

The Origin of Lactation as a Water Source for Parchment-Shelled Eggs

Olav T. Oftedal¹

Available evidence indicates that mammary gland secretions first evolved in synapsids that laid parchment-shelled eggs. Unlike the rigid-shelled eggs of birds and some other sauropsids, parchment-shelled eggs lose water very rapidly when exposed to ambient air of lower vapor pressure, whether due to differences in relative humidity or to differences in temperature. This precludes endothermic incubation of parchment-shelled eggs in an open nest. Synapsids may have avoided egg desiccation by incubating eggs in a pouch, but this would limit maternal activity. Parchment-shelled eggs are able to take up liquid water across the eggshell. I propose that mammary secretion originally evolved as a means of supplying water to eggs, and as such was essential to the evolution of endothermy among the egg-laying cynodonts that were ancestral to mammals. It is possible that synapsid eggs, like parchment-shelled squamate eggs, were also capable of uptake of some nutrients, such as sodium and ionic calcium. Living monotremes still produce parchment-shelled eggs. The porous eggshell and bilaminar yolk sac membrane of these eggs permit substantial uptake of uterine secretions during the intrauterine period, and might also facilitate uptake of mammary secretions during egg incubation. In its simplest form, mammary secretion may be an ancient trait of egg-laying synapsids, having had an important role long before milk became obligatory for suckling young.

KEY WORDS: lactation; egg physiology; water balance; endothermy; evolution; monotreme.

INTRODUCTION

Monotremes (the echidnas and platypus) have retained a primitive component of mammalian reproduction: the laying of shelled eggs containing extraembryonic membranes similar to those of other amniotes (1). Lactation evolved prior to the divergence of monotremes and therian mammals (i.e. marsupials plus eutherians), at a time when the young developed within, and hatched from, large-yolked eggs (2,3). Most evolutionary biologists have assumed that there must have been some benefit to the eggs or hatchlings that favored the hypertrophy of skin glands and the secretion of an evolutionary precursor of milk (2–4), but there is little agreement what

that benefit may have been. For example, it has been suggested that cool eggs benefited by warming (5,6), that hot eggs benefited by cooling (7), that water-stressed eggs benefited from moisture (4,7), that incubated eggs benefited by adhering to a sticky abdominal patch (6), and that eggs vulnerable to microbial or fungal attack benefited from antimicrobial compounds in secretions (2). In addition, dependent hatchlings might have benefited from encountering or ingesting supplemental water (7), nutrients (2,4,6), antimicrobials (2), or pheromones (8) in skin secretions.

Given this perceived central role of eggs and recently hatched young in the origin of lactation, it is surprising that little effort has been made to determine how the anatomy, physiology, and nutrient stores in eggs may have shaped their needs. Were the eggs of mammalian ancestors similar to bird eggs, or to eggs of other taxa such as squamates (lizards and snakes), crocodylians or turtles? Did any particular aspects of

¹Department of Conservation Biology, Conservation and Research Center, Smithsonian National Zoological Park, Washington, District of Columbia 20008; e-mail: oftedal@nzp.si.edu or ooftedal@att.net.

eggs play a particular role in the early evolution of lactation?

To answer such questions, it is important to understand the differences in eggshell structure among amniotes, to connect these differences to the physiological needs of eggs, and to determine if it was in fact possible to apply a secreted fluid to the surface of eggs without suffocating them. I will develop the argument that mammalian ancestors produced eggs that had a permeable shell and were thus prone to desiccation. Such eggs could not be incubated at an elevated temperature, as required for the evolution of metabolically-sustained high body temperatures (or endothermy), unless they were protected from excessive water vapor loss. Alternatively, they required a supplemental source of liquid water. An ability of synapsid eggs to absorb moisture and perhaps other constituents is suggested by comparative data on other taxa, particularly squamates that produce eggs with parchment-like eggshells. Particular attention will be given to what is known about egg anatomy and incubation in monotremes, the only surviving synapsid group that still produces eggs.

See the accompanying review on mammary gland origins (3) for a summary of synapsid evolution and for definitions of key taxonomic and zoological terms that may not be familiar to mammary gland biologists.

EGG STRUCTURE AND WATER BALANCE

The Structure of Synapsid Eggs

Synapsids appear in the fossil record more than 300 million years ago (3). These early amniotes presumably differed from earlier tetrapods (9) in having relatively large-yolked eggs in which specialized extraembryonic membranes assisted in nutrient mobilization, respiratory gas exchange, storage of embryonic waste products, and protection of the embryo within a fluid-filled compartment (1,10,11). Collectively, these new features freed eggs from a need to be immersed in water and permitted an increase in maternal nutrient investment per egg. As a consequence, the developmental period became prolonged, the larval stage was deleted, and relatively large, self-feeding hatchlings emerged into terrestrial habitats.

Early amniote eggs apparently lacked a calcified eggshell, however. From a phylogenetic analysis of the eggshells of extant amniotes, Stewart (11) concludes that deposition of calcium carbonate crystals over the fibrous eggshell membrane is a derived

character of sauropsids (extant "reptiles" and birds), and not a primitive amniote feature. A brittle calcified eggshell has evolved independently in archosaurs (dinosaurs, crocodylians, birds), chelonians, and some gekkonid lizards, leading to a variety of eggshell structures and differences in crystalline form (e.g., Fig. 1). However, most lizards and snakes have a parchment-like eggshell, with scattered and variable sites of calcium deposition, and although monotreme eggshells are multilayered they apparently lack a distinct calcareous layer (12,13).

The conclusion that early amniote eggs were not rigidly calcified is consistent with the absence of fossil eggs from the Carboniferous, Permian, and early Triassic (14), a period of about 100 million years in which many synapsid radiations occurred (3). Eggshells lacking calcification are soft and pliable, collapse and shrivel if the contents disintegrate or desiccate, and have little structure that can be fossilized. There are no positively identified fossil eggs for any of the lizards, snakes or tuataras that lay parchment-shelled eggs (14). By contrast, the distinctive patterns seen in the crystalline columns and cones of highly calcified eggshells (Fig. 1) are well preserved in fossils, providing evidence not only that an egg-like object was in fact an egg, but also the type of eggshell and its likely source. For example, at least eight distinctive morphotypes of dinosaur eggshells have been recognized, but dinosaur eggs (and dinosaur fossils) are not found until the middle/late Triassic (14–16). Fossilized eggshells of other sauropsids (including birds) appear even later (14). An early report of a Permian egg attributed to an early synapsid or "pelycosaur" (17,18) has since been discounted as one of the many egg-like objects easily mistaken for fossil eggs, including concretions within sediments, calculi formed in the digestive tracts of animals, and fossilized insect cocoons (14,19).

The monotremes produce eggs with a parchment-like eggshell, which may be regarded as a primitive synapsid trait (11–13,20). However, monotreme eggs are quite small and contain little yolk, as the tiny, immature hatchling is dependent on maternal milk for many months (21). Early synapsids undoubtedly produced relatively large, yolk-rich eggs prior to the evolution of nutrient-rich mammary secretions.

Water Vapor Loss Across Various Types of Eggshells

By laying eggs with a parchment-like eggshell, synapsids risk egg mortality due to the rapid flux of

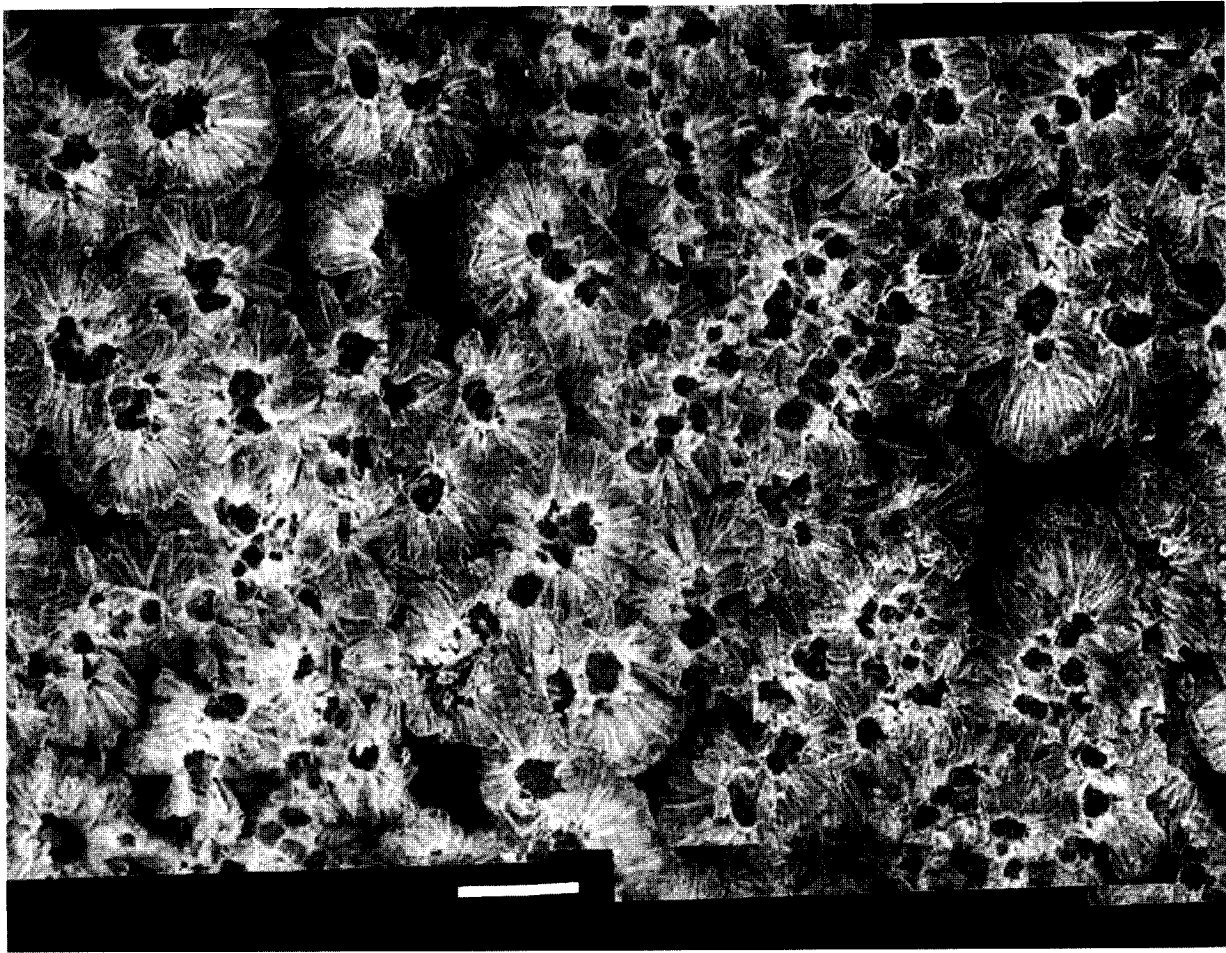


Fig. 1. Composite scanning electron micrograph of the inner surface of the rigid, calcified eggshell of an unincubated egg of a desert tortoise, *Gopherus agassizii*. The shell units of calcium carbonate radiate in somewhat irregular fashion from attachment areas (dark ovoid areas) to the underlying fibrous membrane. Note most shell units are tightly appressed, but occasional gaps represent pores. Several scans are stitched together in this view, as evident by horizontal or vertical lines in some places white bar = 50 μ . (540 \times magnification)

water vapor that may occur across this relatively permeable barrier. Although water vapor loss through monotreme eggshells has not been measured, there are many studies of the parchment-shelled eggs of lizards and snakes that may be compared to data on the calcified eggshells of turtles, crocodylians, and birds (22–25). Water vapor transport across the eggshell of any egg is a function of egg surface area, the difference in water vapor pressure between the interior (egg contents) and exterior (surrounding air) and a water vapor transport coefficient (k_v) (22). The coefficient k_v is a property of the eggshell that determines the amount of water lost by an egg in a given set of environmental conditions. In most squamates with parchment-shelled eggs, k_v ranges from about 120 to 320 $\text{mg H}_2\text{O} \cdot \text{d}^{-1} \cdot \text{kPa}^{-1} \cdot \text{cm}^{-2}$ ($n = 28$), but is

only about 2–7 ($n = 5$) in turtles and crocodylians with rigid, calcified eggshells (24); similar or even lower k_v characterize rigid-shelled bird eggs. Thus for a given egg size and surface and a particular ambient vapor pressure, a parchment-shelled squamate egg will lose water at about 50–150 times the rate of a rigid-shelled crocodylian or turtle egg.

With their high k_v parchment-shelled eggs have great difficulty retaining water in the face of a vapor pressure differential across the eggshell. Even a small differential causes great water loss, whether due to reduced relative humidity in ambient air or a difference in temperature between egg and environment (Table I). For a given k_v small eggs lose a greater percent of water per day due to their larger surface area per unit egg volume.

Table I. Predicted Water Loss Rates of Parchment- and Rigid-Shelled Eggs^a

Egg mass (G)	Temp. egg (°C)	Ambient temp (°C)	Ambient R.H. (%)	Vapor pressure difference (kPa)	Parchment-shelled egg			Rigid-shelled egg		
					Water loss (mg/d)	Percent loss (%)	Days for 33% loss (d)	Water loss (mg/d)	Percent loss (%)	Days for 33% loss (d)
<i>Eggs incubated at ambient temperature, two humidities</i>										
5	28	28	99	0.0376	84.6	2.45	14	0.43	0.012	2663
50	28	28	99	0.0376	293	0.85	39	2.58	0.007	4420
500	28	28	99	0.0376	1017	0.30	112	15.5	0.004	7336
5	28	28	95	0.1880	423	12.3	2.7	2.14	0.06	533
50	28	28	95	0.1880	1466	4.25	7.8	12.9	0.04	884
500	28	28	95	0.1880	5085	1.47	22	77.6	0.02	1467
<i>Eggs incubated at two elevated temperatures, 99.5% relative humidity</i>										
5	30	28	99.5	0.4609	1037	30.1	1.1	5.24	0.15	217
50	30	28	99.5	0.4609	3595	10.4	3.2	31.6	0.09	361
500	30	28	99.5	0.4609	12466	3.61	9.1	190	0.06	598
5	32	28	99.5	0.9700	2182	63.3	0.5	11.0	0.32	103
50	32	28	99.5	0.9700	7567	21.9	1.5	66.5	0.19	171
500	32	28	99.5	0.9700	26237	7.60	4.3	400	0.12	284

^a Water loss was predicted using equations relating vapor conductance to egg mass for parchment-shelled squamate eggs and for rigid shelled bird eggs (24). The relative humidity (R. H.) within the egg was assumed to be 99.5%, attributable to a water potential of -600 kPa (25). The eggs were assumed to contain 69% water, by analogy to extant sauropsids with precocial young, including crocodiles (69-70%), turtles (66-72%), and precocial birds (mean 68%, range 57-75%) (26,27). Lower values for squamate eggs (28) are biased by oviductal eggs that had not attained full hydration.

As most embryos cannot survive a loss of more than a third of egg water content (29,30), small parchment-shelled eggs (5 g) are probably unable to survive much longer than 2 weeks even at 99% relative humidity at 28°C (Table I). Larger eggs, such as were probably produced by the relatively large (mostly 20–330 kg) synapsids in the Pennsylvanian and early Permian (17), fare better: a 500-g parchment-shelled egg is predicted to require 112 days to lose one third of its water under these conditions (Table I). This is within the range of incubation lengths in “reptiles” (ectothermic sauropsids) (31). By comparison, rigid-shelled eggs lose very little water under these conditions and could survive for prolonged periods (Table I). Reduction of water vapor flux in rigid eggshells reflects the effective barrier presented by the interlocking columns and cones of calcium carbonate crystals. Gas exchange in rigid-shelled eggs is largely restricted to diffusion through the relatively narrow pores that penetrate the eggshell (32).

Parchment-shelled squamate eggs are normally buried in damp soil where the air is in equilibrium with soil water, and hence relative humidity approaches 100% (22). For example, soil at a water potential (a measure of its energy state) of -600 kPa will contain air at a relative humidity of about 99.5%, equivalent to that within the egg. If early synapsids did not have calcified eggshells, they had no choice but to select very moist environments for egg deposition, or their eggs would have succumbed to desiccation. Among extant squamates and chelonians, substrates that are too dry cause egg mortality, reductions in hatchling size, and reduction in hatchling vigor, with parchment- and flexible-shelled eggs being most affected (33–35). The progressive aridity that developed at the end of the Permian and through the Triassic may have favored the evolution of rigid-shelled eggs, which first appear with the emergence of dinosaurs in the Triassic (14,36,37).

Another factor that may strongly influence egg water vapor loss is a temperature gradient between egg and environment. This occurs because the saturated vapor pressure of air increases markedly with a rise in air temperature. Table I illustrates the vapor pressure difference, and the predicted water losses, across eggs that are incubated at temperatures 2°C and 4°C above ambient. Note that the vapor pressure difference associated with a 2°C temperature gradient is about three times that associated with an ambient relative humidity of 95% (under isothermic conditions). The predicted daily water losses are as much as 30–60% in small parchment-shelled eggs (Table I).

Even large parchment-shelled eggs could not tolerate a temperature gradient of 4°C for more than a few days without becoming desiccated. However, such temperature gradients present no particular problem for rigid-shelled eggs, which at most lose a few tenths of a percent of egg water per day. Thus the development of endothermic incubation, in which the egg is maintained at an elevated temperature, poses a special problem of water loss for parchment-shelled eggs but not for rigid-shelled eggs (such as bird eggs).

Note that the range of modelled egg masses, 5–500 g, encompasses much, but not all, of the range of egg mass seen in extant squamates (0.06–303 g), turtles (2.6–107 g), crocodylians (52–113 g), and birds (0.3–1480 g) (38). Even larger egg masses have been estimated for eggs of extinct birds [*Aepyornis*, 4 kg (39)] and dinosaurs [up to ca. 5.5 kg (40)]. Although early synapsids likely had large eggs, the only extant synapsids, the monotremes, produce small-yolked eggs of about 1.5–2 g [calculated from egg volume (21)].

Egg Water Uptake

The extreme vulnerability of parchment-shelled eggs to water vapor loss is offset by an ability to absorb liquid water from surrounding soil. Liquid water transport through an eggshell depends on the surface area exposed to water, the difference in energy state of water (termed the water potential) between egg contents and the immediate environment, and a coefficient (k_l) indicating the liquid water transport properties of the eggshell (22). Liquid water transport requires the presence of a continuous water column between egg contents and surrounding medium.

Many studies have shown the importance of variation in soil water potential on egg water uptake in sauropsids with parchment- or flexible-shelled eggs, although interactions with variables such as soil structure and temperature may be complex (35,41). The water potential of soil is affected by a variety of factors, including dissolved osmolytes, capillary effects, and the surface properties of soil particles (42). Primarily due to water uptake, parchment-shelled eggs of lizards and snakes may increase in mass by 10–100% after they have been laid, with increases as high as 360% being reported for small eggs (43,44). In fact, a hatchling may be heavier than its egg was at oviposition (44). The advantage of a parchment-like eggshell is that it can accommodate such expansion, whereas a rigid-shelled egg could not (35). Although studies

have not been done on water uptake by monotreme eggs after egg-laying, it is likely that water uptake was important to early synapsid eggs, as it still is in parchment-shelled squamate eggs.

By contrast, water uptake appears to be minimal in rigid-shelled eggs. Rigid-shelled eggs rely on diffusion of O₂ and CO₂ through narrow pores that must stay open to allow respiration, especially in later developmental stages when demands of the embryo are high. Egg swelling due to water uptake may cause the eggshell to crack, opening an entry for microbial invasion (26,35). Birds such as grebes that routinely incubate eggs in wet environments have eggshells that are impervious to liquid water (45). Minimal fluid uptake has been reported in some rigid-shelled eggs (45,46), but is relatively unimportant to egg water balance.

Egg Water Stores

A rigid eggshell presents a barrier to movement of both water vapor and liquid water, requiring that the egg be laid containing the water it will need for development. Such eggs are termed endohydric, in contrast to ectohydric eggs that must take up water from the environment to complete embryogenesis (30). In endohydric eggs, water is initially stored in an albumen layer, and subsequently mobilized into the yolk; ectohydric eggs usually have little albumen (30). Birds produce endohydric eggs, in which the proportion of albumen varies in relation to water needs for development (23,27). The rigid-shelled eggs of crocodylians, many turtles and some geckos are also endohydric, while the parchment-shelled eggs of squamates are mostly ectohydric.

Were the parchment-shelled eggs of early synapsids also ectohydric? It may have been influenced by egg size. Larger eggs have a favorable ratio of volume to surface area, and thus contain more water relative to the propensity for water loss at the egg surface (30). The largest extant parchment-shelled eggs are those of pythons (230 g, *Python molurus*), which have a low water vapor conductance due both to large size and a low k_v (47 mg · d⁻¹ · kPa⁻¹ · cm⁻²) (24,47). The reason for this low coefficient, the lowest measured for any parchment-shelled egg, is not known. Large egg size may help explain how some iguana eggs can survive in quite dry soil (30), and how python eggs can be incubated above ground (48). The increasing aridity due to climate change in the late Permian and Triassic (36,49) would have favored large eggs in synapsids.

ENDOTHERMY AND EGG INCUBATION

The Contrast Between Egg Incubation in Birds and Mammals

Endothermy, or the maintenance of an elevated body temperature by virtue of a high metabolic rate (and increased surface insulation), evolved in two phylogenetically distant egg-laying lineages: the sauropsid lineage leading to birds and the synapsid lineage leading to mammals. Endothermy provides the opportunity to incubate eggs at an elevated temperature and thereby promote rapid growth and development and shorter incubation times. Even ectotherms such as squamates, turtles, and crocodiles employ a variety of strategies to maintain eggs at temperatures that are higher and more stable than the general environment (50). These include selection of sunny nest sites, burying eggs at depths where temperatures are less variable, use of fermenting vegetation for mound nests, and basking in sunshine by females that have retained developing eggs in the oviduct. Some pythons coil tightly about their eggs and use shivering thermogenesis to keep eggs warm (48,51).

True endothermic incubation requires eggs to be isolated from the substrate. Eggs in contact with soil rapidly reach thermal equilibrium with the soil because of its high heat conductance (ten to several hundred times that of air) and high heat capacity (several thousand times that of air) (23). It is difficult to maintain a large thermal gradient between an egg and environmental heat sinks unless the egg is enclosed by an insulating barrier such as a nest. However, a thermal gradient from egg to environment also creates a large vapor pressure gradient, because a 10°C temperature rise nearly doubles the saturation vapor pressure for water.

Birds evolved from an archosaurian lineage that includes crocodylians and dinosaurs, and that produced eggs with rigid, highly calcified eggshells. The immediate ancestors of birds had rigid-shelled eggs, whether these ancestors were endothermic or not (52). As Ar (23) notes, "it seems that the rigid eggshell provides, above all, a solution to the water budget of the developing embryo." In terms of eggshell structure, bird eggs were preadapted to endothermy and its challenge of increased water loss. Subsequent avian evolution has included a fine tuning of eggshell vapor conductance so that water loss of eggs averages about 15% of initial egg mass [range 10–23% (53)], even though eggs face a wide variety of ambient temperatures, altitudes and relative humidities, all of

which affect the vapor pressure differential across the eggshell (54–58).

Synapsids did not have the advantage of rigid-shelled eggs, so a temperature-driven vapor pressure gradient from egg to environment would cause severe water loss from eggs (Table I), leading to embryonic mortality. How then could synapsids, including early mammals, incubate eggs at elevated temperatures? The apparent conflict between the advantages of egg incubation at elevated temperature and the large water cost of a thermal gradient across the eggshell had to be resolved by synapsids as they evolved high metabolic rates and body temperatures.

When Did Endothermy Evolve Among Synapsids?

The synapsid lineage that led to mammals was characterized by a gradual acquisition of mammalian characters over successive radiations [see accompanying review (3)], including those associated with elevated respiratory rates, high levels of energy expenditure and endothermy. This was most apparent in the three therapsid groups [therocephalians, dicyonodonts (Anomodontia), and cynodonts] that survived the Permian–Triassic mass extinction. These characters include:

1. Water-conserving maxilloturbinals in the anterior nasal chamber of therocephalians and cynodonts; these structures correlate to high ventilation rates in extant vertebrates (59,60),
2. Well-vascularized and presumably rapidly-grown fibrolammellar bone in many therocephalians, dicyonodonts, and cynodonts; such bone structure is typical of large rapidly-growing endotherms, but rarely found in ectotherms (61–63).
3. A bony secondary palate in some therocephalians, dicyonodonts, and cynodonts; this not only strengthens the skull during feeding, but by segregating the dorsal part of the oral cavity as a respiratory pathway allows respiration while the mouth is full (64,65). A secondary palate is probably also be a prerequisite to suckling (65).
4. Reduction of the lumbar ribs in cynodonts, which is believed to indicate differentiation of function between thoracic and lumbar ribs during the evolution of diaphragmatic breathing (64).

5. Changes in the pelvis and hind limb bones produced a transition from a sprawling to upright posture among therocephalians, dicyonodonts, and cynodonts; the more erect gait probably involved greater neuromuscular control, maneuverability and running speed (64,66), as might be expected in animals of increased metabolic scope
6. Restructuring of the jaw bones and increasingly diversified dentition in cynodonts suggest effectiveness as active predators (64).

While none of these features represents an iron-clad demonstration of elevated metabolic rate, in aggregate they suggest that some Triassic therapsids, and especially nonmammalian cynodonts, were active, rapidly-growing animals with enhanced respiratory function, improved locomotor ability, and specializations for feeding. Based primarily on the presence of maxilloturbinals, Hillenius (60) and Ruben (67) argue that elevation of metabolic rate began with Late Permian therocephalians, but achieved mammal-like endothermy among advanced nonmammalian cynodonts in the Triassic. Thus, egg incubation at above-ambient temperature might have appeared as early as the Late Permian or during the Triassic.

The Pouch as a Means of Restricting Water Loss by Parchment-Shelled Eggs

Early synapsids may have buried eggs in moist ground to minimize egg water loss (or promote egg water uptake), but this would be incompatible with the need of endothermic therapsids for an insulated nest. Among Triassic therapsids, some taxa were apparently burrow-dwelling, including some dicyonodonts and some cynodonts, and may have used burrow nest-sites where relative humidity was high (68). Yet this is not likely to be a general solution to the problem of egg water loss.

In this context the success of pythons (*Python molurus*) in incubating parchment-shelled eggs at temperatures up to 8°C above ambient is instructive (48,69,70). The female completely surrounds the eggs in a tight coil, and warms them from without by heat generated by shivering thermogenesis. The entire “nest” is warmed, so a thermal gradient does not develop between egg and nest. As ambient temperature drops the coils become tighter, restricting exchange of air between nest and environment, as indicated by elevated CO₂ within the “coil-nest” (48).

Unfortunately, water vapor pressure within the nest has not been measured, nor has the loss of water from nest to environment.

Most synapsids have not had a body form that would allow such a tight enclosure of eggs, but various types of pouches found among extant monotremes and marsupials may achieve a similar result: a “nest” environment of homogeneous temperature and high relative humidity (71). Incubated eggs in a pouch would have a temperature and relative humidity similar to the pouch, and so long as maternal water replaced moisture lost from the pouch opening, egg water loss would be minimal.

Water loss from eggs incubated by living monotremes has not been measured. Among extant monotremes, short-beaked echidnas (*Monotremata: Tachyglossus aculeatus*) incubate their eggs in a well-developed pouch at 32°C, the normal body temperature of echidnas living in their thermoneutral zone (21). However, the aquatic platypus is pouchless (21). The incubating platypus nests near the edge of the water in a burrow that she plugs with nest material, presumably to maintain high humidity (72). She apparently lies on her side or back with the eggs on her abdomen, covered by her recurved flat tail (72), thereby forming a “incubatorium” or pouch-like enclosure. The ventral surface of the tail is mostly naked, and in a resting platypus has a surface temperature of 30–32°C when the burrow temperature is 20°C (21). Thus incubation temperature is about the same for both species, and in both “nest” humidity must approach saturation. Eggs are incubated for about 10–11 days in both species.

The pouch or an equivalent “incubatorium” appears to be a physiologic necessity created by the susceptibility of parchment-shelled eggs to dehydration, and this necessity must have applied to endothermic incubation in Triassic therapsids. If so, a functional pouch was one of the crucial mammal-like traits that evolved among therapsids, and to which novel support structures, the epipubic bones, were later committed (73,74).

The role of a pouch in the evolution of lactation has been long debated. Charles Darwin (75) proposed that primitive mammalian neonates were carried in a pouch and began to imbibe skin secretions therein. The notion that a pouch is a primitive mammalian trait has since fallen out of favor, because 1) phylogenetic analyses suggest that pouches evolved independently among various marsupial taxa, 2) some of the pouchless marsupials (including daryurids and didelphids) are thought to be anatomically “primitive,”

and 3) other functions (besides pouch support) have been attributed to epipubic bones (76–78). However, pouches are not obligatory for neonates that can obtain fluid via milk ingestion, and thus pouches may have evolved and regressed repeatedly in marsupials without compromising offspring survival.

It is possible that pouch use, rather than burying of eggs, may be a primitive form of egg care in synapsids. Many extant anurans (frogs and treefrogs) with moist, glandular skin carry eggs and or tadpoles attached to skin, or imbedded in cutaneous “pouches,” and most terrestrial salamanders curl around their eggs and thereby help keep them moist (9). If egg transport in moist pouch-like structures was a norm among early synapsids, there would have been little selective pressure favoring calcified eggshells or other means of protecting eggs from desiccation.

SKIN SECRETIONS AND EGG WATER

Can Egg Water Loss be Replaced by Skin Secretions?

Eggs in a pouch are protected from moisture loss, but they severely constrain the mobility of the parent. If eggs could be periodically rehydrated by absorbing water from skin secretions, they could be parked in a nest during foraging or other activities. Clutch size could be expanded beyond the number of eggs that could fit in a pouch (or pouches) at once, and large eggs, that would be cumbersome to carry in a pouch, would be easier to care for. These and other advantages may have accrued to synapsids that developed glandular secretions to minimize pouch dependence.

There is precedence for egg moistening via cutaneous contact, not only in amphibians (79) but also in birds. King penguins (*Aptenodytes patagonica*) incubate a single large egg within a brood “pouch” that covers most of the egg; the egg also rests on the feet of the parent, preventing contact with the cold substrate in subantarctic rookeries (80). The upper part of the egg is in continuous contact with a warm, wet brood patch. Water loss of the king penguin egg may be described by a two part model in which water vapor loss from most of the egg is offset to a small degree (ca. 1%) by water uptake by the wet portion in contact with the brood pouch (81). As rigid-shelled bird eggs are unable to absorb much liquid water, it is assumed that this uptake is as water vapor.

Water uptake occurs in many squamate eggs, including those retained in the oviduct until hatching.

Egg retention in squamates typically involves simple apposition of maternal epithelial tissues to the eggshell and underlying extraembryonic membranes (82). The thickness and calcification of the eggshell also diminish with increase in egg retention (83). The simple chorioallantoic and yolk sac placentas thus formed facilitate gas exchange, but also participate in egg uptake of water, as well as uptake of ions from maternal fluids, including sodium, calcium, and in some species potassium (82).

The mechanism by which these constituents are absorbed or transported into the egg have not been studied. However, endocytosis has been observed in the chorial (external) epithelium of the chorioallantoic membrane in chicken eggs, suggesting that targeted mechanisms exist for recovery of water and ions external to the chorioallantois but within the eggshell (84,85). If similar mechanisms occur in parchment-shelled eggs, oviposited eggs may augment water flux into the egg via ionic uptake. Of particular interest is the recent observation that oviposited skink eggs (*Lampropholis* spp.) appear to take up environmental sodium, since the hatchlings contain nearly twice as much sodium as do freshly laid eggs (86).

Thus it appears feasible that cutaneous fluids applied to an eggshell could be absorbed. For example, egg dehydration would cause an increase in osmotic concentrations and hence a drop in water potential, creating a water potential gradient for uptake of liquid water. Net uptake of sodium, potassium, or other ionic constituents would also facilitate inward movement of water, especially if ion gradients developed among egg compartments. In the freshly laid chicken egg, most of the sodium and potassium are in the albumen layer, but these move into other compartments, including the allantois that serves for waste storage, during embryonic development (87). Other small organic molecules that might be absorbed and utilized by eggs include sugars and amino acids. Tracer studies have demonstrated the uptake of glycine and leucine by retained squamate eggs, but which membranes and structures are involved is debated (82,88,89).

An objection to fluid-coating of eggs is that the surface available for respiratory gas exchange would become restricted by fluid-filled pores. In oviposited squamate eggs most water uptake occurs in the first half of egg incubation, before embryonic respiratory needs are high (43,44). Studies on developing flexible-shelled eggs of sea turtles indicate that partial covering of the eggshell with petroleum jelly leads to an increase in embryo mortality, particularly if the affected

surface is that immediately over the developing embryo (90). The much greater oxygen consumption and chorioallantoic vascular density in endotherms (91) increases the importance of unimpeded gas exchange through pores overlying the chorioallantois. This suggests an advantage for regional specialization of eggshell surfaces, such as occurs in King penguins (see above) and in retained squamate eggs in which different extraembryonic membranes participate in a variety of structures involving apposition to maternal tissues (82). Even the relatively nonvascular abembryonic end (bilaminar omphalopleure) of squamate eggs may play a substantial role in nutrient uptake (88,92).

Evidence of Fluid Absorption by Monotreme Eggs

The eggs of extant monotremes may provide insight into ancestral synapsid eggs, so aspects of their structure and development will be summarized. Following ovulation, the monotreme ovum receives a thin overlay of mucoid glycoproteins (an albumen layer) as well as a compact basal layer and a fibrous rodlet layer, which comprise the inner layers of the eggshell (12,13). These layers are added as the egg passes through the infundibulum and Fallopian tube and then enters the uterus. At this stage the eggs are about 4–5 mm in diameter. Following development of a blastodisc at the embryonic pole, blastoderm spreads out to encircle the yolk mass; it is initially unilaminar but by cell migration and division becomes bilaminar, with overlying ectoderm and underlying endoderm (93). The overlying ectoderm layer is homologous to the trophoblast in marsupials and eutherians, and appears to play a key role in uptake of uterine secretions (13,93). Remarkably, the entire egg swells rapidly in size, apparently due to uptake of uterine secretions, and attains a diameter of about 15–17 mm prior to egg-laying. This represents a volume increase of about 33 fold. The eggshell copes with such swelling both by stretching and by incorporation of additional protein into the rodlet layer (13).

The constituents derived from uterine secretions, and absorbed during this phase of egg swelling, are believed to be the primary source of nutrients for the developing embryo during egg incubation (13,21,93,94). The embryo itself has reached a developmental stage of about 19–20 somites at oviposition. The third and thickest matrix layer of the eggshell is deposited towards the end of the intrauterine period, after most of the swelling of the egg is complete. However, this

final layer is also quite porous, containing clefts or channels that penetrate to the surface (12).

In the latter part of the intrauterine period, mesoderm spreads out between the two layers of the bilaminar blastocyst in the vicinity of the developing embryo, converting the membrane to a trilaminar vitelline membrane (1,13). Prior to egg laying, this trilaminar omphalopleure or vitelline membrane is restricted to the area proximal to the embryo, but during the incubation period it extends progressively over most of the yolk surface, except at the abembryonal pole where bilaminar omphalopleure persists. Luckett (1) considers the bilaminar omphalopleure as histologically specialized for nutrient uptake, because the ectodermal layer is columnar and contains both pinocytotic vesicles and larger vacuoles. By contrast, the surface ectoderm layer in the trilaminar vitelline membrane is thin and squamous, apparently to facilitate respiratory gas exchange, although the inner endoderm layer is columnar and vacuolated, reflecting its involvement in phagocytosis and absorption of the yolk (1). Vascularization develops only in the middle or mesoderm layer, and hence does not penetrate into the bilaminar layer at the abembryonal end. Thus monotreme eggs retain regional specialization of extraembryonic surface membranes throughout incubation.

A parallel situation occurs in marsupials, in which a similar bilaminar omphalopleure persists at the abembryonal end of the developing egg and is implicated in endocytosis and nutrient uptake from uterine secretions (1,77). The vascular trilaminar omphalopleure is the primary site of respiratory gas exchange.

During monotreme egg incubation, the trilaminar vitelline layer is split by encroachment of the exocoelomic cavity, and separates into the chorion (ectoderm plus nonvascular mesoderm) and the definitive yolk sac or splanchnopleure (vascular mesoderm plus endoderm). The expanding allantoic vesicle merges with the chorion to form the chorioallantoic respiratory structure (1,13). However, as neither this structure nor the vitelline membrane penetrate into the bilaminar omphalopleure at the abembryonal end of the egg, it appears that this part of the eggshell is relatively unimportant to respiratory gas exchange.

I conclude that monotreme eggs have a porous eggshell, regional functional specialization, and appropriate extraembryonic membranes for uptake of fluids, not only during the intrauterine period, but also during much or most of egg incubation. Whether they actually absorb fluids during egg incubation is

not known. Although data on the size of incubated eggs are limited, there does not appear to be a substantial expansion in volume or mass of eggs during incubation (13,21).

Incubated monotreme eggs are sometimes found to be covered with moist, sticky material of unknown origin, causing the eggs to adhere to mammary hair, and in the case of platypus eggs, to stick together after laying (21,72). Eggs obtained in utero, even if fully developed, contain only three eggshell layers (12,13,20,21,94). However, after laying a thin, superficial "fourth layer" comprised of organic matter with embedded foreign material has been observed on the outer surface of both platypus and echidna eggs (12). Hill in 1933 noted that "the material forming the superficial layer [of a laid platypus egg] is quite distinct from that of layer 3, and the fact that it contains foreign particles and that it sometimes passes continuously over the openings of the pore-canals suggest that it is laid down in a semi-fluid condition on the surface of the outer zone, and only hardens after the egg is laid." In echidnas, this layer is discontinuous and irregular, and contains foreign particles "so that it looks like a secondary deposit on the surface of the shell proper" (12). These potentially significant observations have been overlooked by more recent investigators (13,21).

Although this outermost layer may simply represent dried residual uterine fluids, an alternative explanation, that these materials derive from secretions of glands in the mammary areola, warrants investigation. Monotreme mammary glands attain a tubular structure prior to egg laying, and in this state are fully secretory, although relatively little milk is produced (95). Milk samples obtained from tubular-grade mammary glands before egg-laying, during incubation and shortly after hatching are dilute, containing about 88% water, little fat, and a variety of whey proteins (21,95,96).

If the mammary areola or patch of monotremes doubles as an egg-moistening area, the presence of mammary hairs and the absence of nipples may be of functional significance, since hairs would wick fluids onto eggs whereas nipples might inhibit fluid transfer from one surface (mammary areola) to another (eggshell) (3). In any event, monotremes represent a derived rather than primitive state relative to the origin of lactation, as monotreme eggs have very small yolks, the incubation period is very short (10–11 days), and the hatchlings are extremely altricial, being dependent on milk nutrients for a long period of posthatching development (21).

Is it possible that monotreme eggs absorb nutrients, as well as fluids, from applied glandular secretions during the incubation period? By analogy to squamate eggs, one might expect monotreme eggs to absorb constituents such as sodium, calcium, and possibly potassium from surface fluids, but this has not been investigated. Noting that glucose has been reported to cross the inner fibrous membrane of chicken eggs, Hughes (94) suggested that glucose and other macromolecules might have crossed a shelled egg in ancestral mammals as a first step in the evolution of viviparity.

CONCLUDING SPECULATION

The water needs of parchment-shelled synapsid eggs appear to preclude endothermic incubation in the absence of a warm, moist pouch environment that is close to vapor pressure equilibrium with egg contents, and/or regular replacement of egg water loss by surface-applied fluids. However, egg incubation in a pouch would place major locomotor constraints on incubating parents. It is hard to imagine that active and often predatory Triassic therapsids (64) could have carried eggs about in a pouch, without their being liable to crushing. It is more likely that egg moistening via lactation was already established at this point, allowing eggs to be left in a nest.

If so, how ancient is the origin of enhanced cutaneous secretion for parental care? If early glandular-skinned synapsids (3) kept in close body contact with eggs, as do most extant amphibians with terrestrial eggs (9), provision of cutaneous gland secretions to eggs could have been an early synapsid trait, dating back 300 million years or more. This span is consistent with date estimates for the origin of lactose synthesis (3), and may suggest that a primitive form of lactation is very ancient indeed. The ability of mothers to keep eggs moist while guarding them, and to replenish egg moisture losses after foraging trips, would have been advantageous long before the evolution of endothermy. Thus synapsids might have been preadapted to the evolution of endothermy by virtue of an established system of egg supplementation, just as the ancestors of birds were preadapted to endothermy by the presence of a rigid, calcified eggshell. Although lactation is a defining trait of extant mammals, it may have played a central role in synapsid evolution long before mammals came into existence.

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