

RESISTANCE OF THE SHELL MEMBRANE AND MINERAL LAYER TO DIFFUSION OF OXYGEN AND WATER IN FLEXIBLE-SHELLED EGGS OF THE SNAPPING TURTLE (*CHELYDRA SERPENTINA*)¹

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Abstract. At oviposition, flexible eggshells of many turtles have an outer mineral layer and an inner membrane layer of approximately equal thickness. We measured conductances of both layers to H₂O and O₂ at various levels of eggshell hydration.

Both the mineral layer of the eggshell and the shell membrane offer significant resistance to diffusion of water vapor and oxygen in eggshells of the snapping turtle, *Chelydra serpentina*. Conductance to water vapor increases in both the membrane and mineral layer with increasing hydration of the eggshell, but conductance to oxygen decreases under similar conditions. Removal of the mineral layer increases conductance to oxygen in moist and dry eggshells, but decreases conductance at intermediate levels of dehydration. Removal of the mineral layer consistently increases conductance to water vapor. The eggshell membrane accounts for 24–76% of overall resistance to diffusion of water vapor. These results suggest that bulk flow of H₂O or physical changes in the shell may interact with diffusion to limit gas exchange through the turtle eggshell.

Conductance	Gas exchange
Diffusion	Permeability
Eggshell	Turtle eggs
Eggshell membrane	Water loss

At oviposition, the eggshells of many species of turtles include an outer mineral layer and an inner shell membrane of approximately equal thickness*; such eggshells are termed 'flexible' in contradistinction to the predominantly membranous eggshells of lepidosaurs and the rigid, predominantly mineral eggshells of other turtles, crocodilians and birds (Solomon and Baird, 1976; Packard, 1980; Packard and Packard, 1980). In the laboratory, flexible eggs undergo gross changes in mass,

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* In common usage, 'eggshell' signifies the mineral layer exclusive of the shell membrane(s). We use the term 'eggshell' to denote the outer covering of the egg, including both the mineral layer (if present) and eggshell membrane.

shape and volume throughout incubation, depending on their hydric environment (Tracy *et al.*, 1978; Packard *et al.*, 1980, 1981a,b). In those species that have been studied, the outer mineral layer of flexible eggs gradually exfoliates late in incubation and comes to account for only a small portion of eggshell thickness at hatching (Packard, 1980; S. Satel, unpublished data). In each of these respects, flexible eggs of turtles differ from avian eggs (Wangensteen *et al.*, 1970/71; Rahn *et al.*, 1979; Packard, 1980; Packard and Packard, 1980).

Because of its relatively great thickness, the eggshell membrane of flexible eggs may play an important role in limiting gas exchange between the embryo and the environment, either directly or through interaction with the mineral layer. Packard (1980) has speculated that conductance of flexible eggshells is a complex function of egg hydration, eggshell membrane structure, and acute alterations in the arrangement of adjacent mineral shell units as an egg swells. However, most examinations of eggshell permeability to gas exchange in flexible eggs (Ackerman and Prange, 1972; Packard *et al.*, 1979) have neglected the eggshell membrane, either explicitly or in calculations of results. To explore the role of the eggshell membrane in gas exchange, we measured O_2 conductance (G_{O_2}) and H_2O conductance (G_{H_2O}) in flexible eggshells of *Chelydra serpentina*, the snapping turtle. We characterized resistance to diffusion of gas in both the eggshell membrane and the mineral layer by examining eggshells in which the mineral layer was removed experimentally. Also, we observed the effect of shell hydration on conductance.

Materials and methods

Freshly laid eggs of *Chelydra* were held at room temperature on water-saturated vermiculite for 1–2 weeks before experimentation and between measurements. Each egg underwent six experimental treatments sequentially. Experimental treatments were as follows: (1) Control egg (intact). (2) A small hole (less than 1 cm²) was cut in the shell, and then sealed with paraffin. (3) The hole was opened, contents of the egg were extracted, the inside of the egg was rinsed several times with distilled water, and the egg was filled with albumen from an infertile hen's egg. The hole was resealed. (4) The hole was opened; the egg was rinsed, filled with distilled water, and resealed. (5) The hole was opened; the egg was rinsed, left empty, and held in water-saturated air overnight. The egg was filled with distilled water, and the hole was resealed. (6) Similar to 5, except that the rinsed, empty egg was held in dry air overnight.

Water loss was measured with the method of Packard *et al.* (1979). After each experimental treatment, eggs were placed above dehydrated indicating Drierite (W.A. Hammond Drierite Co., Xenia, OH) in a desiccator at 23.8 °C. Eggs were weighed ± 0.1 mg at 0.5–1 h intervals. Water loss during the first 2 h was high and variable, presumably due to evaporation of water on the outer surface of the egg; these measurements were omitted from subsequent calculations. Thereafter, water

loss was determined for 4–11 intervals; variation in water loss was random with respect to time. These measurements were averaged to calculate flux ($\dot{M}_{\text{H}_2\text{O}}$).

In a second experiment, the *Chelydra* eggshells used above were cut approximately in half, and these halves were mounted on one end of Plexiglas cylinders (4 cm × 2.5 cm i.d.; 3.1 cm o.d.) using epoxy. The other end of each cylinder was sealed with a rubber stopper. Cylinders were placed in individual desiccators at 24.2 °C with the eggshell end up 2 cm beneath a screen container of dehydrated Drierite, and $\dot{M}_{\text{H}_2\text{O}}$ determined as above by repeated weighings of the cylinder plus eggshell. Cylinders plus eggshells underwent three successive experimental treatments as follows: (1) Cylinder filled with distilled water up to 3 cm below the eggshell. (2) Similar to 1, except that a small paper wick ran from the water to the inner surface of the eggshell. (3) Cylinder and inside of eggshell filled with distilled water. After these measurements, eggshells were decalcified in 5% EDTA while still attached to cylinders, and measurements were repeated.

Eggshell halves not used in the above experiment were stored in distilled H₂O for 8–10 weeks. Shell membrane and the thickness of the mineral layer were unchanged during this period. For measurements of O₂ diffusion, fragments from each of four eggshells were mounted in epoxy on one end of Plexiglas cylinders. Oxygen flux through a shell fragment was measured according to the technique of Wangenstein *et al.* (1970/71). A No. 730 O₂ electrode (Transidyne General Corp., Ann Arbor, MI) was mounted in a smaller cylinder; when the cylinders were nested, the tip of the electrode protruded into the space enclosed by the shell fragment. Hypodermic needles mounted in the inner cylinder allowed flushing of the enclosed space with various gas mixtures. The surface areas of the shell fragments were 1–5 cm²; the enclosed space was approximately 1 cm³. The electrode-bearing cylinder could be removed during experimental manipulations and then replaced in exactly its previous position.

Cylinders bearing eggshell fragments were stored in water-saturated air with water on the inner surface of the eggshell membrane. Prior to the initial determination of O₂ flux, all obvious liquid water was carefully removed from the membrane surface with an absorbent paper towel. The electrode was inserted, the electrode–eggshell assembly was placed in water-saturated air at 25 °C, and the enclosed space was flushed with water-saturated N₂. As oxygen diffused through an eggshell fragment, the subsequent rise in the P_{O₂} in the chamber was recorded on a chart recorder (Linear Instruments, Irvine, CA). The P_{O₂} increased logarithmically with time as in previous reports that employed this protocol (Wangenstein *et al.*, 1970/71; Ackerman and Prange, 1972). The time required for the P_{O₂} in the enclosed space to reach 50% of atmospheric levels ($t_{0.5}$) was calculated from the chart record. After 2–5 determinations of $t_{0.5}$, the electrode was removed and the cylinder bearing the eggshell fragment held in a desiccator containing Drierite for 4 successive 15 min periods. The $t_{0.5}$ was re-measured after each period of desiccation. Afterwards, eggshell fragments were decalcified in EDTA (see above) while still attached to the cylinders, and the entire sequence of $t_{0.5}$ measurements repeated.

An intact and a decalcified eggshell half were placed in the desiccator until completely dry, and rates of water loss calculated from repeated measurements of mass.

Eggshell thicknesses of freshly laid eggs were determined with an ocular micrometer and a dissecting microscope.

The following equations, taken from Wangenstein *et al.* (1970/71), Ar *et al.* (1974), or modified from these authors, were used to calculate permeability and conductance:

$$G_{O_2} = (0.249 \cdot V)/(t_{0.5} \cdot T)$$

$$K_{O_2} = G_{O_2} \cdot A^{-1}$$

$$G_{H_2O} = \dot{M}_{H_2O}/\Delta P_{H_2O}$$

where G_{O_2} = O_2 conductance ($\text{cm}^3 O_2 \text{ STP} \cdot \text{sec}^{-1} \cdot \text{cm}^{-2} \cdot \text{torr}^{-1}$)

K_{O_2} = O_2 permeability ($\text{cm}^3 O_2 \text{ STP} \cdot \text{sec}^{-1} \cdot \text{torr}^{-1}$)

G_{H_2O} = H_2O conductance ($\text{g } H_2O \cdot \text{d}^{-1} \cdot \text{torr}^{-1}$)

V = enclosed volume (cm^3)

$t_{0.5}$ = time required for P_{O_2} in enclosed space to reach 50% of ambient P_{O_2} (sec or min)

A = area of shell (cm^2)

T = absolute temperature ($^{\circ}\text{K}$)

\dot{M}_{H_2O} = rate of water loss ($\text{g} \cdot \text{d}^{-1}$)

ΔP_{H_2O} = difference in vapor pressure across shell (torr)

Resistance to H_2O flux was calculated as $G_{H_2O}^{-1}$ for both intact (membrane plus mineral layer) and decalcified (membrane alone) eggshells. Resistance of the mineral layer alone was calculated by subtraction. The proportion of O_2 resistance due to the membrane was computed as $t_{0.5}$ for decalcified eggshell divided by $t_{0.5}$ for intact eggshell.

Most prior studies (*e.g.*, Wangenstein *et al.*, 1970/71; Ackerman and Prange, 1972) reported O_2 conductance or permeability as calculated from the above equations. We have not calculated the G_{O_2} for each egg because the epoxy used to mount the shell fragments penetrated them laterally to an unknown extent; thus measurements of A and V were only approximate. Instead, we present the O_2 data in terms of $t_{0.5}$, which is inversely proportional to G_{O_2} providing that V , A and T are constant. Because A and V were fixed at mounting and remained constant for individual shell fragments, the $t_{0.5}$ accurately reflects changes in resistance to O_2 diffusion in repeated measurements of the same shell fragment.

Mixed model analyses of variance were used to analyze the effect of experimental treatments and variation among eggs in G_{H_2O} (Snedecor and Cochran, 1967). The procedure of Snedecor and Cochran (1967, p. 320) was employed to replace two missing values for eggshell halves before performing the analysis of variance; degrees of freedom and mean squares were adjusted appropriately before computation of F statistics. The Student–Newman–Keuls procedure was used to distinguish between means at the 0.05 level of significance. Friedman's analysis of variance (Siegel, 1956) was used to examine changes in $t_{0.5}$.

Results

WATER CONDUCTANCE

In measurements of whole eggs, the experimental treatments increased the mean G_{H_2O} by no more than 17% ($\bar{x} = 10\%$) above the G_{H_2O} of control eggs (table 1). This change was not significant. For five eggs for which complete data were available for all six experimental treatments, neither experimental treatment ($F_{(5,20)} = 0.001$; $P = 0.999$) nor variation among eggs ($F_{(4,20)} = 0.001$; $P = 0.999$) significantly affected G_{H_2O} .

An additional one way analysis of variance was performed that included measurements for the five eggs described above and for three additional eggs for which only partial data were available (table 1). Although the results are potentially misleading because this procedure cannot account for individual variation among eggs, the larger sample size in this procedure may increase the likelihood of finding a significant difference among treatments if one exists. Even so, experimental treatment had no significant effect upon the G_{H_2O} of whole eggs according to this analysis ($F_{(5,34)} = 1.144$; $P = 0.36$). In particular, handling and drying of shells between measurements did not decrease the G_{H_2O} .

Freshly laid eggs of *Chelydra serpentina* included a mineral layer that averaged 50% of the total shell thickness. For 5 eggs, the mean total thickness was $360 \mu\text{m} \pm 20 \mu\text{m SE}$.

TABLE 1
Effect of experimental treatments on G_{H_2O} of freshly laid *Chelydra* eggs

	Experimental treatment ^a					
	1	2	3	4	5	6
G_{H_2O} ($\text{g} \cdot \text{d}^{-1} \cdot \text{torr}^{-1}$)						
Complete eggs ^b						
Mean	0.249	0.261	0.289	0.277	0.264	0.264
Standard error	0.037	0.008	0.005	0.016	0.011	0.006
Incomplete and complete eggs ^b						
Mean	0.248	0.269	0.291	0.279	0.265	0.264
Standard error	0.025	0.009	0.004	0.014	0.009	0.006
Sample size	7	8	7	7	6	5

^a (1) Intact egg. (2) Hole in eggshell sealed with paraffin. (3) Egg contents replaced with albumen. (4) Egg contents replaced with H_2O . (5) Egg in moist air overnight and then filled with H_2O . (6) Egg in dry air overnight and then filled with H_2O . See Materials and methods.

^b Eggs were exposed to 6 different treatments. 'Complete eggs' refers to 5 eggs for which data were available for all 6 treatments. 'Incomplete eggs' refers to 3 additional eggs for which only partial data were available.

The experimental treatments, shell hydration and removal of the mineral layer, significantly affected the $G_{\text{H}_2\text{O}}$ of shell halves ($F_{(5,18)} = 72.439$); $P < 10^{-5}$). Shell halves had a greater $G_{\text{H}_2\text{O}}$ when filled with water than when filled with H_2O -saturated

TABLE 2
Effect of internal hydration and decalcification on $G_{\text{H}_2\text{O}}$ of freshly laid *Chelydra* eggshell halves

Treatment ^a :	Intact			Decalcified		
	1	2	3	1	2	3
Sample size ^b	5	4	4	5	5	5
$G_{\text{H}_2\text{O}}$ ($\text{g} \cdot \text{d}^{-1} \cdot \text{torr}^{-1}$)						
Mean	0.023	0.043	0.119	0.101	0.113	0.162
Standard error	0.002	0.005	0.005	0.007	0.006	0.010
Significance ^c						

^a (1) Dry eggshell enclosing H_2O -saturated air. (2) Moist eggshell enclosing H_2O -saturated air. (3) Moist eggshell enclosing H_2O . See Materials and methods.

^b Repeated measurements were made on 5 eggs; values less than 5 indicate that some measurements were missing.

^c Results of Student–Newman–Keuls procedure. Samples not bracketed by the horizontal line differed at the 0.05 level of significance.

TABLE 3
Resistance of the mineral layer and eggshell membrane to gas exchange at various levels of eggshell hydration in eggs of *Chelydra*

Treatment	Resistance ($\text{d} \cdot \text{torr} \cdot \text{g}^{-1}$)			$\frac{R_{\text{membrane}}}{R_{\text{total}}}$
	Mineral layer	Membrane	Total	
$G_{\text{H}_2\text{O}}$:				
Dry shell enclosing H_2O -saturated air ($n = 5$)	32.18 ± 3.24^a	9.59 ± 0.67	41.78 ± 3.15	0.24 ± 0.03
Moist shell enclosing H_2O -saturated air ($n = 4$)	14.38 ± 2.51	8.42 ± 0.55	22.80 ± 2.27	0.39 ± 0.06
Moist shell enclosing liquid H_2O ($n = 4$)	2.29 ± 0.56	5.69 ± 0.38	7.98 ± 0.33	0.72 ± 0.06
G_{O_2}				
0 min desiccation ^b ($n = 3$)				0.76 ± 0.05
15 min desiccation ($n = 4$)				3.73 ± 1.35
30 min desiccation ($n = 4$)				1.27 ± 0.14
45 min desiccation ($n = 4$)				1.22 ± 0.13
60 min desiccation ($n = 4$)				0.72 ± 0.18

^a Values are mean \pm standard error.

^b Omits value for eggshell 'b', fig. 1.

air (table 2). The removal of the mineral layer increased the G_{H_2O} by 69-324% (table 2).

Resistance to H_2O flux of the shell membrane, the mineral layer, and the composite eggshell each decreased with increasing hydration (table 3). Changes in G_{H_2O} were due primarily to changes in the resistance of the mineral layer. Thus, even though membrane resistance varied relatively little, the membrane accounted for a steadily increasing proportion of overall resistance (from 24% to 72%) with increasing hydration.

OXYGEN CONDUCTANCE

For the half shells dried in conjunction with the G_{O_2} measurements, the decalcified shell lost water at proportionately higher rates than the intact shell (fig. 1). The wet

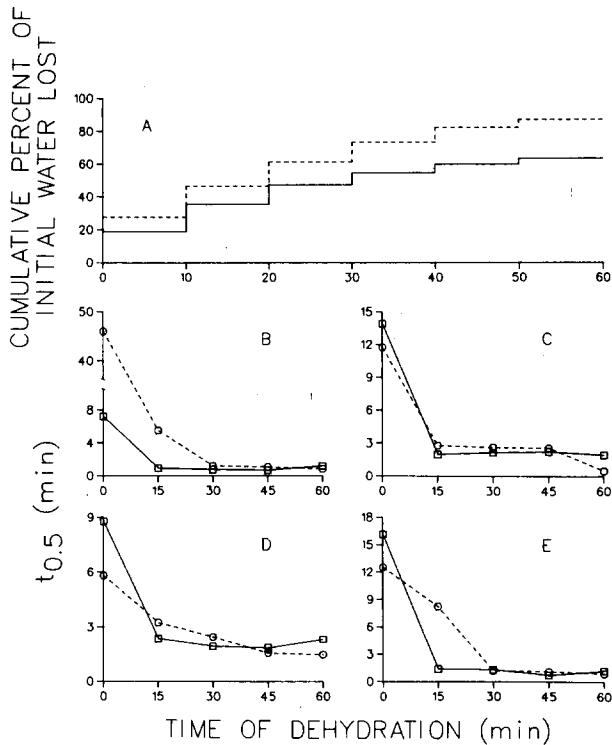


Fig. 1. Effect of dehydration on rates of water loss and $t_{0.5}$ of O_2 diffusion in intact and decalcified fragments of freshly laid *Chelydra* eggs. The $t_{0.5}$ is inversely proportional to G_{O_2} and directly proportional to resistance to diffusion. A: Cumulative water loss in an intact (solid line) and decalcified (broken line) eggshell fragment. Mass of water and total wet mass are provided in text. B-E: Effect of dehydration on $t_{0.5}$ of shell fragments from 4 separate eggs. Each graph is for the same shell fragment measured multiple times; each point is the mean of 2-5 determinations of $t_{0.5}$. Decalcified and intact eggshells are signified as in A. Thickness of intact eggshell (μm : eggshell membrane, mineral layer): B, 145, 170; C, 135, 140; D, 155, 140; E, 170, 170.

mass of the decalcified fragment (261 mg) was 56% water, and the wet mass of the intact fragment (719 mg) was 22% water.

In general, dehydration reduced the $t_{0.5}$ (and hence increased the O_2 conductance) of shell fragments (fig. 1). The effect of dehydration on the $t_{0.5}$ was similar in fragments with and without a mineral layer, and was significant in both cases (Friedman's analysis of variance, 4 df; $\chi^2 = 10$, $P < 0.05$ for shells with a mineral layer; $\chi^2 = 16$, $P < 0.01$ for shells lacking a mineral layer). The decline in the $t_{0.5}$ in general resembled the decline in water loss from other shell fragments under similar conditions (fig. 1a).

Two aspects of the change in the $t_{0.5}$ were unexpected. Removal of the mineral layer did not always decrease the $t_{0.5}$; in fact, it frequently increased the $t_{0.5}$. In 12 of 20 trials, the $t_{0.5}$ of shell fragments lacking a mineral layer was greater than the $t_{0.5}$ of fragments with an intact mineral layer (fig. 1). Hence, the membrane alone often had a greater resistance to O_2 diffusion than the mineral layer plus membrane, and the resistance of the membrane alone never averaged less than 72% of the resistance of the intact shell (table 3). A second unexpected aspect was that the $t_{0.5}$ of 3 out of 4 fragments with a mineral layer present increased between 45 and 60 min of dehydration (fig. 1). This increase was evident in none of the fragments that lacked a mineral layer.

Discussion

CRITIQUE OF METHODS

Our procedures included the decalcification and the repeated dehydration or hydration of eggshells. The measurements of G_{H_2O} for whole eggs suggest that these experimental manipulations affected conductance negligibly. In the measurements of whole eggs, the replacement of egg content and drying did not alter the rates of water loss significantly; even the application of paraffin to the shell did not decrease the G_{H_2O} . Taigen *et al.* (1978) have reported similar results for avian eggs.

Given the difficulties in measuring A and V (see Materials and methods), we can say little about variation in conductance among eggs. For example, differences in $t_{0.5}$ among the shell fragments (fig. 1) may have been due to actual differences in K_{O_2} or only to differences in the exposed surface area. Our primary focus, however, was on differences *among* experimental treatment *within* individual eggshells.

The measurements of G_{H_2O} were done in still air. The G_{H_2O} of *Chelydra* eggshell probably is sufficiently high to result in a boundary layer that offers significant resistance to diffusion of water (Tracy and Sotherland, 1979; Packard *et al.*, 1979). Thus our measurements may underestimate changes in eggshell G_{H_2O} due to experimental treatments, but nonetheless are indicative of changes in total G_{H_2O} (eggshell plus boundary layer). Also, air is probably still in the natural nest environment (Ackerman, 1977).

Eggshells of 4 eggs of *Chelydra* after 5 days incubation on damp peatmoss contained 28–44% H₂O by mass (G. C. Packard, pers. comm.). These values are higher than for our wet eggshell fragment, 22%.

COMPARATIVE AND ENVIRONMENTAL ASPECTS

Packard and Packard (1980) and Packard (1980) have reviewed the distinctive features of flexible-shelled eggs of turtles as compared to avian eggs; our results are in substantial agreement with their conclusions. Measures of G_{H_2O} (tables 1, 2) are consistent with the literature (Packard *et al.*, 1979; Thompson, 1981) and are much greater than expected for avian eggs of the same size (Packard *et al.*, 1979). Assuming average values of 1.54 cm² for A and 1 cm³ for V and using the mean $t_{0.5}$ from fig. 1, we calculate a K_{O_2} of 2.07×10^{-6} cm³ · sec⁻¹ · cm⁻² · torr⁻¹ for intact moist shells and 1.69×10^{-5} cm³ · sec⁻¹ · cm⁻² · torr⁻¹ for intact dry shells of freshly laid *Chelydra* eggs. These values of K_{O_2} are consistent with the literature in that they bracket the range of K_{O_2} reported for other turtle eggshells (Ackerman and Prange, 1972; Thompson, 1981) and are not nearly so different from avian levels as are values for G_{H_2O} . Thus water and O₂ permeabilities are not coupled in flexible eggs of turtles in the same way as in avian eggs (Ackerman, 1980). The relatively high K_{H_2O} of turtle eggs is thought to be correlated with the humid environment within turtle nests; the lower relative K_{O_2} may reflect the lower temperatures and O₂ demands of reptilian embryos (Ackerman, 1980).

Another difference between avian eggs and flexible eggs of turtles is in the proportional contribution of the shell membrane to overall shell thickness. In the chicken egg, the inner and outer shell membranes may be 55–90 μm thick and the mineral shell 250–350 μm thick (Kutchai and Steen, 1970/71); hence the membranes represent 11–30% of the shell thickness. In *Chelydra* eggs, the mineral layer and the membrane were each 180 μm thick. These differences are reflected in the relative resistance to O₂ diffusion in the shell membrane and mineral layer. The resistance due to the membranes was greater than 72% of total resistance to O₂ diffusion in eggs of the snapping turtle (table 3), and ranged between 0% and 50% in chicken eggs (Kutchai and Steen, 1970/71; Wangensteen *et al.*, 1970/71; Kayar *et al.*, 1981). The membrane is equally significant in resisting H₂O loss from hydrated eggs of the snapping turtle (table 3); no comparable data are available for avian eggs.

Late in incubation, the proportional resistance of the mineral layer to O₂ diffusion probably declines in both avian eggs and flexible eggs of turtles. In the chicken egg, the resistance of the mineral layer plus the outer shell membrane decreases to 36% of total (Piiper *et al.*, 1980). In the eggs of snapping turtles, the mineral layer exfoliates during incubation (Packard, 1980) and presumably comes to account for a miniscule resistance to diffusion. We suggest that exfoliation of the mineral layer during incubation of flexible eggs of turtles may augment O₂ supply to the embryo in the same manner as internal pipping in avian eggs (Rahn *et al.*, 1979).

POSSIBLE MECHANISMS OF GAS EXCHANGE LIMITATION

Certain interactions among the mineral layer, the membrane, and shell hydration were unexpected and complex. For example, shell halves enclosing H₂O showed a greater $G_{\text{H}_2\text{O}}$ than shell halves enclosing H₂O-saturated air (table 2), even though the vapor pressure gradient across the eggshell was presumably identical in both cases (Lomholt, 1976; Taigen *et al.*, 1978; Tracy *et al.*, 1978). After the mineral layer was removed, the $t_{0.5}$ of O₂ diffusion remained high and was sometimes greater than when the mineral layer was present. Eggshells had lower $G_{\text{H}_2\text{O}}$ when the mineral layer was present than when it was removed.

We speculate that two phenomena may account for these unusual results: bulk flow of H₂O through the eggshell and mechanical alterations of pore size and shape. A complex array of fibers comprises the eggshell membrane (Packard, 1980); spaces between the fibers (*i.e.*, pores) are small (<4 μm , see fig. 16 in Packard, 1980). Pores this small exert sufficient capillary pressure to raise a column of water to a height many times the thickness of the eggshell ($h_c = 741$ cm assuming a contact angle of 0°; see Hillel, 1980) with a negligible reduction in vapor pressure. Even the larger pores that traverse the mineral layer (ca. 100 μm diameter, see figs. 3–4 in Packard, 1980, and fig. 1c of Packard and Packard, 1980) can sustain a column of water equal to eggshell thickness. Hence capillarity of pores may diminish the effective thickness of the membrane by leading liquid water to the external surface of the eggshell, and thereby may increase $G_{\text{H}_2\text{O}}$. Furthermore, the structure of the membrane (Packard, 1980) might permit capillary transport of liquid circumferentially as well as radially; *i.e.*, the inner surface of the eggshell membrane need contact only a small area of liquid to saturate the membrane (*cf.* differences between treatment 1 and 2, table 2). In liquid-filled eggs, the hydrostatic pressure is added to the capillary pressure in all but the uppermost portions of the egg (*cf.* differences between treatments 2 and 3, table 2).

Kutchai and Steen (1970/71), Wangensteen *et al.* (1970/71), Lomholt (1976) and Kayar *et al.* (1981) have suggested previously that the filling of pores with liquid reduces G_{O_2} , principally because the diffusion of O₂ is less rapid through liquid than through air. The diminution in the $t_{0.5}$ of O₂ diffusion in both turtle eggs (fig. 1) and chicken eggs (Kayar *et al.*, 1981) with drying of the eggs is consistent with this proposal, as are differences in reports of K_{O_2} among studies in which avian shell membranes were allowed to dry to different extents (Kayar *et al.*, 1981). In the eggs of snapping turtles, the G_{O_2} in general was inversely related to $G_{\text{H}_2\text{O}}$ (fig. 1, table 2). Thus flexible eggshells may undergo major changes in both G_{O_2} and $G_{\text{H}_2\text{O}}$ as a function of egg hydration. The Packards and their co-workers have gathered extensive laboratory data and limited field evidence that flexible eggs vary considerably in hydration as a function of external conditions. Moreover, Kutchai and Steen (1970/71), Lomholt (1976), and Tullett and Board (1976) have suggested that changes in internal conditions (colloid osmotic pressure) result in withdrawal of liquid water from membrane pores in avian eggs. It is conceivable that similar phenomena might occur in turtle eggs.

Alternatively, changes in the size and shape of pores may account for changes in conductance with dehydration. As flexible eggs of turtles gain or lose water, they undergo significant changes in mass, volume, and shape (Tracy *et al.*, 1978; Packard, 1980; Packard *et al.*, 1980, 1981a,b). Although configuration of pores in flexible-shelled eggs has not been examined in relation to eggshell hydration, it seems likely that pores in both the mineral layer (Packard, 1980) and the eggshell membrane alter in shape with the hydric state of the egg. Tullett and Board (1976) have described decreases in the size of the pores in the membrane associated with wetting of the inner shell membrane in the chicken egg. Given that conductance is in part a function of pore geometry (Board and Scott, 1980), changes in pore shape, length, and diameter should modify conductance. Moreover, apposition of a relatively rigid layer (the mineral layer) and a compliant layer (the membrane) may enable the compliant layer to resist changes in pore deformation associated with hydration or dehydration.

The changes in G_{O_2} during eggshell dehydration (fig. 1) were also consistent with these proposals. The increase in $t_{0.5}$ at 60 min dehydration may, for example, have resulted from the closing of pores as the shell dried. When the mineral layer was removed from shell fragments, the resistance of the shell to O_2 diffusion remained high and was frequently greater than the resistance of the shell when the mineral layer was present. This anomalous result may have stemmed from adjacent pillars in the mineral layer anchoring pores in the membrane in an open conformation; when the mineral layer was removed, the pores may have diminished. By contrast, dispersal of adjacent pillars as a flexible egg hydrates may account for its ability to swell (Packard, 1980).

In egg capsules of three congeneric species of gastropods (*Thais*), K_{H_2O} and wall thickness are positively related, but both K_{H_2O} and thickness are negatively related to wall stiffness (T. Daniel and J. Pechenik, pers. comm.). Daniel and Pechenik propose that the greater stiffness of the thin walls necessitates greater pressure to induce deformation as an egg capsule dries; this pressure may reduce the water potential of the egg capsule, and thus may reduce \dot{M}_{H_2O} and G_{H_2O} . Furthermore, the stiffness of a snail egg capsule is proportional to its osmotic pressure, which increases as a capsule dehydrates. Thus any initial dehydration should result in a compensatory reduction in permeability to water loss. Such mechanisms may also limit the conductance of flexible eggshells of turtles, which deform as a turtle egg dehydrates (Packard *et al.*, 1981a); however, the magnitude of these forces and their effect on water potential and G_{H_2O} of turtle eggs await measurement.

Other mechanisms may also account for the observed patterns. For example, movement of liquid out of membrane pores, its adsorption onto calcareous surfaces (Hillel, 1980), and its subsequent evaporation may account for increases in G_{O_2} as membranes dry. Indeed, our observations are too few to establish the relative importance (if any) of bulk flow and pore deformation in regulating conductance. Nonetheless, patterns of gas exchange through flexible eggshells of turtle eggs are clearly more complex than indicated in the early diffusion models proposed for avian eggs (Wangensteen *et al.*, 1970/71; Rahn *et al.*, 1979), and invite further study.

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References

- Ackerman, R. A. and H. Prange (1972). Oxygen diffusion across a sea turtle (*Chelonia mydas*) eggshell. *Comp. Biochem. Physiol.* 43A: 905-909.
- Ackerman, R. A. (1977). The respiratory gas exchange of sea turtle nests (*Chelonia*, *Caretta*). *Respir. Physiol.* 31: 19-38.
- Ackerman, R. A. (1980). Physiological and ecological aspects of gas exchange by sea turtle eggs. *Am. Zool.* 20: 575-583.
- Ar, A., C. V. Paganelli, R. B. Reeves, D. G. Greene and H. Rahn (1974). The avian egg: water vapor conductance, shell thickness and functional pore area. *Condor* 76: 153-158.
- Board, R. G. and V. D. Scott (1980). Porosity of the avian eggshell. *Am. Zool.* 20: 339-349.
- Hillel, D. (1980). *Fundamentals of Soil Physics*. New York, Academic Press, 413 p.
- Kayar, S. R., G. K. Snyder, G. F. Birchard and C. P. Black (1981). Oxygen permeability of the shell and membranes of chicken eggs during development. *Respir. Physiol.* 46: 209-221.
- Kutchai, H. and J. B. Steen (1970/71). Permeability of the shell and shell membranes of hen's eggs during development. *Respir. Physiol.* 11: 265-278.
- Lomholt, J. P. (1976). The development of the oxygen permeability of the avian eggshell and its membranes during incubation. *J. Exp. Zool.* 198: 177-184.
- Packard, G. C., T. L. Taigen, M. J. Packard and R. D. Shuman (1979). Water vapor conductance of testudinian and crocodylian eggs. *Respir. Physiol.* 38: 1-10.
- Packard, M. J. (1980). Ultrastructural morphology of the shell and shell membrane of eggs of common snapping turtles (*Chelydra serpentina*). *J. Morphol.* 165: 187-204.
- Packard, G. C. and M. J. Packard (1980). Evolution of the cleidoic egg among reptilian antecedents of birds. *Am. Zool.* 20: 351-362.
- Packard, G. C., T. L. Taigen, M. J. Packard and T. J. Boardman (1980). Water relations of pliable-shelled eggs of common snapping turtles (*Chelydra serpentina*). *Can. J. Zool.* 58: 1404-1411.
- Packard, G. C., M. J. Packard and T. J. Boardman (1981a). Patterns and possible significance of water exchange by flexible-shelled eggs of painted turtles (*Chrysemys picta*). *Physiol. Zool.* 54: 165-178.
- Packard, G. C., M. J. Packard, T. J. Boardman and M. D. Ashen (1981b). Possible adaptive value of water exchanges in flexible-shelled eggs of turtles. *Science* 213: 471-473.
- Piiper, J., H. Tazawa, A. Ar and H. Rahn (1980). Analysis of chorioallantoic gas exchange in the chick embryo. *Respir. Physiol.* 39: 273-284.
- Rahn, H., A. Ar and C. V. Paganelli (1979). How bird eggs breathe. *Sci. Am.* 240: 46-55.
- Siegel, S. (1956). *Nonparametric Statistics for the Behavioral Sciences*. New York, McGraw-Hill Book Company, 312 p.
- Snedecor, G. W. and W. G. Cochran (1967). *Statistical Methods*, 6th Edn. Ames, The Iowa State University Press.
- Solomon, S. E. and T. Baird (1976). Studies on the eggshell (oviducal and oviposited) of *Chelonia mydas*. *J. Exp. Mar. Biol. Ecol.* 22: 145-160.

- Taigen, T. L., G. C. Packard, P. R. Sotherland and L. R. Hanka (1978). Influence of solute concentration in albumen on water loss from avian eggs. *Auk* 95: 422-424.
- Thompson, M. (1981). Gas tensions in natural nests and eggs of the tortoise *Emydura macquarii*. In: Proceedings of the Melbourne Herpetological Symposium, edited by C. B. Banks and A. A. Martin. Parkville, Victoria, Australia, The Zoological Board of Victoria, pp. 74-77.
- Tracy, C. R., G. C. Packard and M. J. Packard (1978). Water relations of chelonian eggs. *Physiol. Zool.* 51: 378-387.
- Tracy, C. R. and P. R. Sotherland (1979). Boundary layers of bird eggs: do they ever constitute a significant barrier to water loss? *Physiol. Zool.* 52: 63-66.
- Tullett, S. G. and R. G. Board (1976). Oxygen flux across the integument of the avian egg during incubation. *Br. Poult. Sci.* 17: 441-450.
- Wangensteen, O. D., D. Wilson and H. Rahn (1970/71). Diffusion of gases across the shell of the hen's egg. *Respir. Physiol.* 11: 16-30.