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Reduction of pore area of the avian eggshell as an adaptation to altitude

(water vapor permeability)

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ABSTRACT Standard measurements of water vapor conductance in units of mg of $H_2O \cdot day^{-1} \cdot torr^{-1}$ (SI equivalent is mg of $H_2O \cdot day^{-1} \cdot pascal^{-1}$) of fresh eggs of the red-winged blackbird (*Agelaius phoeniceus*) and the native chicken of India (*Gallus gallus*) collected at altitude are significantly less than those of eggs of the same species collected near sea level. This decrease is caused by a reduction of the total effective pore area of the eggshell at altitude. It appears to be proportional to the reduction in barometric pressure and the simultaneous increase in the diffusion coefficient of water vapor. Thus, reduction in pore area offsets increased diffusivity at altitude, and water vapor loss through the eggshell at any altitude remains the same as at sea level. The data suggest a structural adaptation of the shell to altered diffusivity of gases at altitude in order to prevent excessive water loss of eggs during natural incubation.

During normal incubation bird eggs continuously lose water. The amount lost is proportional to egg weight and inversely proportional to incubation period (1–3) and appears to be regulated in such a fashion that the typical egg loses about 15% of its initial weight prior to pipping. The volume of water lost is replaced by an equivalent gas volume, the air cell, which provides gas for initial inflation of the lungs and the rebreathing maneuvers prior to pipping of the eggshell (4). The water vapor transport across the eggshell takes place by molecular diffusion (5), and shell permeability is determined by the pore geometry (area and length), which is fixed when an eggshell is formed. Thus, a sea level egg transferred to altitude, under otherwise similar nest conditions, should lose water more rapidly in direct proportion to the increase of the diffusion coefficient of water vapor. This was demonstrated by Paganelli *et al.* (6) by placing eggs in a desiccator and exposing them to various simulated altitudes.

Reduction of pore area of the shell would prevent excessive dehydration at altitude. Such a reduction was observed by Wangensteen *et al.* (7), who showed that the water vapor conductance of chicken eggs acclimated for many generations at an altitude of 3800 m was reduced compared with their sea level controls. More recently Packard *et al.* (8) reported a decrease in water vapor permeability of eggs of the barn swallow (*Hirundo rustica*) collected at increasingly higher altitudes, while Ledoux (9) demonstrated an increase in eggshell permeability of White Leghorn hens after their transfer from high altitude (3800 m) to an intermediate level at 1200 m. In this study we extend similar observations to two native species, the red-winged blackbird of North America and the native chicken of India. From the measurements of water vapor conductance of freshly collected eggs at altitude and sea level and measurements of shell thickness we have calculated the total effective pore area of the eggshell. Our results indicate that the shell pore area is reduced in approximate proportion to the reduction in barometric pressure and in proportion to the concomitant increase of the diffusion coefficient of water vapor. In such a case the water vapor flux at altitude (for a given partial pressure difference across the eggshell) remains the same as at sea level and, hence, excessive dehydration due to the increased diffusivity of water vapor at altitude is prevented.

METHODS

Fresh eggs of the red-winged blackbird (*Agelaius phoeniceus*) were collected at an altitude of 2400 m in the Rocky Mountains near Gunnison, Colorado, during the first 2 weeks of June 1976. Another group of eggs was collected from the same species at an altitude of 215 m, near Albany, NY, during the last 2 weeks of May. These eggs were shipped to the laboratory and placed in desiccators provided with silica gel at a constant temperature of 25.5° and weighed daily or every other day to the nearest 0.1 mg, over a period of 8 days. Fresh eggs of the native chicken of India (*Gallus gallus*) were obtained at an altitude of 2500 m in the Ladakh region in the State of Janu and Kashmir and transported to New Delhi, India, for comparison with eggs obtained locally. These eggs were placed in an incubator maintained at a temperature of 37.0° and a relative humidity of 20%. Weight loss was measured daily over a 10-day period. Later all eggs were emptied, the shells were dried, and shell thickness was determined by suitable calipers as described (10). The shell thickness is a convenient measure of average pore length and is used in calculating pore area. For the red-winged blackbird, egg volumes were also determined by weighing eggs dry and submersed. Surface areas were computed as described (10).

From the daily weight loss of eggs in the desiccator and the water vapor pressure difference that exists across the gas-filled pores of the eggshell one can calculate the water vapor conductance by the method of Ar *et al.* (11). The water vapor pressure difference across the eggshell is essentially equal to the saturation vapor pressure at 25.5° [24.5 torr (3.27 kPa)] since the osmotic pressure of the albumen does not depress the vapor pressure within the shell by more than a fraction of a torr, and the vapor pressure in the desiccator is essentially zero. The water vapor pressure difference across the shell of the chicken eggs maintained at 20% relative humidity at 37° was (0.8 × 47.0) or 37.6 torr. Thus, the conductance, G_{H_2O}, is equal to the vapor flux $\dot{M}_{H_2O}$ (mg·day^{-1}) divided by the vapor pressure difference ΔP_{H_2O} (torr) and has units of mg of $H_2O \cdot day^{-1} \cdot torr^{-1}$ (SI equivalent is mg of $H_2O \cdot day^{-1} \cdot pascal^{-1}$) or

$$G_{H_2O} = \dot{M}_{H_2O}/\Delta P_{H_2O} \qquad [1]$$

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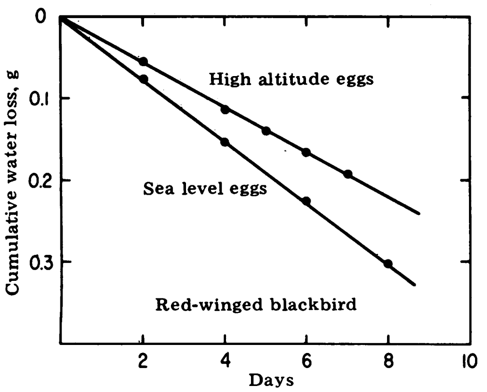

FIG. 1. Cumulative water loss of eggs of the red-winged blackbird collected at altitude (2400 m, $n = 15$) and sea level ($n = 15$), during exposure in a desiccator at 25.5°.

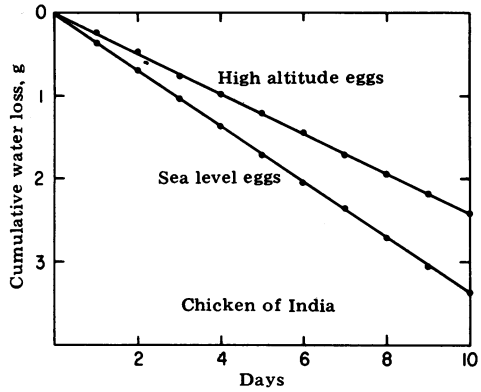

FIG. 2. Cumulative water loss of eggs of the native chicken of India collected at altitude (3500 m; $n = 30$) and at sea level ($n = 29$), during exposure in an incubator at 37° at 20% relative humidity.

Since the transport of water vapor across the gas-filled pores of the eggshell occurs by molecular diffusion (5), the conductance can be described in terms of Fick's first law of diffusion (6, 12). Thus:

$$G_{H_2O} = (A_p/L)(D_{H_2O}/RT)(6.94 \times 10^3) \quad [2]$$

where A_p = effective pore area of the eggshell (mm^2); L = length of pore or shell thickness (mm); D_{H_2O} = diffusion coefficient of water vapor in air (cm^2·sec^{-1}); RT = gas constant × absolute temperature (torr); and 6.94×10^3 = constant to adjust units on both sides of the equation.

Since the last two bracketed terms are constants, it can further be seen that A_p/L, the pore geometry, is the basic property that determines the conductance of the eggshell and that the effective pore area of an eggshell, A_p, can be calculated if the water vapor conductance, G_{H_2O}, and the shell thickness, L, are known. At sea level (barometric pressure = 760 torr), where the diffusion coefficient of water at 25° is 0.268 cm^2·sec^{-1} (3):

$$A_p = 0.447\, G_{H_2O} \cdot L \quad [3]$$

RESULTS

The mean cumulative weight loss of the red-winged blackbird eggs collected at altitude and at sea level, when placed in a desiccator at 25.5° and weighed periodically over a period of 8 days, is plotted in Fig. 1. The altitude eggs had a mean weight loss of 27.2 mg·day^{-1} (SE ± 2.0), the sea level eggs, 37.8 mg·day^{-1} (SE ± 1.6). Fig. 2 provides similar information for chicken eggs in an incubator at 37° and a relative humidity of 20%. The altitude eggs had a mean weight loss of 247 mg·day^{-1}, the sea level eggs, 346 mg·day^{-1}. The standard error of each daily mean weight loss was 5% of the mean.

The physical dimensions of the eggs of the red-winged blackbird collected at the two altitudes indicate that the altitude eggs are significantly larger than those collected near sea level, while the shell thickness is essentially the same (Table 1). Of particular interest is the observation that water vapor conductance of altitude eggs is reduced to 72% of the sea level value. Since shell thickness for both altitudes is essentially the same, effective pore area of the altitude eggs calculated from Eq. 3 is reduced in similar proportion to shell conductance. The permeability of the shell to water vapor, cm^3 (STP) sec^{-1}·cm^{-2}·torr^{-1}·10^6, can also be derived by dividing the conductance by the surface area, converting the weight of water vapor to volume, and adjusting the time dimensions. This dimension allows one to make comparisons between sea level and altitude eggs when differences in surface area exist.

The results for the chicken eggs of India are shown in Table 2. The initial egg weight and surface area of the high altitude eggs are not significantly different from those of the sea level eggs. The conductance, however, is significantly less and is reduced to 71% of the sea level value. Since the shell thickness at altitude is slightly smaller, the calculated pore area of the altitude eggs is 65% of the sea level value.

Table 1. Physical dimensions, water vapor conductance of egg, water vapor permeability, and calculated pore area of red-winged blackbird eggs collected at 87 and 2410 m altitude

	Altitude, 87 m*			Altitude, 2410 m†			
	Mean	SE	*n*	Mean	SE	*n*	*P*
Egg							
Weight, g	4.11	0.06	19	4.49	0.05	39	<0.001
Area, cm^2	12.29	0.12	19	13.04	0.10	39	<0.001
Volume, cm^3	3.91	0.05	19	4.27	0.05	39	<0.001
Shell							
Thickness, mm	0.098	0.002	16	0.095	0.001	18	<0.1
Conductance, mg·day^{-1}· torr^{-1}	1.55	0.07	16	1.11	0.08	15	<0.01
Permeability, cm^3 (STP) sec^{-1}·cm^{-2}·torr^{-1}·10^6	1.82	0.08	16	1.23	0.09	15	<0.01
Pore area, mm^2	0.0678	0.003	16	0.0472	0.003	15	<0.01

* Albany, NY. Barometric pressure = 752 torr.

† Gunnison, CO. Barometric pressure = 567 torr.

Table 2. Physical dimensions, water vapor conductance of egg, water vapor permeability, and calculated pore area of eggs of native chicken of India collected at 220 and 3500 m altitude

	Altitude, 220 m*		Altitude, 3500 m†		
	Mean	SE	Mean	SE	*P*
Egg					
Weight, g	42.45	0.93	40.05	0.63	N.S.‡
Area, cm^2	57.8	1.88	55.6	0.97	N.S.
Shell					
Shell thickness, mm	0.325	0.009	0.297	0.006	<0.05
Conductance, $mg \cdot day^{-1} \cdot torr^{-1}$	9.22	0.58	6.57	0.24	<0.001
Permeability, cm^3 (STP) $sec^{-1} \cdot cm^{-2} \cdot torr^{-1} \cdot 10^6$	2.30	0.14	1.70	0.06	<0.001
Pore area, mm^2	1.34	0.09	0.87	0.04	<0.001

* Delhi. Barometric pressure = 736 torr. $n = 29$.

† Himalayas. Barometric pressure = 505 torr. $n = 30$.

‡ Not significant.

DISCUSSION

During natural incubation, egg temperature as well as the humidity of the nest's microclimate is maintained at relatively constant levels, which establishes a constant water vapor pressure difference between the inside of the eggshell and the microclimate, and accounts for the constant rate of water loss from the egg, $\dot{M}_{H_2O}$ (3, 12), as may be seen from Eq. **1**. The conductance, G_{H_2O}, is determined by the ratio of A_p/L, which is established during eggshell formation in the shell gland of the hen. However, for a given pore geometry, A_p/L, the conductance is proportional to the diffusion coefficient D (see Eq. **2**) and must increase with altitude since D is inversely related to the barometric pressure (13). If the rate of water loss, egg temperature, and nest humidity are to remain the same at altitude as at sea level for a given species, then the ratio A_p/L must be reduced in direct proportion to the change in barometric pressure. Our results suggest that this has occurred in our two altitude populations, primarily by a reduction in the total effective pore area, A_p.

While this provides an attractive hypothesis as far as water conservation is concerned, one must also consider the effects of a conductance change upon the behavior of metabolic gases. In this case, considerable advantage could be gained by maintaining a sea-level pore geometry, thus allowing eggshell conductance to increase. The same O_2 flux could then be maintained at altitude for a smaller O_2 pressure difference across the eggshell barrier than at sea level, making this difference available to the embryo. Wangensteen *et al.* (7) observed that at 3800 m the ΔP_{O_2} across the eggshell was, indeed, greatly reduced at all stages of development when compared with similar values at sea level. But this was in spite of a reduced pore area which kept the conductance at altitude essentially the same as at sea level. In this case embryonic weight and metabolic rate were less at comparable stages of development and accounted for the smaller ΔP_{O_2} and ΔP_{CO_2} across the eggshell.

Table 3 presents a summary of our data and includes similar data recently reported by Ledoux (9), who followed the conductance changes of eggs of White Leghorn hens after transfer from the Barcroft Laboratory of the University of California at 3800 m to 1200 m. For each species the relative changes in barometric pressure are similar to the relative changes in water vapor conductance and total effective pore area. When allowances are made for the larger eggs of the red-winged blackbird at altitude, the relative change in pore area, expressed as pore area per cm^2 of shell surface, is not appreciably altered. The egg weights of the native chicken of India and the White Leghorns were the same at both altitudes. The results of Ledoux's observations are of particular interest since they reflect conductance changes in the eggs of the same hen after transfer to the lower altitude. The changes took between 1 and 2 months

Table 3. Summary of absolute and relative barometric pressure, water vapor conductance, and total effective pore area in eggs of various species

	Barometric pressure		Shell conductance		Total effective pore area	
Species	torr	Relative	$mg \cdot day^{-1} \cdot torr^{-1}$	Relative	mm^2	Relative
Red-winged blackbird						
87 m	752	1.00	1.50	1.00	0.066	1.00
2410 m	567	0.75	1.11	0.74	0.047	0.72
Native chicken						
220 m	736	1.00	9.14	1.00	1.34	1.00
3500 m	505	0.69	6.57	0.72	0.87	0.65
White Leghorn*						
3800 m	480	1.00	10.3	1.00	1.32	1.00
1200 m	657	1.37	13.4	1.30	1.86	1.42

* Data from Ledoux (9).

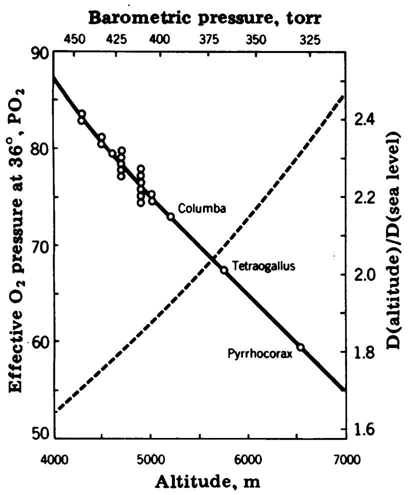

FIG. 3. The effective O_2 pressure surrounding eggs incubating at altitudes above 4000 m and the relative increase in the diffusion coefficient of gases. Nesting records from the reports of Groebbels (16), Hingston (17), Longstaff (18), Noel (15), Swan (19, 20), and O. P. Pearson (personal communication).

to occur. This observation provides the first indication of the rate at which this adaptation occurs and raises the question of the signals that are responsible for the change in pore geometry of the shell. These signals may well be associated with the known changes in blood gas tensions of O_2 and CO_2 when birds become acclimated to altitude (14). The typical sea level chicken egg has approximately 10,000 pores, each with an average radius of 7–8 μm. How is the total pore area reduced at altitude? Is it a change in pore radius, in the number of pores, or both?

The data so far suggest that total effective pore area of a species becomes smaller in direct proportion to the relative increase in the diffusion coefficient or decrease in barometric pressure to altitudes up to 3800 m. However, there are many species known to breed at higher altitudes in the Andes and Himalayas, including the highest breeding record at 6550 m (15), where the barometric pressure is 328 torr and the diffusion coefficient of gases 2.3 times the sea level value. The nesting records for 21 species above 4000 m are plotted in Fig. 3 and show not only the effective O_2 pressure surrounding the incubating egg, but also the relative change of the diffusion coefficient. It is pertinent to ask whether at these very low ambient O_2 pressures some other way than pore area reduction has developed for preventing dehydration. One possible solution would be to provide eggs with a greater-than-normal water content but to retain the sea level pore geometry. Thus, an egg at one-half atmosphere, or 5500 m, might lose 30% of its initial egg weight instead of 15%. However, the same oxygen flux as at sea level could now be delivered at half the O_2 partial pressure loss across the eggshell and account for a saving of 20–30 torr O_2 at the end of the incubation period (21).

Packard *et al.* (8) recently reported observations that are qualitatively similar to ours, but that differ in an important quantitative aspect. They showed that the decrease in eggshell conductance of barn swallows at increasing altitudes was very much larger than the proportional decrease in barometric pressure. While this would more than compensate for the increased diffusivity of water vapor at altitude, it would also increase the partial pressure difference for O_2 and CO_2 across the eggshell and thus provide a relatively lower O_2 and higher CO_2 pressure for the embryo.

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