incubation period, hatchling mass, and clutch sizes among collecting locations. Significant differences were accepted at $P < 0.05$.

Oxygen consumption.—Eggs were collected near the Columbia National Wildlife Refuge, Grant and Adams counties, Washington (200 m); Lawrence, Douglas County, Kansas (290 m); Boulder (1,600 m); Gunnison (2,400 m); and Gothic (2,900 m) between Day 7 of incubation and hatching. The eggs were transported quickly to a laboratory at or near the altitude of the collection site and were placed in forced-air incubators or small boxes heated with an external heater. Oxygen consumption ($\dot{M}_{O_2}$) was measured between 3 and 48 h after eggs were removed from the nest. The method of Vleck and Kenagy (1980) was used to measure $\dot{M}_{O_2}$ in embryos collected in Washington. In this method, a closed-system apparatus was used in conjunction with an Applied Electrochemistry oxygen analyzer. The eggs were placed in the closed system and held at 36°C. At intervals determined by the age of the embryo, air samples were taken from the chamber and injected into the analyzer. Oxygen levels in this system never fell below 20%.

Metabolic rates of embryos collected at other locations were measured at 36°C in a modified Scholander device. Eggs were placed individually over KOH in a 2.5-cm-diameter and 5-cm-long plexiglass chamber that was connected to an equilibration chamber by a manometer consisting of small-bore glass tubing. The metabolism chamber was well aerated with room air before the egg was placed inside. Oxygen was replaced as it was consumed during the

TABLE 1. Mean ± standard error of the mean hatchling mass, incubation period of the first egg to hatch, incubation period of all other eggs, and clutch sizes of nests of Red-winged Blackbirds at various altitudes. Sample sizes are in parentheses. Degrees of freedom, F-ratio, and F-probabilities from analysis of variance are presented in each column.

Location and (altitude)	Hatchling mass (g)	Incubation period of first egg (days)	Incubation period of all other eggs (days)	Clutch size
Tallahassee, Florida (30 m)	3.51 ± 0.15 (14)	11.33 ± 0.21 (6)	12.22 ± 0.40 (9)	3.21 ± 0.08 (57)
Olema, California (30 m)	3.38 ± 0.03 (3)	—	—	3.38 ± 0.12 (16)
Lake Itasca, Minnesota (460 m)	2.92 ± 0.17 (10)	11.50 ± 0.50 (2)	11.33 ± 0.33 (3)	3.65 ± 0.21 (20)
Boulder, Colorado (1,600 m)	3.47 ± 0.12 (23)	11.00 ± 0.30 (10)	11.41 ± 0.13 (39)	3.86 ± 0.08 (38)
Gunnison, Colorado (2,400 m)	3.51 ± 0.10 (41)	11.00 ± 0.31 (7)	11.38 ± 0.18 (26)	4.21 ± 0.08 (48)
Gothic, Colorado (2,900 m)	3.53 ± 0.09 (21)	10.50 ± 0.29 (4)	11.13 ± 0.24 (15)	4.04 ± 0.12 (22)
df	5,111	4,28	4,91	5,200
F-ratio	1.97	0.86	2.21	17.257
F-probability	0.09	0.50	0.07	0.00

equilibration period with injection of O_2 by a 1-cc syringe. The chambers were submerged throughout the tests in a circulating water bath. After embryonic $\dot{M}_{O_2}$ had stabilized, measurements of the time required for the embryo to consume 0.5 ml O_2 were repeated three times and the results averaged. The data for $\dot{M}_{O_2}$ are presented as a function of embryonic mass rather than day of incubation to minimize scatter in the results: because adults often begin incubation before the clutch is complete, within-clutch development is asynchronous, and masses of embryos can vary 50% on the same day of incubation. Embryonic mass was measured following tests of $\dot{M}_{O_2}$. The embryo was removed from the egg, blotted dry, and then weighed on a Mettler or Torbal balance, precise to 0.001 or 0.005 g, respectively. External yolk masses were removed before weighing.

Least-squares regression lines were calculated to define the relation between log-transformed $\dot{M}_{O_2}$ and embryonic mass. Analysis of covariance was used to detect significant differences in slopes and intercepts of the regression among populations.

RESULTS

Incubation period, hatchling mass, and embryonic mortality.—Mean incubation period of the first egg to hatch varied between 10.5 and 11.5 days at our locations (Table 1). No significant differences existed among these means compared among collecting locations ($P = 0.50$) nor among mean incubation periods calculated for all other eggs ($P = 0.07$) (Table 1). These data, compared with incubation periods at other sites available from the literature (see Discussion), indicate that incubation periods of Red-winged Blackbirds are independent of altitude over a 2,900-m gradient. Similarly, no significant differences existed ($P = 0.09$) among average hatchling masses compared among populations (Table 1). Mean clutch sizes of the three Colorado populations were significantly ($P < 0.05$) larger than those at other locations.

Hatchability varied between 89 and 95% in the Boulder, Gunnison, and Gothic populations and did not vary significantly among groups. Eggs that did not hatch either were infertile or lacked a distinct embryo. No unhatched eggs contained an advanced embryo that had died in the latter stages of development.

Oxygen consumption.—A linear relation exists between log-transformed values for $\dot{M}_{O_2}$ and body mass of embryos between 0.6 and 3.6 g (Fig. 1). The regression equations describing the relations between $\dot{M}_{O_2}$ and body mass at each location are listed in Table 2. The existence of any effect of hypoxia on metabolism might be demonstrated in two ways. First, be-
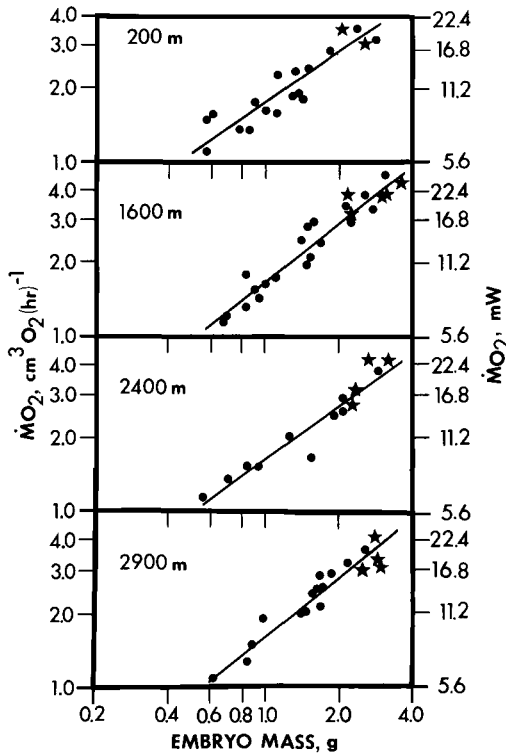
Fig. 1. Relation of oxygen consumption ($\dot{M}_{O_2}$ in cm³ $O_2 \cdot$ h⁻¹ and mW) to body mass (g) in embryos of Red-winged Blackbirds measured at altitudes at which the eggs were laid. The locations are: Columbia Refuge, Washington (200 m) and Lawrence, Kansas (290 m); Boulder, Colorado (1,600 m); Gunnison, Colorado (2,400 m); and Gothic, Colorado (2,900 m). Circles and stars represent values of embryos with unpipped and pipped shells, respectively. The equations for the solid lines representing the linear regressions best describing the relation between log-transformed $\dot{M}_{O_2}$ and body mass are listed in Table 2.

Table 2. Equations for least squares regression lines describing the relation between oxygen consumption ($\dot{M}_{O_2}$ in mW) and body mass (g) of Red-winged Blackbird embryos measured at the altitudes at which the eggs were laid. Conversion of cm³ $O_2 \cdot$ h⁻¹ to mW was made according to the relation: 1 cm³ $O_2 \cdot$ h⁻¹ = 4.8 cal $\cdot$ h⁻¹ = 5.6 mW (kJ $\cdot$ h⁻¹).

Altitude	Equation
200–290 m	$\log \dot{M}_{O_2} = 0.994 + 0.676 \log$ Mass $n = 19$; $r^2 = 0.84$; $S_{y \cdot x} = 0.065$
1,600 m	$\log \dot{M}_{O_2} = 0.967 + 0.792 \log$ Mass $n = 22$; $r^2 = 0.94$; $S_{y \cdot x} = 0.046$
2,400 m	$\log \dot{M}_{O_2} = 0.965 + 0.723 \log$ Mass $n = 14$; $r^2 = 0.93$; $S_{y \cdot x} = 0.052$
2,900 m	$\log \dot{M}_{O_2} = 0.958 + 0.791 \log$ Mass $n = 18$; $r^2 = 0.94$; $S_{y \cdot x} = 0.042$

ther, although data are limited, the $\dot{M}_{O_2}$'s of the pipped embryos do not vary significantly from the values of the largest unpipped individuals in the montane groups. This evidence indicates that $\dot{M}_{O_2}$ of the Red-winged Blackbird embryos is independent of altitude to 2,900 m.

DISCUSSION

Oxygen is required by avian embryos for maintenance of existing tissues and for synthesis of new cells from raw materials contained within the egg before laying. Therefore, restriction of O_2 below critical levels should retard growth and reduce survival. Several lines of evidence suggest that hatchling mass, duration of incubation period, and survival to hatching can provide indirect evidence of the level of O_2 availability to the embryo. First, patterns of O_2 uptake during incubation are closely correlated with patterns of embryonic growth in altricial and precocial species (Vleck et al. 1979, D. Vleck et al. 1980). Second, reduction of gaseous exchange by application of an impermeable material over portions of the eggshell of domestic chickens results in retardation of embryonic growth, prolongation of incubation period, and reduction in hatching success (Tazawa et al. 1971). Although such manipulation would be expected to affect diffusion of O_2, CO_2, and water vapor between the embryo and the environment, restriction of O_2 availability to the embryo was thought to have caused these abnormalities (Tazawa et al. 1971). Finally, incubation of chicken eggs in a 60% O_2 atmosphere results in larger embryos

cause the effect of hypoxia would be expected to be most pronounced at larger body masses just before pipping of the shell, the slope of the regression line of the montane embryos might be lower than those of the lowland groups. Second, the metabolic rate of pipped individuals in montane populations might be greater than those of unpipped embryos dependent on O_2 diffusion through the shell. Neither of these possibilities is supported by the data. Analysis of covariance indicates no significant differences among the slopes of the regression lines of the four populations. Fur-

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TABLE 3. Average incubation period and hatchling mass of White-crowned Sparrows (*Zonotrichia leucophrys*) and Horned Larks (*Eremophila alpestris*) breeding at various altitudes.

Location	Altitude (m)	Incubation period (days)	Hatchling mass (g)	Reference
Zonotrichia leucophrys				
Berkeley, California	46	12.3	2.4	DeWolfe (1968), Banks (1959)
Fairbanks, Alaska	123	11.9–12.1	2.7	King and Hubbard (1981)
Hart Mountain, Oregon	1,890	12.2	2.7	King and Hubbard (1981)
Tioga Pass, California	3,000	10.9–13.4	2.8	Morton et al. (1972), Morton and Carey (1971)
Niwot Ridge, Colorado	3,290	11.9	2.6	King and Hubbard (1981)
Niwot Ridge, Colorado	3,475	12.2–13.0	2.7	King and Hubbard (1981)
Eremophila alpestris				
Ithaca, New York	122	—	2.85	Pickwell (1931)
Evanston, Illinois	183	11	—	Pickwell (1931)
Macomb, Illinois	216	—	2.4	Beason and Franks (1973)
Teton City, Montana	1,128	10–11	—	DuBois (1935)
Dacono, Colorado	1,530	11	—	MacDonald (1916)
Beartooth Butte, Wyoming	3,200	11	2–3	Verbeek (1967)
Guanella Pass, Colorado	3,600	11.3	2.6	Conry (1978)

and more advanced morphological development at comparable ages than incubation of embryos in room air (Temple and Metcalfe 1970).

The incubation period of populations of Red-winged Blackbirds at low altitudes is generally 11–12 days (Allen 1914, J. Bart pers. comm.). This information, coupled with results from our populations laying at higher altitudes (Table 1), indicates that incubation period and hatchling mass do not vary significantly between sea level and 2,900 m, despite a 28% reduction in O_2 tension at the highest site. Similarly, hatching success of Red-winged Blackbird embryos did not appear to be affected by such hypoxia. These results agree with observations that incubation periods and hatchling masses are independent of altitude in populations of White-crowned Sparrows (*Zonotrichia leucophrys*) and Horned Larks (*Eremophila alpestris*) breeding from low altitudes to 3,475 and 3,600 m, respectively (Table 3). These data for three species of wild birds suggest that adequate levels of O_2 are obtained by embryos for normal metabolism and growth in hypoxic conditions extending to at least 3,600 m. This contention is supported by the lack of variability of O_2 consumption of Red-winged Blackbird embryos at altitudes up to 2,900 m (Fig. 1) and the fact that no embryos died in the latter stages of development when O_2 demand inside the

egg would be most intense. These results are in striking contrast to the reduction in metabolism, hatchability, growth rate, and hatchling mass noted in chicken embryos subjected to moderate hypoxia (79.9–53.3 kPa), equivalent to that found between 1,700 and 3,800 m (Wangensteen et al. 1974, Rahn 1977, Visschedijk 1980, Visschedijk et al. 1980).

The ability to maintain normal metabolism at low ambient O_2 tensions will depend in part on the degree of resistance posed to O_2 diffusion between the atmosphere and the embryonic blood and in part on the ability of the tissues to function at low internal O_2 tensions. Oxygen diffuses through the pores of the eggshell, the outer and inner shell membranes, and the chorioallantoic membrane before it contacts embryonic blood. Considerable resistance to O_2 diffusion is initially posed by the wet outer shell membrane (Kutchai and Steen 1971, Lomholt 1976, Tullett and Board 1976). After this membrane dries in the first few days of incubation, about $1/3$ of the resistance to O_2 flux is posed by the "outer barrier," comprised of the shell and outer shell membrane, and the remaining $2/3$ by the "inner barrier," consisting of the inner shell membrane and the chorioallantoic membrane (Bissonnette and Metcalfe 1978, Piiper et al. 1980). Oxygen consumption of altricial embryos rises exponentially throughout incubation in direct correlation with

TABLE 4. Estimation of ΔP_{O_2} and $P_{A_{O_2}}$ in eggs of Red-winged Blackbirds laid at various altitudes and incubated at 36°C.

Altitude (m)	P_B (kPa)	G_{H_2O}[a] (mg·day^{-1}· kPa^{-1})	G_{O_2}[b] (cm^3·day^{-1}· kPa^{-1})	$P_{I_{O_2}}$[c] (kPa)	ΔP_{O_2}[d] (kPa)	$P_{A_{O_2}}$[e] (kPa)
200–290	97.9	11.1	12.3	19.2	7.3	11.9
1,600	83.5	9.7	12.6	16.2	7.1	9.0
2,400	75.6	8.7	12.5	14.6	7.2	7.4
2,900	71.0	9.1	13.9	13.6	6.5	7.1

[a] Carey et al. (in press). Values corrected to 25°C and 101.3 kPa.
[b] $G_{O_2} = 1.071 \cdot G_{H_2O} \cdot (101.3/P_B)$ (Hoyt et al. 1979, Visschedijk et al. 1980).
[c] $P_{I_{O_2}} = (P_B - 6.0) (0.209)$ (Wangensteen and Rahn 1970/71).
[d] $\Delta P_{O_2} = \dot{M}_{O_2}/G_{O_2}$ (Rahn et al. 1974).
[e] $P_{A_{O_2}} = P_{I_{O_2}} - \Delta P_{O_2}$ (Hoyt et al. 1978).

growth of body mass (C. Vleck et al. 1979, 1980). Because the resistance of the outer barrier is fixed after drying of the membrane (Wangensteen and Rahn 1970/71) and that of the inner barrier increases only slightly during incubation (Tazawa 1980), the O_2 tension in the egg progressively decreases throughout incubation as metabolic consumption increases (Wangensteen and Rahn 1970/71, Wangensteen 1972, Rahn et al. 1974, Tazawa 1980). Just before the embryo pips the inner shell membrane and begins pulmonary respiration, the oxygen tension in the air cell ($P_{A_{O_2}}$) varies between 10.0 and 16.6 kPa and averages about 13.8 kPa in eggs incubated at low altitudes (Rahn et al. 1974; Hoyt et al. 1978, 1979; Vleck et al. 1979; Hoyt and Rahn 1980; D. Vleck et al. 1980).

The effect of progressively lower P_B on the $P_{I_{O_2}}$ of Red-winged Blackbird eggs laid at various altitudes can be estimated using the data in this study (Table 4). The values needed to calculate $P_{A_{O_2}}$ are: metabolic rate ($\dot{M}_{O_2}$) just before internal pipping, the conductance of the egg to O_2 (G_{O_2}), the effective O_2 tension outside the egg ($P_{A_{O_2}}$) and the O_2 difference between the inside and outside of the egg (ΔP_{O_2}). The $\dot{M}_{O_2}$ of a 3.0-g embryo is predicted to be 90 cm^3·day^{-1} STPD (500.4 mW) at all altitudes (Table 2). Because O_2 and H_2O share the same diffusion path through the eggshell (Paganelli et al. 1978), G_{O_2} can be calculated from the conductance to water vapor (G_{H_2O}) measured by the method of Ar et al. (1974) using eggs collected at each location. The calculation of G_{O_2} from G_{H_2O} requires corrections for the incubation temperature, the difference between diffusion coefficients of O_2 and H_2O, and the effects of the decrease of P_B on diffusion at each

location (Paganelli et al. 1978; Hoyt et al. 1979; Visschedijk et al. 1980).

The following equations are used for calculation of $P_{A_{O_2}}$ at any altitude:

$$G_{O_2} = G_{H_2O} \cdot 1.071 \cdot \frac{101.3}{P_B}$$
(see Hoyt et al. 1979,
Visschedijk et al. 1980)   (2)

$$P_{I_{O_2}} = (P_B - 6.0)(0.209)$$
(Wangensteen and Rahn 1970/71)   (3)

$$\Delta P_{O_2} = \dot{M}_{O_2}/G_{O_2}$$
(Rahn et al. 1974)   (4)

$$P_{A_{O_2}} = P_{I_{O_2}} - \Delta P_{O_2}$$
(Hoyt et al. 1978)   (5)

where $G_{O_2} = $ cm^3·day^{-1}·kPa^{-1}, $G_{H_2O} = $ mg·day^{-1}·kPa^{-1}, P_B at any location $= $ kPa, $P_{I_{O_2}} = $ kPa, $\Delta P_{O_2} = $ kPa, $\dot{M}_{O_2} = $ cm^3 STPD·day^{-1}, and $P_{A_{O_2}} = $ kPa. The term 6.0 in Eq. 3 is the saturated water vapor (kPa) at the measurement temperature of 36°C (see Wangensteen and Rahn 1970/71, for discussion).

The $P_{A_{O_2}}$ of advanced Red-winged Blackbird embryos incubated between 200–290 m is estimated to be 11.9 kPa torr (Table 4), a value within the range determined for other small, altricial eggs at low altitudes (Vleck et al. 1979). The $P_{A_{O_2}}$ at 2,900 m is reduced to 7.1 kPa. This decline is significant for embryonic development, because the $P_{A_{O_2}}$ forms the upper end of the diffusion gradient for O_2 through the inner barrier into the blood. The rate of O_2 diffusion into the blood at the low $P_{A_{O_2}}$ of the montane eggs would be expected to be lower than at sea level, unless the P_{O_2} gradient across the inner barrier is maintained by equivalent decreases

in blood and tissue P_{O_2} or the conductance of the inner barrier to O_2 is lower in eggs laid at high altitudes than at lower ones. Because no information exists concerning normal values of blood and tissue P_{O_2} of altricial embryos nor their tolerances to low tissue P_{O_2}, we cannot speculate about the mechanisms they might use to grow normally at low $P_{A_{O_2}}$.

At least two factors affect the $P_{A_{O_2}}$ in montane eggs. The most obvious one is the reduction in $P_{I_{O_2}}$ that is a natural consequence of the decrease in P_B (Table 4). Because the $P_{I_{O_2}}$ forms the upper end of the ΔP_{O_2} across the eggshell, the $P_{A_{O_2}}$ must decrease with P_B.

The second factor involves the interrelation among the eggshell conductance to gaseous diffusion (G), P_B, and the diffusion coefficient (D). Because the D_{O_2} increases with decreasing P_B (Reid and Sherwood 1966, Paganelli et al. 1975), O_2 diffuses more readily at low P_B than at 101.3 kPa. Because $P_{I_{O_2}}$ decreases with P_B, however, there are fewer molecules to diffuse at high altitudes. Therefore, the increase in D_{O_2} can compensate only in part for the decrease in $P_{I_{O_2}}$ (Visschedijk et al. 1980). One possible solution to the problems associated with reduced $P_{I_{O_2}}$ would involve increasing the eggshell conductance to gas by enlarging the pore area (Ap). This solution would facilitate the flux of O_2 into the shell but, due to the increases in D_{H_2O} and D_{CO_2}, would also create a hazard of excessive losses of CO_2 and water vapor during incubation (Rahn 1977). The few data available indicate that an increase in Ap does not occur, at least in species breeding to 3,000 m (see Carey 1980, for review). In actual fact, the Ap of Red-winged Blackbird eggs, estimated by measurement of G_{H_2O} (standardized to 101.3 kPa), decreases in approximate proportion to P_B and the increase in D (see Table 4), with the result that daily water loss during natural incubation is independent of P_B to 3,000 m (Carey 1980, Carey et al. in press). When G_{O_2} is calculated from G_{H_2O} and corrected for the effect of P_B on D (see Eq. 2), G_{O_2} remains constant from low altitude to 2,400 m (Table 4). The reduction in G at 2,900 m, however, undercompensates for D at that altitude, resulting in a greater G_{O_2}, a smaller ΔP_{O_2}, and higher $P_{A_{O_2}}$ than would be anticipated if eggshell conductance were fully compensated for P_B. Therefore, a slight modification in G_{O_2} at 2,900 m may contribute in a small manner to the es-

tablishment of adequate O_2 levels for normal growth and development.

Experiments on embryonic domestic chickens indicate that control levels of O_2 consumption and growth are not maintained, even in moderately hypoxic circumstances above 1,700 m or below 80 kPa (Wangensteen et al. 1974, Rahn 1977, Visschedijk et al. 1980, Visschedijk 1980). Although this reduction in O_2 consumption has been interpreted as an adaptive mechanism to reduce the ΔP_{O_2} and to attain levels of arterial O_2 and CO_2 typical of acclimatized adults (Wangensteen et al. 1974, Rahn 1977), the low rate of metabolism may simply represent an inability of the embryo to obtain sufficient O_2 to sustain normal rates. One factor that could contribute to O_2 deficiency is the considerable resistance posed by the inner barrier to O_2 diffusion. The difference between the $P_{A_{O_2}}$ and arterial blood $(P_{a_{O_2}})$ (13.8 and 7.0 kPa, respectively) represents a resistance equivalent to 6.8 kPa (Wangensteen 1972, Tazawa 1980). Although this resistance can apparently be adjusted to about 3.2 kPa in hypoxia, it may contribute to the observed effects of hypoxia in chicken embryos. It is clear that the resistance of the inner barrier to O_2 diffusion in Red-winged Blackbird eggs at 2,900 m must be substantially below the 6.8 kPa value of chicken eggs; otherwise, $P_{A_{O_2}}$ would approximate 0.3 kPa. Therefore, modification of the resistance posed by the inner barrier could contribute to the success of wild birds breeding at high altitudes. Such modification of O_2 diffusion could substantially improve O_2 diffusion without causing detrimental increases in flux of CO_2 and water vapor from the egg (see Piiper et al. 1980).

Populations of birds breeding successfully at high altitudes have been confronted with several conflicting requirements: maximization of O_2 availability despite a reduction in $P_{I_{O_2}}$ and minimization of excessive losses of CO_2 and water vapor despite increases in D_{CO_2} and D_{H_2O}. The solution of Red-winged Blackbird populations breeding at 2,900 m involves the evolution of an egg structure that controls water and CO_2 losses at the expense of maximizing O_2 availability. This compromise was achieved without detrimental effects on embryonic development and hatching, perhaps by modification of the resistance of the inner barrier to O_2 diffusion, increased tolerance of tissue to

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