EFFECT OF EGGSHELL THINNING ON WATER VAPOR CONDUCTANCE OF MALLEEFOWL EGGS[1]

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Abstract. The water vapor conductance of eggs of mound-nesting Malleefowl (*Leipoa ocellata*) increases about three-fold during the 60-day incubation period. As the embryo absorbs calcium from the inside of the eggshell, mainly from the shoulder and equator, the pore length decreases about 21% and the narrowest part of each pore is lost. Because the inner 21% of a pore accounts for about 85% of the resistance to gas diffusion in unincubated eggs, its loss greatly increases conductance. The increase in conductance of Malleefowl eggs is greater than that recorded for eggs of other birds, and appears to be a requirement for successful gas exchange in an underground environment.

Key words: Conductance; eggshell; mass loss; water vapor.

INTRODUCTION

Avian embryos exchange respiratory gases and water vapor by diffusion through pores in the eggshell (Wagensteen and Rahn 1970–1971, Wagensteen et al. 1970–1971, Rahn and Paganelli 1982). The pores and underlying shell membranes form a resistance to gas exchange, the reciprocal of which is termed "conductance." The conductance of an eggshell depends on the number, surface area, and length of pores (Ar et al. 1974). The boundary layer over the surface of the egg is a negligible component of overall conductance (Spotila et al. 1981). The number and structure of pores are fixed when the eggshell is formed. Because pore structure does not change much during incubation in domestic fowl, *Gallus domesticus*, eggs (Simkiss 1961), it has been generally assumed that conductance of an egg remains essentially constant throughout incubation (Rahn et al. 1974; Visschedijk and Rahn

1981, 1983; Rahn and Paganelli 1982). Constant conductance should result in a constant rate of water vapor loss throughout incubation (Ar et al. 1974, Rahn and Ar 1974). However, some studies have found the rate of mass loss to increase as incubation proceeds (Kendeigh 1940; Carey 1979; Sotherland et al. 1980; Grant et al. 1982; Seymour et al., in press). This increase has been attributed to changes in shell conductance, egg temperature, and ambient humidity. The mechanism that causes conductance to increase during incubation is unknown.

During development, the avian embryo uses the eggshell as a source of calcium for the ossification of the developing skeleton (Romanoff 1967, Packard and Packard 1984). The removal of calcium results in a thinning of the eggshell (Vanderstoep and Richards 1969, Kreitzer 1972, Sotherland et al. 1980, Finnlund et al. 1985). Eggshell thinning in domestic fowl occurs by absorption of calcium from the ends of mammillary cores (the mammillary knobs), and does not greatly affect pore length (Simkiss 1961). However, this may not be true in all species.

Malleefowl, *Leipoa ocellata,* are megapode birds which incubate their eggs in mounds con-

structed from sand and decaying vegetation. The underground nesting environment provides incubation conditions different from those of other avian eggs. Oxygen tensions are lower, carbon dioxide tensions are higher, and the atmosphere is high in humidity (Seymour and Ackerman 1980; Seymour et al. 1986, in press). These environmental factors select for a high eggshell conductance which facilitates oxygen and carbon dioxide exchange without the danger of excessive water loss (Seymour and Rahn 1978, Seymour 1985). In previous studies (Seymour et al., in press), we discovered that the rate of mass loss in megapode eggs increased up to five times that measured just after laying. Because these changes could be fully accounted for only by large changes in shell conductance, we measured the water vapor conductance of fragments of Malleefowl shells before and after incubation.

MATERIALS AND METHODS

SOURCE OF EGGS

Malleefowl eggs were collected under South Australia National Parks and Wildlife permits from natural mounds at Calperum Station, near Renmark, South Australia. Incidental to other studies, a collection of shell fragments of undeveloped, infertile, and hatched eggs was made. The eggs had been incubated in the laboratory at a constant temperature of 34°C. They were buried in boxes of natural mound sand that were kept at a high humidity to retard evaporation of water from the sand.

WATER VAPOR CONDUCTANCE

Water vapor conductance (G_{H_2O}) was measured on a series of shell fragments from infertile eggs and from eggs at hatching. The fragments were boiled for 20 min in 5% NaOH to remove organic material, including shell membranes (Tyler and Greake 1953). Fragments from the pole, shoulder, and equator of each egg were rinsed, dried, and sealed to the tops of 30-ml glass vials with a hot glue applicator (Bostik). The surface area of the shell fragments was 4.5 cm². The vials contained 8 ml of water and a wad of cotton. The seals were inspected for holes and the vials placed in a desiccator containing silica gel at 34°C overnight. Then they were transferred with gloved hands to an electronic balance (Mettler AE 163), weighed to 0.01 mg, replaced in the desiccator for 3 to 5 days, and reweighed. G_{H_2O} per m² was

calculated from mass loss and an internal P_{H_2O} of 39.9 torr and an external P_{H_2O} of 0 torr.

Water vapor loss ($\dot{M}_{H_2O}$) was measured in three infertile eggs and in three fertile eggs that contained advanced, but dead, embryos. Because these three pairs of eggs were taken from the same mounds, they were assumed to have been laid by the same hens. These eggs were removed from the sand daily, brushed free of sand, and weighed to 1 mg on an electronic balance (Sartorius 12645 MP). Because the eggs were neither living nor rotten, mass loss was assumed to be due to water vapor only.

EGGSHELL THICKNESS

Eggshell thickness was measured in representative fragments with a micrometer (Mitutoyo 0–25 mm) fitted with a curved surface on one jaw. These measurements were confirmed by measuring scanning electron micrographs of casts of fragments from the same eggs.

EGGSHELL STRUCTURE

Shell fragments of undeveloped and hatched eggs treated with NaOH (as above) were coated with 15 nm of carbon and 20 nm of gold-palladium, and the inside and outside surfaces viewed with a scanning electron microscope (ETEC Autoscan). Other fragments of the same eggs were treated with NaOH, placed in Spurr's medium (R1032 Agar Aides, U.K.) under vacuum for 24 hr and then cured. After curing, the imbedded shell was cut radially and the block placed in concentrated HCl which dissolved the calcium carbonate, leaving a cast of the pores in the block. Casts were rinsed, dried in a desiccator, coated with carbon (15 nm) and gold-palladium (20 nm), and viewed with the scanning electron microscope.

RESULTS

WATER VAPOR CONDUCTANCE

G_{H_2O} of shell fragments from the pole were 24% greater than for fragments from the shoulder or equator in unincubated eggs (Table 1). G_{H_2O} of shell fragments from the shoulder and equator increased significantly during incubation by factors of 4.1 and 4.3, respectively, while G_{H_2O} of those from the poles did not increase significantly. Thus, by the end of incubation, mean G_{H_2O} at the shoulder and equator was approximately 1.8 times higher than at the poles. Assuming that each region represented a third of

TABLE 1. Water vapor conductance and thickness of fragments of Malleefowl eggshell, before and after incubation. Pole, shoulder, and equator indicate regions of the shell.

		Conductance			
	Pole	Shoulder [mg/(d·torr·cm²)]	Equator	Whole egg[a] [mg/(d·torr)]	Thickness (µm)
Before					
Mean	0.156	0.125	0.126	20.2	279
SD	0.072	0.059	0.075	8.5	21
n[b]	5	4	5	5	8
After					
Mean	0.291	0.506	0.544	64.2	221
SD	0.161	0.257	0.211	25.7	14
n	7	10	7	9	8
Difference					
Mean	0.135	0.382	0.418	44.0	58
t[c]	1.96	4.41	4.83	3.56	6.50
P	0.11	<0.01	<0.01	<0.01	<0.001

[a] Conductance of whole egg calculated assuming a 170-g egg which has a surface area of 144.9 cm² (Paganelli et al. 1974) and that each part of the egg contributes equally to overall conductance. In one egg before incubation and in two after, both shoulder and equator fragments were not available so conductance of shoulder and equator were assumed equal.
[b] n = Number of eggs.
[c] t-test for unequal sample size and unequal variance (Sokal and Rohlf 1969).

the entire shell, an estimate of whole egg G_{H_2O} was made. Whole egg G_{H_2O} increased approximately threefold from 20.2 mg/(d·torr) to 64.2 mg/(d·torr) during incubation (Table 1).

The rate of water loss was approximately three times higher in three developed, but dead, eggs than in three infertile eggs taken from the same mounds (Table 2).

EGGSHELL THICKNESS

Shell thickness was measured, without reference to site, in eight unincubated eggs (119 fragments) and in eight hatched eggs (81 fragments). Incubation resulted in a 21% decrease in shell thickness (Table 1).

EGGSHELL STRUCTURE

We could not detect any changes in the structure of the outside surface during incubation, but the inside surface changed greatly. The inside of unincubated eggs showed a rough field of mammillary knobs with interspersed pores where the columns fail to meet (Fig. 1A), a structure typical of unincubated avian eggs in general (Tyler 1969). The tops of the knobs sometimes showed impressions of the fibers of the outer shell membrane. After hatching, the inner surface had assumed a homogeneous etched appearance, and the pore openings became more obvious (Fig. 1B). There was no evidence of mammillary knobs.

Casts of unincubated eggs revealed pores having multiple openings to the outside surface (Fig.

1C). Wide gas spaces in the outer and middle layers of the shell gradually narrowed into a single thin opening near the bases of the mammillary knobs. There was no evidence of pores which did not completely pierce the shell.

Casts of hatched eggs showed that the multi-branched pore structure near the outside surface retained its structure during incubation, but the narrow segment of the single pore canal was considerably shortened (Fig. 1D).

Shell thickness, measured from casts of 11 fragments from five unincubated eggs, averaged 274 µm. In 22 fragments from eight hatched eggs, the mean thickness was 205 µm. These values are similar to those measured on the same eggs with the micrometer (Table 1).

DISCUSSION

Measurements of G_{H_2O} have classically been made by the method of Ar et al. (1974) in which whole eggs are placed in desiccators at zero humidity

TABLE 2. Rates of mass loss of infertile and developed but dead Malleefowl eggs in sand at 34°C.

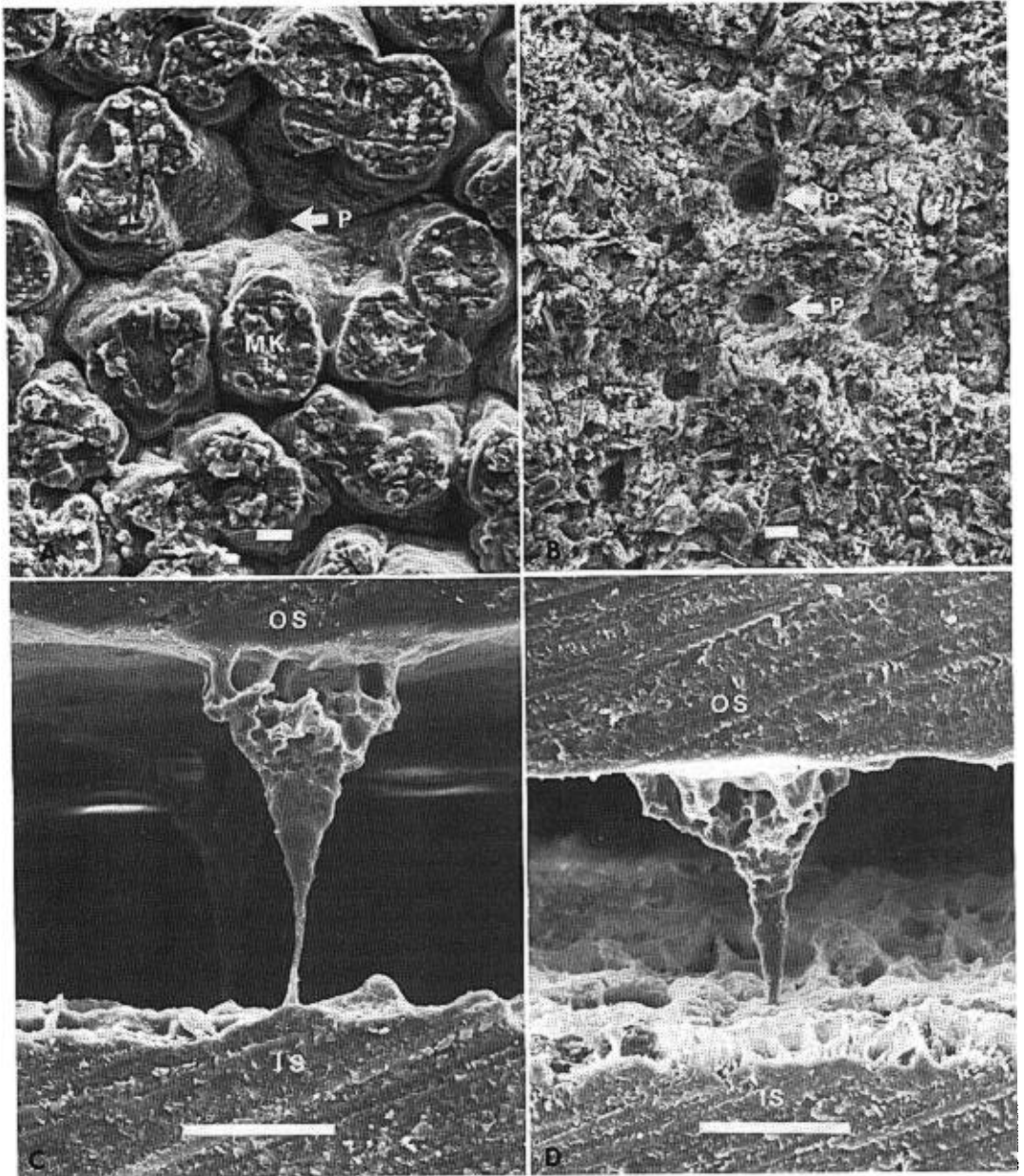| Mound no.	Mass loss (mg/day)		Ratio developed/ infertile
	Infertile	Developed	
40	178	521	2.92
17	240	984	4.09
6	252	479	1.90
Mean	223	661	2.97

FIGURE 1.   A. Scanning electron micrographs ($\times 300$) of inside surface of Malleefowl eggshell before incubation. B. The same, after incubation. MK = mammillary knobs, P = pores. Scale bar = 10 μm. C. SEM ($\times 150$) of Spurr's casts of pores in unincubated eggshell. D. The same, after incubation. OS = outside surface, IS = inside surface. Scale bar = 100 μm.

and their mass loss measured with time. By measuring G_{H_2O} of eggshell fragments from hatched eggs we were able to estimate G_{H_2O} of a full-term Malleefowl egg. Our estimate of G_{H_2O} for a 170-g unincubated egg [20.2 mg/(d·torr) at 34°C, Table 1] is close to the reported value measured by the standard method [21.4 mg/(d·torr) at 25°C = 21.7 mg/(d·torr) at 34°C; Seymour and Ackerman 1980]. This observation indicates that an accurate estimate of whole egg G_{H_2O} is possible using shell fragments. Furthermore, the observed threefold increase in $\dot{M}_{H_2O}$ of advanced but dead

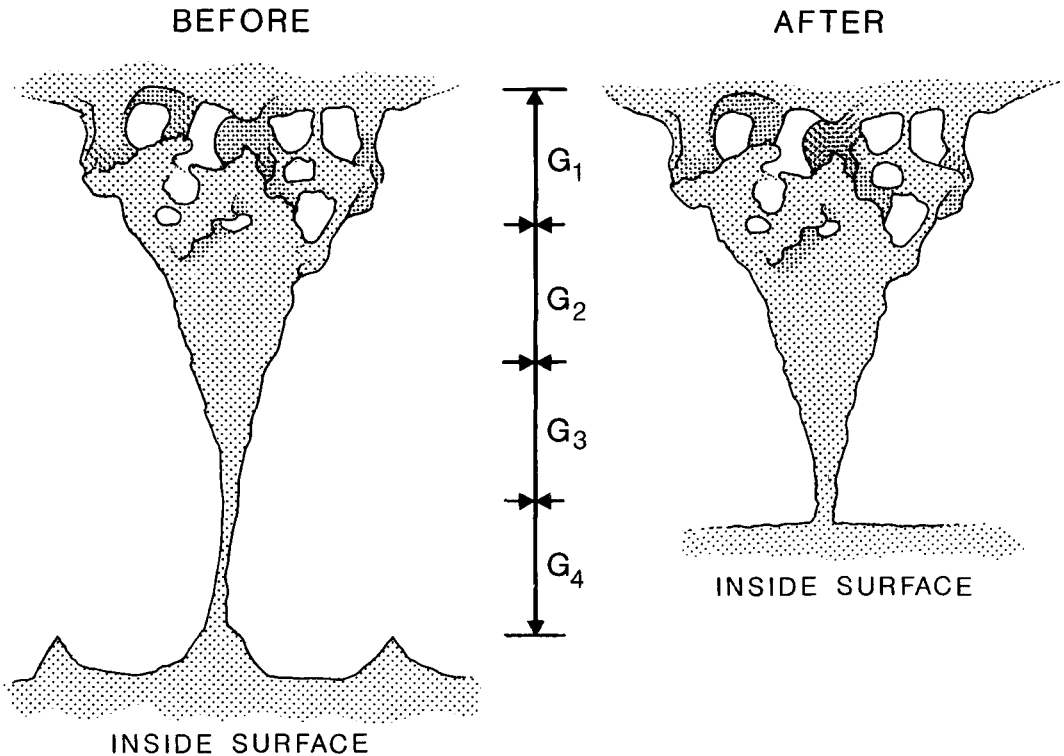## BEFORE   AFTER

FIGURE 2. Schematic diagram of a pore before and after incubation, showing arbitrary divisions into four segments of different relative gas conductances.

eggs compared to infertile ones incubated under identical conditions (Table 2) can only be explained by a threefold increase in G_{H_2O}. This is in agreement with the increase measured in shell fragments.

A large increase in $\dot{M}_{H_2O}$ occurs over the last third of incubation in Malleefowl eggs (Seymour et al., in press), so the observed increase in G_{H_2O} must also occur during this period. The change in G_{H_2O} over the last weeks of incubation is caused by progressive absorption of calcium from the eggshell and consequent thinning of the eggshell and shortening of the pores. A 21% thinning of the eggshell can account for a 27% increase in conductance if, as is usually assumed, the pores are straight tubes of uniform diameter. However, the resistance to gas diffusion in funnel-shaped pores is not uniform and the narrow end accounts for most of the resistance (Tøien et al. 1987). Malleefowl pores have relatively large funnel-shaped structures opening to the outside surface. The mouth of the funnel is partially plugged with inorganic material (Board et al. 1982) so that the gas diffusion path is made up of branching sub-

channels within the funnel (Fig. 1C). The structure of the outside of the pore remains constant throughout incubation (Fig. 1D), but, when the inside region is eroded away by the embryo, the largest proportion of the total diffusion resistance is removed and gas conductance greatly increases.

The increase in G_{H_2O} in the shoulder and equator regions compared to the pole region (Table 1) suggests that shell thinning is not uniform around the egg. We cannot suggest why this is so. It does not result from unequal distribution of the chorioallantois due to formation of an air-cell at one end of the egg. In some birds, calcium absorption does not occur over the air-cell (Simkiss 1975), but in megapodes the shell membranes do not divide and an air-cell does not form (Baltin 1969, Seymour and Ackerman 1980).

To analyze the effect of shell thinning further, the pore can be divided arbitrarily into a series of segments (Fig. 2), each of which has a resistance that can be calculated from its length and diameter. The conductance of each segment is

proportional to its average cross-sectional area divided by its length (Tøien et al. 1987). Conductance is the inverse of diffusion resistance, and total resistance of a pore is equal to the sum of the resistances of all segments: $1/G_{tot} = 1/G_1 + 1/G_2 + 1/G_3 + 1/G_4$. As an example, G_{tot} for the pore shown in Figure 1C was calculated and then recalculated assuming that pore length is shortened by 21% during incubation (Table 2, Fig. 2). In this case, G_{tot} increases by a factor of 3.5. Other pores yielded increases of between 2.7 and 3.9. These rough estimates are weakened by our inability to measure effective radius in the plugged segments of the pores accurately. They nonetheless indicate that conductance increases measured in shell fragments are reasonably well explained by erosion of the bottom of pores.

Previously reported increases of G_{H_2O} of avian eggs during incubation have been of the order of 40 to 100% (Carey 1979, Hanka et al. 1979, Sotherland et al. 1980, Birchard and Kilgore 1980, Rahn et al. 1983, Kern 1986) and occur early in incubation (Carey 1983, Rahn et al. 1983, Kern 1986). The mechanism of this increase is unknown, but it is unlikely to be caused by eggshell thinning as this process occurs later in incubation (Carey 1983). The much larger increase in G_{H_2O} of Malleefowl eggs occurs late in incubation and is clearly related to eggshell thinning.

In conclusion, the conductance changes in megapode eggs facilitate exchange of respiratory gases in the incubation mound where the atmosphere is low in oxygen and high in carbon dioxide. Seymour et al. (1986) showed that the respiratory gas tensions inside buried Malleefowl and Brush Turkey eggs are near the means from normal birds (Paganelli and Rahn 1984), despite the abnormal environment. Furthermore, an initially low conductance in Malleefowl eggs is important as well. If it were high throughout incubation, the eggs would lose about 26% of their initial mass by evaporation which may not be tolerated (Seymour et al., in press).

ACKNOWLEDGMENTS

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