Changes in eggshell thickness, shell conductance and pore density during incubation in the Peking Duck (*Anas platyrhynchos* **f.** *dom*.)

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Shell thickness, shell conductance (water-vapour conductance), and pore density are important properties of eggs that influence the success of embryonic development. We determined these properties in the blunt end, the equator and the sharp end of both unincubated and hatched eggs of Peking ducks. In unincubated eggs, we found that shell thickness increased from the blunt end to the sharp end, water-vapour conductance was the highest in the blunt region and lowest in the equatorial region. Analysis of hatched eggs showed that during incubation the equatorial and sharp end regions became thinner (due to calcium uptake by the embryo), resulting in nearly similar eggshell thickness in all regions after hatching. Pore density and shell conductance, however, increased during incubation both in the equator and blunt end. Our results suggest that the increase in shell conductance during incubation is in this species due to an increase in pore density and not due to thinning of the eggshell.

1. Introduction

The success of embryonic development in birds is related to functional and structural properties of the eggshell (Burton & Tullett 1983, Thompson & Goldie 1990, Soliman *et al.* 1994, Wagner-Amos & Seymour 2002, 2003, Nys *et al.* 2004, Massaro & Davis 2005). All gas exchange between the embryo and external environment in the avian egg occurs by diffusion through thousands of microscopic pores in the chorioallantoic membrane (Wangensteen & Rahn 1970–71, Booth & Seymour 1987, Rokitka & Rahn 1987, Stadelman 2000, Wagner-Amos & Seymour 2002, 2003, Nys *et al.* 2004, Tsai *et al.* in press). The resistance that these pores offer to diffusive gas transport is measured in terms of the shell conductance and is conveniently expressed in terms of the water vapour conductance, $[G_{H_2O} = mgH_2O / (d.Torr)]$ (Wangensteen & Rahn 1970–71, Rokitka & Rahn 1987, Booth & Seymour 1987, Wagner-Amos & Seymour 2003).

In the literature, there are various studies about the regional changes in shell thickness, shell conductance, and pore number during, before and after incubation in avian eggs. In some studies, it has

been assumed that shell conductance and pore density are equally distributed over the entire eggshell surface and remain essentially constant throughout incubation (Rahn & Ar 1974, Visschedijk & Rahn 1983). Other studies provide clear evidence that there are regional changes in shell thickness, shell conductance, and pore structure in the egg (Christensen 1982, Booth & Seymour 1987, Rokitka & Rahn 1987, Booth 1989, Soliman *et al.* 1994), and that these properties change during incubation (Christensen 1982, Booth & Seymour 1987, Booth 1989, Soliman *et al.* 1994).

During development, the avian embryo needs calcium for the ossification of the developing skeleton. Some of this calcium is deposited in the yolk when the egg is laid, but towards the end of incubation additional calcium is mobilized from the eggshell, and the mobilization of calcium causes the eggshell and pore length to thin to some degree (Simkiss 1961, Vanderstoep & Richards 1969, Kreitzer 1972, Sotherland *et al.* 1980, Packard & Packard 1984, Finnlund *et al.* 1985, Nys *et al.* 2004). Therefore, the shell thinning in some avian species results in an increase of shell conductance (Nys *et al.* 2004). For example, in the eggs of Mallee Fowl, *Leipoa ocellata*, shell thickness decreases by 21% during incubation and significantly alters pore structure, resulting in a threefold increase in shell gas conductance (Booth & Seymour 1987).

In other species such as Domestic Fowl *Gallus domesticus*, Quail *Coturnix coturnix*, Cliff Swallows *Petrichelidon pyrrhonota*, and Arctic Terns *Sterna paradisaea*, only 4–8% of the shell is eroded away during incubation (Vanderstoep & Richards 1969, Kreitzer 1972, Sotherland *et al.* 1980, Finnlund *et al.* 1985). In these latter cases, erosion apparently takes place exclusively from the tips of the mammillary knobs (Schwarz & Fehse 1957, Simkiss 1961, Von Doskocil *et al.* 1985) and is thought to have little effect on pore structure and shell gas conductance (Simkiss 1961). Booth (1989) showed that there are regional differences in shell thickness, shell conductance and pore number during incubation in eggs of the Mute Swan *Cygnus olor*. The increase in water-vapour conductance during incubation was directly related to the increase in pore density and shell thinning.

The aim of this study was to determine the shell thickness, shell conductance of water vapour and pore density in three regions (blunt pole, equator and sharp pole) of both unincubated and hatched eggs of the Peking duck (*Anas platyrhynchos f. dom.*). By comparing unincubated with incubated eggs, we can describe the regional changes in egg shell properties that occur during incubation. In particular, we explored whether there is any relation between an increase in water-vapour conductance and shell thinning or change in pore density during incubation.

2. Material and methods

Fresh eggs of the Peking duck were obtained from the Kutluk village (Çınar, Diyarbakır). Eggs (mean mass, 68.37 ± 1.13 g) were collected randomly from the nests and brought to the laboratory. Some of the eggs were artificially incubated at 37.5 °C in incubators.

Shell fragments from the blunt end (BE) (the region over the air cell), equator (Eq), and sharp end (SE) from 15 unincubated and 15 hatched eggs (90 fragments in total) were boiled in a 5% NaOH solution for 15–20 min to remove organic material, including shell membranes (Rokitka & Rahn 1987), rinsed three times in distilled water and left to dry.

2.1. Shell Thickness

Shell thickness was measured in five fragments at 10 areas within each of the three eggshell regions, BE, Eq and SE, from unincubated and hatched eggs, with a micrometer capable of 0.01 mm accuracy. Average shell thickness was calculated for each egg according to the method for the calculation of mean pore number for single eggs.

2.2. Water-Vapour Conductance

Water-vapour conductance (G_{H2O}) was measured in a series of shell fragments of both unincubated and hatched Peking duck eggs. BE, Eq and SE fragments approximately 28 mm in diameter were cut from shell fragments with a dental drill then

rinsed, dried, and sealed, inside surface down, to the tops of 18 ml glass vials (inside mouth diameter 18.1 mm) with a hot glue applicator (Booth 1989). The vials contained 8 ml of water and a wad of cotton. The seals were inspected for holes. Vials were weighed to ± 0.01 mg on an electronic balance (Sartorius A200S) and placed in desiccators containing silica gel at a constant temperature of $30 ± 1°$ C and 31.824 Torr vapour pressure. Vials were weighed daily for 10 d, and the conductance of each shell fragment was determined from its vial's daily mass loss. To calculate vapour pressure difference (Torr), average local temperature and barometric pressure were measured and we determined the water vapour pressure of internal vials (assuming the vial contents to be water saturated) at the average local temperature from a standard water vapour pressure table (Peebles & McDaniel 2004). To obtain the pressure correction factor (ratio), 760 Torr (barometric pressure at sea level) was divided by the average local barometric pressure, then the barometric pressure correction factor multiplied by the recorded internal vial water vapour pressure (at calculated average temperature). It is assumed that water vapour pressure at the outside of the vial in the desiccators is 0 Torr. Because the water vapour pressure gradient is the difference in water vapour pressure between the inside (water saturated) and outside (desiccated) of the vials, the water vapour gradient is represented by the water vapour pressure (adjusted for barometric pressure) of the internal vials. The water vapour conductance for each vial is calculated by dividing average daily water loss (milligrams per day) by the water vapour pressure gradient (Torr) between the inside and outside of the vials as G = rate of water loss (in mg day^{-1})/vapour pressure difference (Torr) (Wangensteen & Rahn 1970, 1971, Rokitka & Rahn 1987).

The surface area of each disk was estimated by covering the fragment with grid paper and then the squares within the effective evapourative area were counted. This area averaged 272 (±9 mm^2 95% confidence interval) and was used to calculate the area-specific conductance of each disk.

In obtaining the mean shell thickness and the mean water vapour conductance (G_{H2O}), the value obtained from the equatorial piece of shell was given twice as much weight as the values obtained from the ends. This was done because the equato-
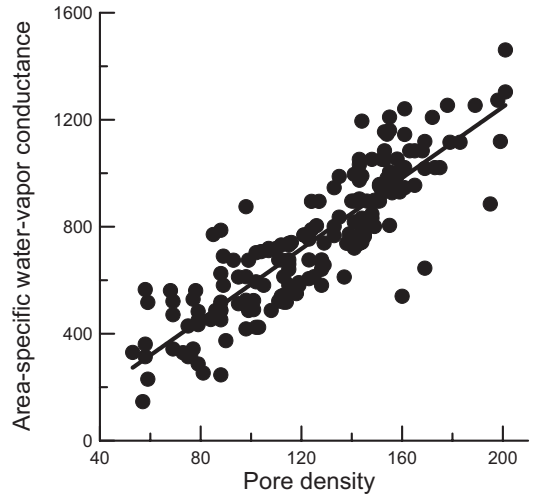

Fig. 1. Relationship between pore density (pores cm^{-2}) and water-vapour conductance (g d^{-1} torr^{-1} cm^{-2}) of Peking duck eggshell. Regression line: $y = 6.05 x$; $R^2 = 0.76$; $n = 180$.

rial piece was taken to represent approximately twice as large a portion of the shell as either of the end pieces.

2.3. Pore Counts

Pore count was measured using the method of Booth (1989). Ninety fragments were removed from the vials, and their inside surfaces were stained with Methylene blue (0.5 g of 89% dye/L of 70% ethanol). The staining solution was taken into pores by capillary action so that each pore appeared as a blue dot on the outside surface. The number of pores per 25 mm^2 was counted for six different areas of each fragment with the aid of a dissecting microscope. The reliability of regional pore counts was increased by treating larger shell fragments cut from areas adjacent to the 90 fragments and counting their pores as previously described.

For calculation of the mean pore density for the entire egg, the mean value for the equatorial region was multiplied twice, added to the values from both ends, and divided by four. This was done because pores are not evenly distributed over the shell, and the equatorial region was taken to represent approximately twice as large a portion of the shell as either of the end pieces (Hoyt *et al.* 1979).

The total number of pores per egg was obtained by multiplying the mean pore count by the total shell surface (A) estimated as $A = 4.835\ M^{0.662}$, with A the shell surface (cm^2) and M the egg mass (g) (Paganelli *et al.* 1974).

Results are presented as means and 95% confidence intervals of the mean. Data from different regions were analyzed with Anova and a significant difference was detected ($P < 0.001$). LSD tests were used to identify differences between groups.

3. Results

3.1. Shell Thickness, Water-Vapour Conductance and Pore Density

We found regional variation of the shell thickness in the eggs of Peking duck (Table 1). In unincubated eggs, shell thickness increased from the blunt end to the sharp end. A significant thinning of shell occurred in both the equator and sharp end regions in hatched eggs. Incubation resulted in an average 6.4% decrease in shell thickness.

In unincubated eggs, shell fragments from the blunt and sharp ends had significantly greater values of G_{H2O} than fragments from the equator regions (Table 2). During incubation there was a significant, 42% increase in G_{H2O} in the equator regions, but G_{H2O} in the blunt ends did not increase significantly, only by 11%, and sharp ends were unaffected by incubation.

During incubation, there was a significant, 35% increase in pore density of the equatorial regions, and the pore density in all three regions averaged 136 ± 5 pores cm^{-2} (Table 3). Pore density in equator regions was lower than that in either the blunt or sharp ends in unincubated eggs, but increased significantly during incubation. Pore density of the blunt and sharp ends did not change significantly during incubation. In hatched eggs, a significant difference in pore density between the blunt and sharp blunt ends occurred.

Mean pore density was 115 ± 5.4 pores cm^{-1} in an unincubated egg and 139 ± 5.6 pores cm^{-1} in a hatched egg. The total number of pores per egg was 9.115 in an unincubated egg and 11.017 in a hatched egg. We estimated the total number of pores (N) in an unincubated egg based on egg mass

M (g) using various equations taken from the literature. Estimates for total number of pores were 8.754 ($N = 1041\ M^{0.504}$, Hoyt *et al.* 1979), 7.766 ($N = 304\ M^{0.767}$, Rahn & Paganelli 1990), and 7.936 ($N = 3520\ M/I$, Tuan 1987).

4. Discussion

4.1. Regional Shell Thickness and Its Relationship with Water-vapour Conductance

We found that shell thickness in Peking duck eggs is quite variable, even within a small area of the same egg. The shell becomes thicker from the blunt end to the sharp end in unincubated eggs and changes in thickness during incubation. Thus, findings in this study confirm quantitatively the differences in shell thickness between different regions of the egg, as also observed by Christensen (1982), Booth & Seymour (1987), Rokitka & Rahn (1987), Booth (1989) Soliman *et al.* (1994), Massaro & Davis (2005). Furthermore, Peking Ducks also show a clear change in shell thickness during incubation as previously documented for some species (Christensen 1982, Booth & Seymour 1987, Booth 1989, Soliman *et al.* 1994). Our findings show the shell thickness increases from the blunt end to the sharp end are compatible with many bird species generally, with a few exceptions (e.g. Thompson & Goldie 1990, Gosler et al. 2005, Massaro & Davis 2005).

We found a decrease in shell thinning of regions of the equator and sharp end during incubation, but the blunt end, where the air cell forms between the inner and outer shell membranes, is unaffected during incubation. The inside surface of unincubated eggs consists of a field of mammillary knobs protruding inward. The tips of the mammillary knobs are eroded away in the equator and sharp end regions during incubation. Therefore, shell thinning occurs only from regions where the chorioallantois and inner shell membrane are in contact with the outer shell membrane (Rokitka & Rahn 1987). Toward the end of incubation, calcium is dissolved from the shell and presumably diffuses through a liquid water film covering the surface of the fibres of the inner and outer membrane to the chorioallantois. Upon reaching the

Table 1. Eggshell thickness (mm) from the blunt end (BE), equator (Eq), and sharp end (SE) of 15 unincubated and 15 hatched Peking duck eggs.

Parameters	Unincubated	Hatched	Difference (%)	P
BE	426 ± 34	423 ± 29	0.7	NS
Eq	445 ± 22	410 ± 20	7	<0.03
SE	473 ± 29	419 ± 40	11	<0.02

Note. Means ± 95% confidence intervals and probability, P, from Student's t test. NS: not significant.

Table 2. Water-vapour conductance ($mg \cdot d^{-1} \cdot Torr^{-1}$ per 10 cm^2) from the blunt end (BE), equator (Eq), and sharp end (SE) of 15 unincubated and 15 hatched Peking duck eggs

Parameters	Unincubated	Hatched	Difference (%)	P
BE	7.71±1.4	8.53±1.8	11	NS
Eq	5.98±0.7	8.95±0.9	42	<0.02
SE	7.53±1.4	7.64±2.1	2	NS

Note. Means ± 95% confidence intervals and probability, P, from Student's t test. NS: not significant.

Table 3. Pore density from (pores/cm^{-2}) the blunt end (BE), equator (Eq), and sharp end (SE) of 15 unincubated and 15 hatched Peking duck eggs.

Parameters	Unincubated	Hatched	Difference (%)	P
BE	131 ± 18	146 ± 22	11	NS
Eq	106 ± 14	149 ± 26	35	<0.01
SE	116 ± 15	117 ± 19	1	NS

Note. Means ± 95% confidence intervals and probability, P, from Student's t test. NS: not significant.

chorioallantois, calcium is actively transported into the blood stream (Tuan 1987), where it is used by the embryo to ossify the developing skeleton (Packard & Packard 1984). The air cell at the blunt end, which is well developed by the end of incubation, forms a barrier to calcium mobilization, presumably by disrupting the continuity of fibres and hence the liquid water interface between the inner and outer shell membranes. Therefore, calcium cannot be mobilized from the shell located over the air cell (Booth 1989). Erosion of the tips of mammillary knobs late in incubation from areas other than the air cell has now been documented in 24 avian species (Rahn & Ar 1974, Von Doskocil *et al.* 1985, Booth & Seymour 1987) and is likely to be a common phenomenon among all bird eggs.

Although a significant thinning of shell occurred in both the equator and sharp end regions in hatched eggs of Peking Duck, water-vapour conductance does not increase in the sharp end, but only in the equator and blunt end regions. These latter regions show a coupled increase in pore density. Therefore, we believe that shell thinning has no effect on shell gas conductance.

4.2. Regional Pore Density and Its Relationship with Water-Vapour Conductance

Pore density in the different regions of eggshell is quite variable in unincubated eggs. Our findings in this study confirm quantitatively the differences in pore density in the egg observed and change in pore density during incubation previously observed by Christensen (1982), Booth & Seymour (1987), Rokitka & Rahn (1987), Booth (1989), Soliman *et al.* (1994).

The pore density is lower in the equatorial region of unincubated eggs compared with that in ei-

ther the blunt or sharp end regions. There is an increase in pore density in this region during incubation. This increase in pore density coincides with an increase in G_{H2O} of the equator region. Pore density is clearly related to G_{H2O} – the greater the pore density, the higher the G_{H2O}. Regional variation in pore density has previously been correlated with regional differences in shell gas conductance in several avian species (Paganelli 1980, Rokitka & Rahn 1987, Booth 1989). Because G_{H2O} per pore remains constant during incubation, a 35% increase in pore number should lead to a 35% increase in G_{H2O}, which is very close to the 42% increase in our observation. Hence, by the end of incubation, pore density and shell conductance in the equatorial region is similar to that of the blunt and sharp ends. How can pore density increase during incubation? One must hypothesize that some of the pores are plugged inside the shell surface when the egg is laid, and that during incubation the shell-thinning process presumably leads to the unplugging of these pores, making them functional. It may be that the inside mouths of plugged pores contain loosely packed crystals of calcium carbonate and that during incubation these crystals are dissolved away (Booth 1989).

The conductance of a typical pore is 6.1 ± 0.3 µg d^{-1} Torr^{-1} (N = 180) in both unincubated and hatched shell fragments. However, these values are considerably greater than the mean value (1.5 µg d^{-1} Torr^{-1}) of birds eggs in general (Ar & Rahn 1985). The reason for this divergence is that the shell fragments used in the current study had most of the cuticle layer removed before G_{H2O} measurements were made. Because this layer plugs pore openings to some extent, its removal probably has the effect of increasing G_{H2O} (Tyler 1963, Booth 1989).

In conclusion, results of this study suggest that there are regional changes in shell thickness, shell conductance and pore density in both unincubated and hatched eggs of the Peking duck and these regions are affected during incubation. Previously reported increases of G_{H2O} of avian eggs during incubation have been of the order of 40–100% (Carey 1979, Rahn & Ar 1980, Sotherland *et al.* 1980). We found a 42% increase in G_{H2O} in eggs of Peking duck during incubation, and showed that this increase is due to increasing pore number in the egg shell.

Pekingin ankan (*Anas platyrhynchos* f. *dom.*) munankuoren paksuuden, kuoren läpäisevyyden ja huokosten määrän muutoksia haudonnan aikana

Kuoren paksuus, läpäisevyys ja huokosten tiheys ovat munan ominaisuuksia, jotka vaikuttavat alkionkehityksen onnistumiseen. Selvitimme nämä ominaisuudet hautomattomien ja jo kuoriutuneiden pekingin ankan munien molemmista päistä ja puolivälistä. Hautomattomien munien kuori oli paksumpi munien terävässä kuin tylpässä päässä. Vesihöyryn läpäisevyys oli suurinta munien tylpässä päässä ja pienintä munien keskiosissa. Kuoriutuneiden munien analysointi osoitti, että haudonnan aikana munien kuori ohenee terävästä päästä ja keskiosista ja johtaa lähes homogeeniseen kuoren paksuuteen kuoriutumiseen mennessä. Huokosten määrä ja kuoren läpäisevyys sen sijaan kasvoivat haudonnan aikana munien keskiosissa ja tylpässä päässä. Tuloksemme viittaavat siihen, että kyseisellä lajilla munan kuoren läpäisevyyden kasvu haudonnan edistyessä johtuu huokosten määrän kasvusta, ei munan kuoren ohentumisesta.

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