

Structural and physiological differences between montane and lowland avian eggs and embryos

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Abstract. A few avian species breed at altitudes up to 6500 m. Embryos in eggs laid at high altitudes are confronted with the problem that gases diffuse more rapidly at low barometric pressure than at sea level. Data on birds breeding up to 4500 m indicate that modifications in eggshell structure and embryonic physiology foster successful development in these groups. At moderate altitudes (up to 3600 m), shell conductance to gases (corrected to 760 torr) is decreased in approximate proportion to the reduction in barometric pressure, thus offsetting the increased tendency of gases to diffuse. At altitudes above 4000 m, the conductance is increased above levels at moderate altitudes, thus fostering improvement in oxygen availability, while increasing rates of water and CO₂ losses. Above 4000 m, embryonic physiological properties become increasingly important for coping with hypoxic, hypocapnic, and dehydrated conditions inside the shell. Nothing is known about characteristics of eggshells and embryos in eggs laid between 4500 and 6500 m. Despite years of artificial selection, domestic fowl do not breed successfully much above 3000 m. Embryos of domestic fowl appear highly sensitive to the effects of hypoxia.

Keywords. Hypoxia; barometric pressure; eggshell conductance; hematocrit; chorioallantoic membrane; oxygen consumption; blood gas tensions.

1. Introduction

The distribution of birds from sea level to the permanent snow line in the mountain ranges of the world provides an opportunity to ask questions concerning how animals respond to changes in the physical environment over geographical gradients. Barometric pressure is a physical feature of the environment which varies continuously with altitude. Other physical features of the high montane environment (hypoxia, cold, intense solar radiation, low water vapour pressure, etc.) vary with altitude as secondary consequences of the variation in barometric pressure. Additionally, certain factors like hypoxia and cold may act synergistically with the result that their interacting effects may pose even greater challenges to organisms than might either factor alone. Despite the potential physiological and ecological hardships posed by these environmental conditions, a few kinds of birds breed successfully in high alpine areas extending to approximately 4900 and 6500 m in the Andes and Himalaya, respectively (Rahn 1977). The purpose of this review is to evaluate the current status of our knowledge about mechanisms which enable birds to breed at higher altitudes than other vertebrates.

2. Gas exchange of avian embryos

Successful reproduction in any habitat requires that embryonic requirements are met within their tolerance limits. Adult birds provide heat, defense from predators,

and periodical turning for the eggs (Drent 1975). Since all nutrients are prepackaged in the egg prior to laying, appropriate levels of gas exchange constitute the only other embryonic requirement for growth and development (Carey 1983). Gases (O₂, CO₂ and Water vapour) are exchanged between the embryo and its environment by the process of diffusion through minute pores in the eggshell (Wangensteen and Rahn 1970/71). Oxygen diffuses into the shell down a partial pressure gradient established by the O₂ consumption of the embryo, whereas CO₂ and water vapour travel out the shell down partial pressure gradients set up by the CO₂ production of the embryo and the humidity of the nest environment, respectively (Wangensteen and Rahn 1970/71). The factors governing the rate of gas diffusion through the shell (M , cm³STPD.s⁻¹) have been described by a modification of the Fick equation (Wangensteen 1972; Paganelli *et al* 1975):

$$M = (D/RT) (Ap/L) \cdot (Ap/L) \cdot \Delta P, \quad (1)$$

where D = binary diffusion coefficient (cm².s⁻¹) RT = gas constant and absolute temperature (cm³ STPD. cm⁻³. torr⁻¹), Ap = effective pore area (cm²), L = length of diffusion path, on shell thickness (cm), and ΔP = partial pressure difference of gas across the shell (torr). The female has control over Ap and L at the time of shell manufacture, although these factors are apparently genetically controlled rather than phenotypically plastic (Carey *et al* 1984), and over T by controlling egg temperature. Furthermore, the incubating parent may have a minor effect on shaping ΔP by trapping gases in the nest microclimate while sitting on the eggs or by ventilation the nest environment when off the nest (Walsberg 1980). All the other components eg. 1 are physical factors over which birds have no control.

The terms $(D/RT) (Ap/L)$ are sometimes combined into the term “ G ” (cm³.s⁻¹. torr⁻¹) representing the conductance, or the diffusive capacity of the eggshell, to each gas (Ar *et al* 1974). Conductance is conventionally corrected to 760 torr and 25°C so that the G of a given species can be compared with that of other species under identical conditions of pressure and temperature. The actual conductance of the egg at the altitude at which it was laid is termed its “effective” conductance.

The average G of a given species appears to have been under selection from mutually antagonistic selective factors. Since O₂ diffuses into the egg and CO₂ and water vapour diffuse in the opposite direction, the conductance must be large enough to allow adequate amounts of O₂ to diffuse in, but it must be small enough to prevent excessive losses of CO₂ and water vapour. Therefore, the average G of a particular species represents a compromise between these mutually antagonistic forces. In addition; G has been selected in relation to the length of the incubation period and egg mass: Ar and Rahn (1978) observed that G is inversely proportional to the incubation period for a given mass of egg. As a result, most eggs incubated at sea level lose about the same proportion of water, in the form of water vapour, as a percentage of initial egg mass during incubation (average = 17%) (Ar and Rahn 1980). Additionally, the interrelation between G , incubation period and egg mass fosters similarities in O₂ and CO₂ exchange. The amount of O₂ consumed per g embryo (Ar and Rahn 1978) and ΔP_{O_2} and ΔP_{CO_2} , across the eggshell at comparative stages of incubation are similar in almost all lowland eggs (Rahn *et al* 1974; Ar and Rahn 1978). Additionally, the final level of O₂ and CO₂ in the air cell at pipping (P_{AO_2} and P_{ACO_2}) of eggs of almost all species fall within rather

narrow limits around 104 and 40 torr, respectively (Rahn *et al* 1974; Hoyt and Rahn 1980) The importance of the latter two observations relates to the fact that the P_{AO_2} and P_{CO_2} prior to the onset of pulmonary respiration closely resemble those found in the lungs of hatchlings and adults (Wangensteen 1972; Rahn *et al* 1974). Therefore, shell conductance not only regulates appropriate levels of gas exchange but also fosters preparation of the embryo for the onset of aerial respiration (Wangensteen *et al* 1970/71; Rahn *et al* 1974).

Since the relation between G , incubation period and egg mass results in relatively similar levels of water vapour, CO_2 and O_2 exchange in most species, it has been hypothesized that G of each species must fall within a narrow range; if not, embryos in eggs with too high or too low G will die before hatching (Ar and Rahn 1980). However, responses of avian embryos to variation in gas exchange have been tested in too few studies thus far to support or refute this hypothesis. Tolerances of embryonic Red-winged blackbirds (*Agelaius phoeniceus*) to variation in water loss, caused by enlarging or restricting G , are fairly broad: embryos hatched and fledged successfully after experiencing a range of water losses from 74 to 33% of the initial egg mass (Carey 1986). Domestic fowl embryos (*Gallus domesticus*) die if exposed to less than 15% O_2 or more than 40% CO_2 (Lundy 1969).

Since conductance of the eggshell (G) is such an important feature for an egg, it is instructive to review how to measure it. Conductance to water vapour G_{H_2O} is the most easily measured because the process requires only simple equipment. If an egg is placed in a desiccator over KOH pellets in a room at a constant, known temperature and its change in mass is measured daily by weighing it on a balance accurate to at least 0.01 g, conductance (G_{H_2O}) can be calculated by the relation:

$$G_{H_2O} (\text{mg} \cdot \text{day}^{-1}, \text{torr}^{-1}) = M_{H_2O} / \Delta P_{H_2O}, \quad (2)$$

where $M_{H_2O}(\text{mg} \cdot \text{day}^{-1})$ is daily weight loss and ΔP_{H_2O} (ton) is the water vapour pressure difference between the inside of the egg (saturation water vapour pressure at known temperature) and the outside of the egg (= zero) (Ar *et al* 1974). Once G_{H_2O} is known, G_{O_2} and G_{CO_2} can easily be calculated (Paganelli *et al* 1978). Since G varies regularly with egg mass for most species, measured G can be compared with the estimated value, derived from allometric equations, for that egg mass. [A number of other egg characteristics, such as shell thickness, number of pores, shell density, water loss, incubation period, etc., can also be predicted if egg mass is known (see Carey 1983)]. These equations can be used to identify eggs of species that do not fit general patterns. For instance, average G of eggs laid in burrows with very humid, hypoxic, or hypercapnic conditions is substantially larger than predicted on the basis of incubation period and egg mass (see Carey 1983 for review).

3. Gas exchange of avian embryos of wild species at high altitudes

Although Aggazzotti (1913) was the first to report that transport to high altitude increased water loss from an egg; Rahn *et al* (1974) were the first to recognize the cause: the diffusion coefficient for gases increases inversely with barometric pressure. If D in eq. 1 is increased, M for any gas will increase and gases will diffuse more readily at low barometric pressure than at sea level (Paganelli *et al*

1975). If eq. 2 is rewritten with P_B representing the barometric pressure (in torr) at the montane location:

$$M = G(760)/P_B \cdot \Delta P_{H_2O}, \quad (3)$$

it becomes clear that the "effective conductance" of the egg (G) for any gas will be higher at altitude than at sea level (figure 1). If an egg were transported from sea level to the elevation where barometric pressure equalled 0.5 atm, gases would diffuse twice as fast as at sea level (Paganelli *et al* 1975). This effect would benefit an embryo by increasing the flux of O_2 inward into the egg, thus in part offsetting the decrease in ambient P_{O_2} (Visschedijk *et al* 1980). However, it would also increase rates of water vapour and CO_2 losses. If such variation in gas exchange caused by increase in D at low P_B would prove detrimental to montane embryos, it is useful to hypothesize what sort of mechanisms for offsetting the effect of D might be employed by birds in order to breed successfully at high altitude.

If maximization of O_2 delivery were the most important priority for embryonic survival at high altitude, several possibilities exist: (i) An increase in G by decreasing

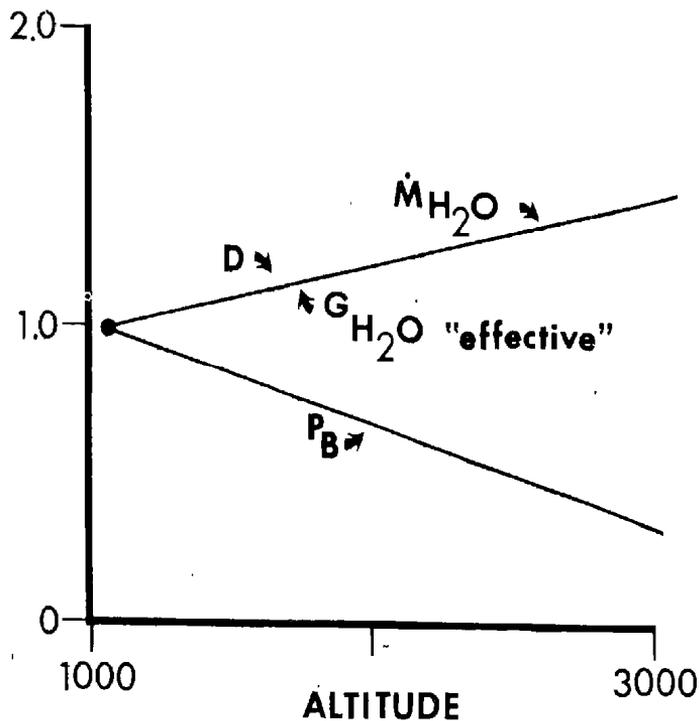


Figure 1. Change in barometric pressure (P_B), diffusion coefficient for gases (D), "effective" G_{H_2O} (actual G_{H_2O} of egg at a given altitude), and daily water loss (M_{H_2O}) as a function of altitude (m). P_B , D , "effective" G_{H_2O} and M_{H_2O} are designated as 1.0 at sea level; the thin lines represent the relative value for each parameter as altitude increases. For instance, imagine that an egg laid at sea level were transported to 2400 m; P_B at that altitude is roughly 0.75 of that at sea level. Since D for any gas varies inversely with P_B , D would be $1.25 \times$ the sea level value. As a result of the effect of D on G_{H_2O} (see equation 1), effective G_{H_2O} , and M_{H_2O} at 2400 m would also be $1.25 \times$ their respective sea level values.

L or increasing Ap , which would decrease the resistance of the shell to diffusion of O_2 , but would also increase the rate of water and CO_2 losses; (ii) an increase in ΔP_{O_2} by decreased nest attentiveness. This option would also enhance losses of CO_2 and water vapour; (iii) an increase in O_2 delivery to the cells by an increase in blood O_2 carrying capacity, variation in haemoglobin oxygen affinity, increased capillarity of the chorioallantoic membrane, or other possible specializations at the cellular and biochemical level.

However, if prevention of excessive water and/or CO_2 losses were the most important priority for successful development and hatching of montane embryos, one or more of these options might be employed: (i) A decrease in G by increasing shell thickness or decreasing Ap , which would have the effect of increasing the resistance of the shell to gaseous diffusion (this option would also increase the resistance of the shell to O_2 diffusion); (ii) A decrease in the ΔP_{H_2O} and ΔP_{CO_2} , by raising P_{H_2O} and P_{CO_2} , in the nest microenvironment. This could be accomplished by trapping these gases through increased adult attentiveness on the nest. (This option would also decrease O_2 availability); (iii) An increase in the initial water content of eggs and a decrease in the plasma $[HCO_3]$. The former mechanism would offset increased water losses by provide additional water to be lost, and the latter would maintain plasma pH at a level similar to that at sea level despite lower P_{CO_2} in the blood.

Over the last 14 years, shell structure and embryonic physiology of 11 species of wild birds breeding between sea level and 4478 m have been compared with lowland congeneric or conspecific groups (Packard *et al* 1977; Rahn *et al* 1977; Sotherland *et al* 1980; Taigen *et al* 1980; Carey *et al* 1983, 1987, 1989a,b, 1990, 1993, 1994). These studies have led to several conclusions about what differences exist between montane and lowland eggshells and embryos and how these differences might contribute toward compensating for increased D at high altitude. First, a few of the alternatives listed above can be ruled out. Water content does not vary significantly with altitude (Carey *et al* 1983, 1987, 1989a,b), nor, with the exception of one species, does shell thickness (Rahn *et al* 1977; Sotherland *et al* 1980; Taigen *et al* 1980; Carey *et al* 1983, 1987, 1989a,b). Since shell thickness is another characteristic that is under mutually conflicting selective requirements (*i.e.*, shell must be thick enough to support the mass of the egg contents and incubating parent, while thin enough to allow the embryo to pip), shell thickness probably cannot be varied significantly without adversely affecting either requirement. It is unknown why water content could not be varied, but both initial and final content of avian eggs appear to be tightly regulated parameters (Ar and Rahn 1980).

Whether or not adult incubation behaviour varies over altitudinal gradients in a manner that would compensate for the effect of D cannot be conclusively determined at this time. Data on incubation behaviour exist on only one species: the pattern of nest attentiveness of adult Red-winged Blackbirds does not vary significantly over a 2900 m altitudinal gradient (Carey *et al* 1983).

Pore area (Ap) and certain aspects of embryonic physiology do vary significantly in birds breeding over altitudinal gradients. These topics merit separate consideration.

3.1 Shell structure

Eggshell conductance of montane eggs varies significantly from that of closely related lowland species, but the exact relation depends on the altitude at which

the eggs are laid. Conductance of eggs laid up to about 3600 m (corrected to 760 torr) significantly declines in approximate proportion to the reduction in barometric pressure at each breeding location (Packard *et al* 1977; Rahn *et al* 1977; Carey *et al* 1983) (figure 2). Taigen *et al* (1980) and Sotherland *et al* (1980) found either

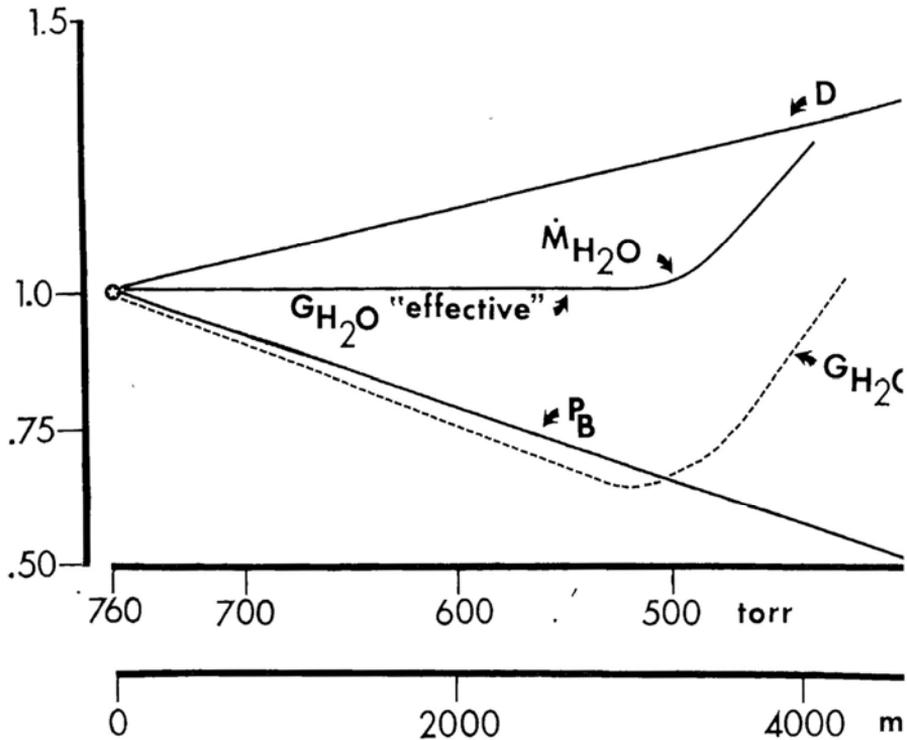


Figure 2. Changes in barometric pressure (P_B), diffusion coefficient for gases (D), G_{H_2O} (standardized to 760 torr and 25°C), “effective” G_{H_2O} (actual G_{H_2O} of egg at a given altitude), and daily water loss (M_{H_2O}) as a function of altitude (m) and barometric pressure (torr). Figure is based on data from 8 species breeding between sea level and 4478 m (Carey *et al* 1983, 1987, 1989a, b, 1990). All parameters are standardized at 1.0 at sea level; lines indicate the relative value for each parameter as altitude increases and barometric pressure decreases. Since the reduction in G_{H_2O} roughly parallels that of P_B , the increase in D is offset and the effective G_{H_2O} and M_{H_2O} are independent of altitude to about 3500 m. Above that altitude, the reduction in G_{H_2O} under-compensates for the reduction in P_B as a result, effective G_{H_2O} and M_{H_2O} rise above levels at more moderate altitudes. G_{H_2O} may even exceed sea level values at altitudes above 4000 m.

no significant relation or a direct relation between G and P_B , but these results were most likely influenced by the age of the egg when measured, rather than a true adjustment to P_B (Carey 1983). Since the reduction in G approximates the reduction in P_B at each location, the increase in D is offset: as a result, water vapour and (presumably) CO_2 losses are the same at all altitudes (Carey *et al* 1983) (figure 2). Because ambient P_{O_2} , at moderate altitudes is lower and Δp is smaller than at sea level, embryos develop in hypoxic environments inside the shell despite the increase in D_{O_2} . However, despite these conditions, embryos apparently receive sufficient O_2 to support normal metabolism: hatchling mass, length of

incubation period, and oxygen consumption at all embryonic masses do not vary significantly with altitude up to 3600 m (Carey *et al* 1982). These data suggest that the priority for shell design at moderate altitudes is to conserve water vapour and CO₂ at the expense of O₂ availability. This “strategy” in shell design succeeds because embryos still can develop normally despite the hypoxic environment in the shell.

Since shell thickness does not vary with altitude in almost all species, the variation in G with altitude is due solely to variation in pore area (A_p). Pore area is a function of both the numbers of pores and their cross-sectional areas. In all cases, G is varied by changing the number of pores, not the dimensions of individual pores (Carey *et al* 1987, 1989a, b). So little is known about the mechanism by which pores are formed in the shell that it is impossible to determine why pore number is varied in preference to pore size.

The average G of birds breeding above 4000 m shows a different relation to P_B than that of eggs laid at slightly lower altitudes (figure 2). Average G (corrected to 760 torr) of eggs laid above 4000 m is not reduced in the same proportion as the reduction in barometric pressure (Carey *et al* 1987, 1989a,b 1990). In fact, average G of some species exceeds the value for eggs of lowland relatives (Carey *et al* 1989a, 1990). The lack of compensation for the reduction in P_B (or the increase in D) causes the “effective” G to be higher at altitude than at sea level (figure 2). As a result, losses of water vapour and CO₂ exceed levels from lowland eggs (Carey *et al* 1987, 1989a,b), although not apparently beyond the tolerance limits of the embryos at altitudes up to 4600 m.

The curvilinear relationship between G and P_B found for eggs laid above sea level (figure 2) is interpreted to result from different priorities for regulation of gas exchange, depending upon the altitude: conservation of water and CO₂ at lower altitudes at the expense of O₂ availability, and improvement of O₂ availability at altitudes above 4000 m at the expense of increased losses of water vapour and CO₂. Adjustments in shell structure at moderate altitudes serve to maintain a hydric and acid-base environment inside the egg similar to that at sea level. However, O₂ tensions inside the egg differ from those at low altitudes because the ambient P_{O_2} , is lower than at sea level. At altitudes above 4000 m, the decrease in ambient P_{O_2} , causes the air cell O₂ tension P_{AO_2} to drop to levels that provide an inadequate pressure gradient for sufficient diffusion of O₂ into the blood, and ultimately into the tissues (Carey *et al* 1989a). Oxygen insufficiency is apparently the selective force that causes shells of eggs laid above 4000 m to be laid with a relatively higher G than at moderate altitudes. However, despite these modifications of shell structure, the resultant increase in G is not sufficient to create gaseous conditions inside the shell that mimic those at sea level. As a result, the gaseous environment inside the shell above 4000 m is hypoxic, hypocapnic, and dehydrated, and would become progressively more so at even higher altitudes. Therefore, embryonic physiological properties that foster growth and development in these extreme conditions become progressively more important at higher altitudes.

3.2 Embryonic physiological properties

Since the higher “effective” G at altitudes over 4000 m causes water vapour to diffuse more rapidly than at sea level, embryos have to deal with dehydration of egg contents. Relative water content of altricial and precocial eggs at laying average

85% and 76%, respectively. Water content of pipped altricial and precocial embryos match those values, respectively (Ar and Rahn 1980). The similarity in water contents of fresh and pipped eggs is due to the fact that water loss during incubation compensates for both the loss of solids through catabolism and metabolic water production (Ar and Rahn 1980). Whether or not embryonic survival depends on tight regulation of final water content is unknown. If eggs lose more water than expected and do not have an initial reservoir of additional water to compensate for higher rates of water loss, the final water content of the pipped embryo might be expected to deviate from the initial water content. However, final water content of coot embryos (*Fulica americana peruviana*) at 4150 m in the Peruvian Andes does not deviate significantly from that of eggs laid by lowland relatives (Carey *et al* 1989a). The apparent reason for this observation is that the montane eggs would lose about 2.4 g more water than lowland eggs. This amount, comprising only 6.5% of the initial water content, is apparently not sufficient to alter significantly the final relative water content of the pipped embryos. No data are available on rates of water loss or water content of eggs laid above 4400 m. Therefore, it is impossible at this time to determine if embryos in eggs laid at altitudes higher than 4400 m experience severe dehydration, and if so, how they deal with it.

Montane embryos develop in progressively more hypoxic conditions inside the shell as altitudes at which eggs are laid increase. The normal air cell oxygen tension (P_{AO_2}) inside a lowland egg falls from about 140 torr in the early stages of incubation to about 104 torr prior to pipping (Wangensteen and Rahn 1970/71). The decrease is due to the increased demand for O_2 by the embryo as it grows and the fixed resistance to O_2 diffusion by the shell (Wangensteen 1972). The P_{AO_2} serves as the upper end of the gradient for diffusion of O_2 into the blood (Wangensteen and Rahn 1970/71; Wangenstein 1972). Therefore, the lower the P_{AO_2} , the more slowly O_2 will diffuse into the blood. Air cell O_2 tensions of coot and Puna teal (*Anas versicolor puna*) eggs are continuously below sea level values, even in a fresh egg, and fall below 45 torr just before pipping (Carey *et al* 1989a 1994). As a result, the O_2 tension in arterialized blood P_{aO_2} approximates 25-30 torr just before hatching in these two species. Surprisingly, the P_{aO_2} of montane coot embryo blood is not substantially below that of lowland coot embryos at similar stages of development. The reason is that the $P_{AO_2} - P_{aO_2}$ difference, which represents the amount of resistance to O_2 diffusion from the air cell through the inner shell membrane and chorioallantoic membrane, is substantially smaller (35 torr) than in lowland eggs (60 torr). Various factors have been hypothesized to account for a similarly large difference existing in sea level eggs of lowland domestic eggs (see below), such as arterio-venous shunts, disequilibria of blood flow to diffusion, and water layers between the inner shell membrane (Piiper *et al* 1980), but no factor has yet been conclusively proven. At this point, we can conclude only that eggs of montane wild species manage to oxygenate arterial blood to a greater extent than would be expected if the same barrier to O_2 diffusion existed in their eggs as it does in lowland ones.

Montane embryos also have higher hematocrits (proportion of blood volume occupied by red blood cells) than lowland ones. While hematocrits of 18-g lowland coot embryos average 31%, that of similarly-sized montane coot embryos averaged 44% (Carey *et al* 1993). Whether additional differences in factors which would maximize O_2 delivery to cells, such as capillary density of the chorioallantoic

membrane, blood oxygen affinity, concentrations of red blood cell phosphorylated intermediates, blood volume, cardiac output, tissue mitochondrial density, etc., exist between montane and lowland embryos remains to be determined. Oxygen affinity of embryos of bar-headed geese (*Anser indicus*, a species which breeds in south-central Asia at altitudes as high as 5600 m), tested on zoo stock maintained at low altitude for a number of years, was significantly greater than that of Canadian goose embryos (*Branta canadensis*), a group that primarily breeds at altitudes below 1700 m (Snyder *et al* 1982). It would be interesting to determine if the magnitude of difference found by Snyder *et al* (1982) would be greater if affinity of bar-headed goose embryos from wild stock could be tested and compared with lowland values.

If physiological adjustments related to O₂ delivery are adequate throughout development, montane embryos should be able to maintain a rate of metabolism similar to that of similarly-sized embryos at sea level. The benefit of maintaining a "normal" metabolic rate is that embryos can then grow, develop and hatch at the same body mass within the same incubation period. Hatching at reduced body masses and prolongation of incubation periods may be associated with increased mortality (Carey *et al* 1989a). Montane Puna teal embryos maintain a high metabolic rate throughout development at 4150 m, higher than even lowland coot embryos of similar size (Carey *et al* 1993, 1994). Metabolism of montane coot embryos at the same altitude, however, is lower than that of similarly-sized lowland coots throughout development. Although montane and lowland coots hatch at similar masses, incubation periods of montane individuals must certainly be longer than those at sea level (Carey *et al* 1989a). Puna teal embryos are able to maintain a higher metabolic rate than montane coot embryos because of less resistance to O₂ diffusion through the inner shell membrane and chorioallantoic membrane (P_{AO_2} — P_{aO_2} difference), higher hematocrits at all stages of development, and probably other undocumented differences in physiological properties (Carey *et al* 1993, 1994).

Venous O₂ tensions of both Puna teal and coot embryos at 4150 m fall below 10 torr (Carey *et al* 1993, 1994). Since venous O₂ tension is thought to reflect tissue O₂ tensions, these embryos are not only maintaining tissue but also growing at tissue O₂ tensions that would be lethal for adult vertebrates for any length of time.

Regulation of the P_{CO_2} of blood is usually important for organisms because of the relationship between pH and P_{CO_2} (Wangensteen and Rahn 1970/71; Wangenstein 1972). The combination of a higher tendency of CO₂ to diffuse from the shell at high altitude and the low metabolic rate produces a 20-torr difference in blood P_{CO_2} , between montane (20-torr) and lowland (40 torr) pre-pipping coot embryos (Carey *et al* 1993). However, pH of the blood of the two groups is statistically indistinguishable (Carey *et al* 1993). The similarity in pH is due to a significant reduction in [HCO₃⁻] in the plasma of the montane embryos. Some of this reduction is clearly due to the low rate of metabolism, but other mechanisms may also be involved. In contrast, Puna teal embryos maintain levels of pH and HCO₃⁻ similar to those of low altitude birds; relatively high rates of metabolism probably serve to supply the HCO₃⁻ (Carey *et al* 1994).

Coots breed over the entire altitudinal gradient from sea level to at least 4150 m in the Peruvian Andes, whereas Puna teal breed strictly high altitudes (Parker *et al* 1982). Although hatchability of coot embryos appears to be high at 4150 m (Carey *et al* 1989a), the prolonged incubation period and depressed metabolic rate of coot embryos suggests that coots at this altitude are breeding near the upper

altitudinal limit of their distribution. Coots at the highest altitudes of their distribution may be prevented from developing adaptations to hypoxia by gene flow from lower altitudes. Because Puna teal breed strictly at high altitude, their isolation at high altitude may have fostered development of specializations permitting maintenance of normal rates of metabolism at extremely low tissue O_2 tensions.

To date, neither shell structure nor embryonic physiology have been studied above 4478 m. It is essential to learn more about the mechanisms used by birds breeding at much higher altitudes in order to understand how structural and functional adjustments can foster successful breeding of birds at such high altitudes.

4. Gas exchange of embryos of domestic fowl at high altitudes

Despite years of artificial selection, hatchability of domestic fowl (*Gallus domesticus*) embryos at high altitude is very low. Even descendants of the chickens introduced to the Andes by the Spaniards 400 years ago produce eggs with very low hatchability above 3000 m (Leon-Velarde *et al* 1984). The causes of low hatchability appear to be embryonic vulnerability and susceptibility to hypoxia (Visschedijk *et al* 1980). Embryos of a group of chickens acclimatized to 3800 m for 15 years exhibited depressed metabolic rate at all developmental stages, low growth rates and hatchling masses, prolonged incubation periods, and low levels of hatchability (Wangensteen *et al* 1974). Embryos of commercial turkey and chicken breeds incubated above 1600 m must be provided with supplemental O_2 for optimal hatchability. These embryos rarely hatch above 3000 m, even with supplemental O_2 (Moreng 1983). Even direct selection for turkey embryos for increased hatchability at moderate altitude (1524 m) resulted in only 69.9% hatchability, compared to 57.7% in controls, after 7 generations (Cramer *et al* 1974).

Since it would be a worthwhile contribution of science to the nutritional status of developing countries with human populations at altitudes above 1600 m to develop a strain of domestic fowl that could produce eggs having high hatchability at high altitudes, it is useful to analyse what is known about how eggs and embryos of domestic fowl differ from those of wild birds breeding at high altitude. Chicken flocks reared between 2000 and 4000 m have evolved shells with an average G that is reduced in approximate proportion to the reduction in barometric pressure and falls within the same range as those of wild birds breeding at comparable altitudes (Wangensteen *et al* 1974; Rahn *et al* 1977; Leon-Velarde *et al* 1984). Therefore, the major unsolved problem for embryonic survival seems to involve mechanisms of O_2 uptake, transport, and utilization. Although much remains to be learned about such matters in embryos of wild birds, at least two obvious differences between characteristics of wild and domesticated embryos have been identified.

First, the resistance of the "inner barrier" (the inner shell membrane and the chorioallantoic membrane) to O_2 diffusion is considerably higher in the chicken than in montane wild birds (Piiper *et al* 1980; Carey *et al* 1993). The ΔP_{O_2} between the air cell O_2 tension (P_{AO_2}) and arterialized blood (P_{O_2}) of pre-internal pipping (pre-IP) chicken embryos is about 47 torr (Piiper *et al* 1980), whereas the approximate value is 10 torr in montane Puna teal embryos at a similar stage of incubation at 4150 m (Carey *et al* 1994).

Secondly, hematocrits of chicken embryos incubated at 3800 m reached 40% just

before hatching (Burton and Smith 1969). This is slightly below the value for Puna teal at 4150 m (Carey *et al* 1994). Therefore, O₂ carrying capacity of embryonic chicken blood is probably lower than of embryos of many wild birds breeding at altitude. Chicken embryos are capable of some phenotypic response to hypoxia. If chicken embryos are incubated in hypoxic conditions (13.5% O₂), they make the transition from embryonic to adult haemoglobin faster than do controls, and improve O₂ affinity of the blood slightly in comparison with controls (Baumann *et al* 1983). However, cardiac output drops in hypoxic embryos (Piiper *et al* 1980) and this response may offset the benefits of the onset of adult haemoglobin synthesis and improved blood O₂ affinity. It is unknown how these characteristics vary from those of wild birds incubated at high altitude,

Chicken eggs can be incubated successfully at a simulated altitude of 5.7 km in incubator consisting of 70% relative humidity, 45% O₂, and 55% N₂ (Visschedijk 1985). These conditions offset the high rates of water loss at that altitude and prevent hypoxia in the embryos. However, it is doubtful that provision of such incubation conditions would be feasible in many developing countries, in which O₂ bottles may not even be available for human use in hospitals in montane regions. Since development of a strain of domestic fowl by artificial selection for high hatchability at high altitudes is unlikely in the foreseeable future, supply of domestic fowl to people living in mountainous regions will likely continue to involve transport of chicks or adults from hatcheries at lower altitudes (Leon-Velarde *et al* 1984).

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