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The Mammary Gland and Its Origin During Synapsid Evolution

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Lactation appears to be an ancient reproductive trait that predates the origin of mammals. The synapsid branch of the amniote tree that separated from other taxa in the Pennsylvanian (>310 million years ago) evolved a glandular rather than scaled integument. Repeated radiations of synapsids produced a gradual accrual of mammalian features. The mammary gland apparently derives from an ancestral apocrine-like gland that was associated with hair follicles. This association is retained by monotreme mammary glands and is evident as vestigial mammary hair during early ontogenetic development of marsupials. The dense cluster of mammo-pilo-sebaceous units that open onto a nipple-less mammary patch in monotremes may reflect a structure that evolved to provide moisture and other constituents to permeable eggs. Mammary patch secretions were coopted to provide nutrients to hatchlings, but some constituents including lactose may have been secreted by ancestral apocrine-like glands in early synapsids. Advanced Triassic therapsids, such as cynodonts, almost certainly secreted complex, nutrient-rich milk, allowing a progressive decline in egg size and an increasingly altricial state of the young at hatching. This is indicated by the very small body size, presence of epipubic bones, and limited tooth replacement in advanced cynodonts and early mammaliaforms. Nipples that arose from the mammary patch rendered mammary hairs obsolete, while placental structures have allowed lactation to be truncated in living eutherians.

KEY WORDS: mammary gland; cutaneous gland; evolution; Synapsida; monotreme; marsupial.

INTRODUCTION

A variety of theories have been put forth to explain the origin of the mammary gland and its secretion. Yet the absence of any structure representing an intermediate grade, the very long period of time over which mammary glands may have evolved, and the lack of any direct fossil evidence of mammary glands, makes it difficult to validate or disprove any theory.

The evolution of lactation has long been shrouded in mystery, even though it is a defining character of all mammals. In 1758 Linnaeus first recognized the uniqueness of mammary glands, and on this basis united terrestrial forms, formerly considered quadrupeds, with the aquatic cetaceans, formerly considered fish, in a new group he called Mammalia, or creatures with mammae or breasts (1). Although animals as diverse as sharks, salamanders, and skinks nourish their young via secretions of the uterus or oviduct, or via placental transfer (2–6), no other animal is known to secrete complex nutritive fluids from elaborate cutaneous glands as a way of feeding the young. How could such an intricate process, involving radical innovation in both mother and suckling young, come into being?

My intent in this review is not to enter into detailed discussion of the strengths and weaknesses of prior hypotheses (7), but to focus on several specific questions:

1. Within the long course of synapsid evolution that led to mammals, is there evidence in support of the appearance of lactation, and in

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which taxa? This approach requires a synopsis of synapsid evolution, so that the evolutionary themes and actors are identified.
2. What types of skin glands characterize synapsids, and could any of these be ancestral to mammary glands?
3. If ancestral skin glands initially evolved to meet the needs of synapsid eggs, as argued in an accompanying article (8), is there a plausible scenario to explain the sequence of events that led to mammary gland secretion as we know it?

This review employs a set of taxonomic terms and conventions that are in widespread use in paleontology and systematics but may be foreign to many neontologists (scientists who work on "new" or living taxa). The discipline known as cladistics or phylogenetic systematics requires any named taxonomic group or clade to be monophyletic, not only in the sense that the group be derived from a single ancestral taxon but also that all descendents of that ancestral taxon be included in that clade. For example, birds are believed to have descended from dinosaurs, and hence they are part of the dinosaur clade: by definition, birds *are* dinosaurs. The Synapsida—which begat Therapsida which begat Mammalia—includes both of the latter: mammals are therapsids and therapsids are synapsids. The terms synapsid or therapsid only exclude more recently evolved mammals if suitably qualified, such as Carboniferous or early synapsids (qualifying by time) or nontherapsid synapsids (qualifying by exclusion). Key taxonomic and zoological terms that may not be familiar to mammary gland biologists are defined, for easy reference, in Table I.

A HISTORICAL PERSPECTIVE ON THE ORIGIN OF LACTATION

Critics of evolutionary theory were quick to point out the difficulty in envisioning the gradual, stepwise evolution of lactation. Charles Darwin himself noted in the first 1859 edition of *On the Origin of Species*: "If it could be demonstrated that any complex organ existed, which could not possibly have been formed by numerous, successive, slight modifications, my theory [of evolution by natural selection] would absolutely break down" (9: p. 189). The eminent St. George Mivart took the bait: "Let us consider the mammary gland, or breast. Is it conceivable that the young of any animal was ever saved from destruction by accidentally sucking a drop of scarcely nutritious fluid from an accidentally hypertrophied cutaneous gland of its mother?" (10:p. 60).

Darwin rose to this challenge, devoting most of a chapter in the sixth 1872 edition of *On the Origin of Species* to Mivart's criticisms, including that of mammary evolution (11). Darwin noted that in seahorses (*Hippocampus* sp.) eggs are hatched and reared in a brood pouch, and he thought the young might be nourished by cutaneous secretions in this pouch. Believing that mammals are descended from animals with a pouch or brood sack, he asked "is it not possible that the young might have been similarly nourished? And in this case, the individuals which secreted a fluid, in some degree or manner the most nutritious, so as to partake of the nature of milk, would in the long run have reared a larger number of well-nourished offspring, than would the individuals which secreted a poorer fluid; and thus the cutaneous glands, which are the homologues of the mammary glands, would have been improved or rendered more effective . . . the glands over a certain space of the sack should have become more highly developed than the remainder; and they would then have formed a breast, but at first without a nipple, as we see in the *Ornithorhynchus* [platypus] at the base of the mammalian series" (11: pp. 295–296). The mammary patch or "areola" of the platypus, and the gross morphology of the platypus mammary gland (Fig. 1) had first been described in 1832 by Richard Owen (12), who was first to prove that monotremes lactated (13).

Although Darwin had seen the platypus in Australia in 1836 while naturalist on the Beagle voyage (13), the notion that this species laid shelled eggs was considered improbable as no other mammal was known to do so. The large paired oviducts and large ova suggested ovi-viviparity: that eggs were retained in utero until they hatch, after which the young emerge, much as in many squamates (lizards and snakes) (12,13). When W. H. Caldwell confirmed in 1884 that the monotremes (platypus and echidnas) were truly egg-laying, it became clear that monotreme reproduction included both egg-incubation and lactation, being a peculiar mix of avian and mammalian traits (14). If lactation could evolve in an egg-laying animal, and if this animal was like a platypus in lacking a pouch, Darwin's theory of the origin of lactation was in shambles. Darwin's theory of natural selection survived the challenge of its critics, but his specific defense of mammary gland evolution did not.

Table I. Reference List of Taxonomic and Specialized Terms Used in This Review

Term	Explanatory comment
Altricial young	Young that are at an immature developmental state, usually referring to the period after hatching or birth.
Amniota (amniotes)	A clade of tetrapods first observed in the Pennsylvanian and characterized by an amniotic egg (and other characters). This clade encompasses both synapsids and sauropsids, and thus includes extant "reptiles" (turtles, crococilians, squamates, tuataras), birds, and mammals.
Apocrine	Secretion in which secretory vesicles bulge out through the apical plasma membrane and break loose, carrying along plasma membrane and cytoplasmic fragments.
Clade	A taxonomic unit that is defined as a group of organisms that share derived characters (i.e. features modified from an original state) by virtue of common ancestry.
Cynodontia (cynodonts)	A clade of therapsids first observed in the late Permian and distinguished by an enlarged dentary bone in the lower jaw, differentiation of thoracic and lumbar vertebrae and other skull and skeletal characters. This clade includes subsequent mammaliaform and mammal radiations.
Diphyodonty	A condition in mammaliaforms (including mammals) in which the number of sets of teeth that develop sequentially is reduced to two: a deciduous set and a permanent set.
Ectotherm	An animal incapable of sustaining an elevated body temperature by internally generated heat, and hence that relies on environmental sources of heat for thermoregulation.
Exocytosis	A secretory process whereby secretory vesicles merge with the apical plasma membrane, causing the vesicular contents to be discharged from the cell surface into the gland lumen.
Endotherm	Animal capable of sustaining an elevated body temperature by internally generated heat (due to a high rate of metabolism).
Eutherian	A clade of extant mammals characterized by elaborate placental structures and thus commonly called placental mammals. This clade is worldwide in distribution and includes all domesticated species and nearly all mammals in North America, Eurasia and Africa. First observed in the Cretaceous.
Holocrine	Secretion in which secretory product accumulates within the cell and is only released into the gland lumen upon death and dissolution of cellular structure.
Mammalia (mammals)	A terminal or crown-group clade encompassing all extant monotremes, marsupials and eutherians, as well as their common ancestor and all of its descendents. Although distinguished from extant sauropsids by the presence of hair and mammary glands, these characters may predate this clade (see text).
Mammaliaformes (mammaliaforms)	A clade of cynodonts first observed in the late Triassic and distinguished by a jaw articulation between the dentary and squamosal bones (and other characters). In traditional usage, all mammaliaforms were considered to be mammals, but herein mammals are considered a subset of mammaliaforms (see text).
Marsupialia (marsupials)	A clade of mammals (including extant possums, opossums, kangaroos and other species) that give birth to very altricial neonates that attach to nipples. Many but not all species have a marsupium or pouch. This clade is largely restricted to Australasia and South America, but opposums venture into North America.
Monotremata (monotremes)	A clade of egg-laying mammals including the extant echidnas and platypus, restricted at present to Australasia but with fossil remains in South America.
Precocial young	Young that are of relatively mature developmental state, usually referring to the period after hatching or birth.
Sauropsida (sauropsids)	A clade of amniotes first observed in the Pennsylvanian and characterized by no or two lateral openings in the temporal region of the skull (and other characters). The term includes all extant "reptiles" and birds.
Squamata (squamates)	A clade of sauropsids first observed in the Jurassic and characterized by a highly flexible skull due to reduction of a variety of skull bones (and other characters). Among extant taxa, this clade includes lizards, snakes, and amphibaenians.
Synapsida (synapsids)	A clade of amniotes first observed in the Pennsylvanian and characterized by a single large opening in the lower temporal region of the skull (and other characters). The clade includes subsequent radiations (e.g. therapsids, cynodonts, mammaliaforms) leading to extant mammals.
Tetrapoda (tetrapods)	A clade of vertebrates first observed in the Devonian and distinguished by enlargement and modification of limb bones for locomotion on land (and other characters). Among extant taxa, the clade includes amphibians and amniotes.
Therapsida (therapsids)	A clade of synapsids first observed in the Permian and distinguished by strengthening of the skull, an increase in surfaces for attachment of jaw musculature, and other skull and skeletal characters. Primitive therapsids are believed to be ancestral to subsequent radiations, including cynodonts.
Turbinal	A scroll-like structure of cartilage and bone found in the nasal airways of some therapsids, including cynodonts and mammals.

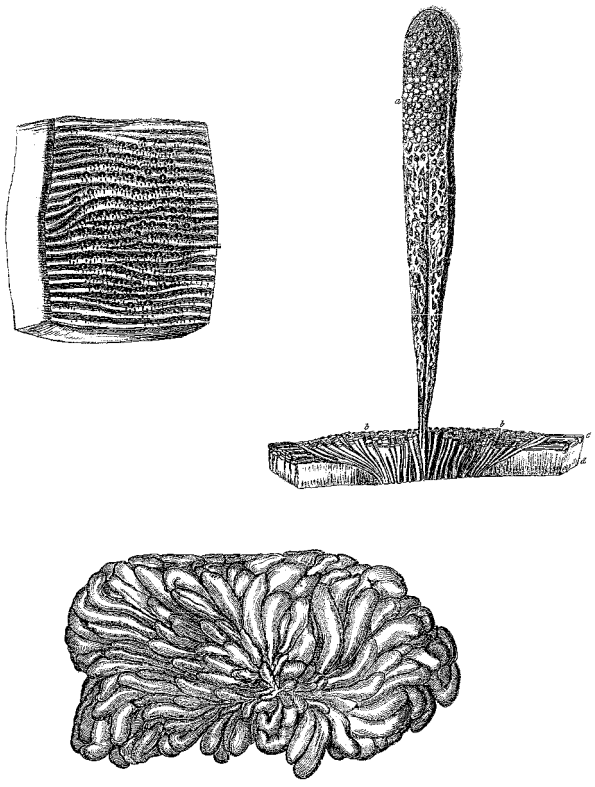

Fig. 1. The mammary gland of the platypus as illustrated in 1832 by Richard Owen (12). This work was known to Charles Darwin (13) but has been overlooked in recent studies of monotreme mammary anatomy (14). (A) Magnified view of the areola, with the mammary hairs removed, showing the orifices of the ducts of the mammary lobules. (B) Magnified view of one of the lobules (a), as well as extremities of the ducts of other lobules (b), muscle fibers (c), and integument (d). (C) Mammary gland in a state of full development. [From Owen (12)]

Since Darwin's time a series of hypotheses have been put forth to explain how lactation might have evolved among egg-laying predecessors of mammals (Table II). Gegenbauer (15) considered monotreme glands to be primitive, resembling sweat glands, but he had only nonlactating animals to examine. In his view monotreme mammary glands evolved from sweat glands while marsupial and eutherian mammary glands evolved from sebaceous glands. Bresslau (16–18) observed that mammary primordia developed very early in embryonic life, and underwent a period of slow development. He took this as evidence of the prior existence of a vascularized brood pouch, like that of birds. Gregory (1) thought that an oily fluid produced by "protolacteal" glands would have kept eggs warm, and if rich in "albuminous" material, might have caused the eggs to adhere to the brooding area. However, Haldane (19,20) suggested that the ancestors of mammals might have needed to keep eggs cool, and could have done this by moistening their fur by bathing, as some Asian birds moisten their feathers. Thirsty hatchlings would benefit from sucking on wet fur, including fur that had become moistened by sweat. And from this sweating, mammary secretions evolved. Long (21,22) pointed to the fact that monotreme eggs absorb uterine secretions prior to egg-laying, and if fluid absorption continued after egg-laying, the eggs of mammalian ancestors might have benefited from nutritive cutaneous secretions. Hopson (23) stressed that late Triassic mammals were so small that they would have had to have small eggs and altricial (immature) young if they were endothermic. Graves and Duvall (24,25) described how pheromones produced by cutaneous glands can induce nuzzling and licking, and suggested that this is how lactation started. Guillette and Hotton (27) regarded both egg retention and skin secretions as essential to survival of altricial young. Building on the work by Brew and others on the molecular similarity of α-lactalbumin and lysozyme, Hayssen and Blackburn (7,27) suggested that the function of cutaneous secretions was initially antimicrobial, and only subsequently became nutritional. More recently, Blackburn (28) argued that mammary glands share features with multiple gland types and could have evolved as a novel mosaic structure incorporating features of multiple types of skin glands, rather than evolving from a single population of glands.

These myriad hypotheses need not be mutually exclusive, and in fact some build on and extend prior approaches. Yet some suggest that mammary glands evolve from sweat glands, some from sebaceous glands and some from apocrine glands; Blackburn's (28) approach was "all of the above." In some scenarios, lactation evolved to assist with egg incubation, in others to provide for live young, and in others no preference is given: either or both are possible. Many scenarios disregard the fossil record, but those that do address mammalian ancestry argue that lactation had evolved in the earliest mammals but without specifying when lactation first appeared. Did lactation suddenly blossom on the evolutionary tree as an evolutionary novelty, or did it evolve gradually and incrementally, as Darwin thought? It is easy to be confused by the plethora of hypotheses, many of which sound attractive but have little predictive value, and cannot be falsified (7).

Table II. Theories on the Origin of Lactation

	Hypothesis and author	Evidence or analogy cited	Brief summary of proposed scenario
1872	Pouch origin [Darwin (11)]	Brood pouch of seahorses, pipefish	Liveborn young reared in marsupial pouch were provided secretions from generalized cutaneous glands, with subsequent specialization into discrete glandular areas within the pouch.
1886	Dual gland origin [Gegenbauer (15)]	Anatomy of pouch and mammary glands	Mammary glands evolved within pouches from "sweat glands" in monotremes but from sebaceous glands in marsupials and placentals. [Anatomy of pouches misinterpreted and nonlactating glands studied, leading to misleading conclusions (14,18).]
1907	Brood patch [Bresslau (16–18)]	Embryology; analogy to bird brood patch	Initial primordia of mammary glands represent brood patches that predate mammary glands; mammary glands derive from cutaneous glands associated with hair follicles but only remnants of follicular primordia still evident in some placentals.
1964	Wet hair [Haldane (19,20)]	Asian birds that provide chicks moisture on feathers	Endothermic "mammal-like reptiles" cooled their eggs with wet hair. Hatchlings sucked sequentially on hair wet from 1) maternal bathing, 2) cutaneous sweat, and 3) nutritious secretions.
1969	Egg supplement [Long (21,22)]	Uterine swelling of monotreme egg	Thin-shelled eggs, in incubatorium anterior to epipubic bones, absorbed moisture and perhaps nutrients secreted by cutaneous glands; secretions later lapped, then sucked, by hatchlings.
1973	Altricial neonate of endotherm [Hopson (23)]	Eggs of small birds; tooth replacement in therapsids and mammals	Endothermy and small body size in late Triassic mammals necessitated small eggs and hatchlings that had to be fed supplemental food. Secretions initially countered egg/neonatal dessication, later major nutrient source for young, leading to reduction in tooth replacement.
1983	Pheromones [Graves and Duvall (24,25)]	Fossil evidence of vomeronasal organs	Cutaneous glands produced pheromones to attract and aggregate neonates; nuzzling and licking of glands led to ingestion and subsequent evolution of nutritive function.
1985	Antimicrobial [Hayssen and Blackburn (7,26)]	Molecular similarity of α-lactalbumin and lysozyme	Survival of incubated eggs or hatchlings was enhanced by lysozyme and other antimicrobial compounds secreted by cutaneous glands. Some milk secretion pathways originated from preexisting antimicrobial constituents.
1986	Egg retention [Guillette and Hotton (27)]	Egg retention and viviparity in lizards and snakes	Egg retention resulted in laying of partly developed eggs or liveborn young. Incubation patch secretions initially protected against dehydration, but subsequently allowed highly altricial state. Monotreme egg represents one of several ancestral states.
1991	Hybrid gland [Blackburn (28)]	Effect of mesenchyme on cytodifferentiation	Regulatory changes in gene expression led to formation of novel mosaic structure incorporating features of multiple skin glands; otherwise as ref. 7.

LACTATION IN A PALEONTOLOGICAL CONTEXT

The Synapsids: The Beginning of the Separate Lineage Leading to Mammals

Any evolutionary scenario to be rigorous should be imbedded within an understanding of the evolutionary history of the organisms, as revealed by the fossil record. Although mammary glands have not been observed in the fossils of early mammals or their predecessors, the remarkable recent discovery of fur in the earliest eutherian fossils, from 125 million years ago (mya) (29), suggests that it could yet occur. Mammary glands have been recovered from extinct woolly mammoths frozen in Siberia (J. Shoshani, pers. comm.), but these are much too recent to reveal anything about the origin of lactation.

The separate ancestral line that would one day produce mammals is first found in fossils of the middle Pennsylvanian, about 310 mya. These small lizard-like creatures had a "window" or fenestra in the temporal region of the skull, and are called synapsids. They were preserved inside stumps of giant lycopodophytes (club mosses) along with other early terrestrial forms from which they did not differ greatly in structure (30,31). The synapsids are one branch of the earliest terrestrial vertebrates, the amniotes, so called in

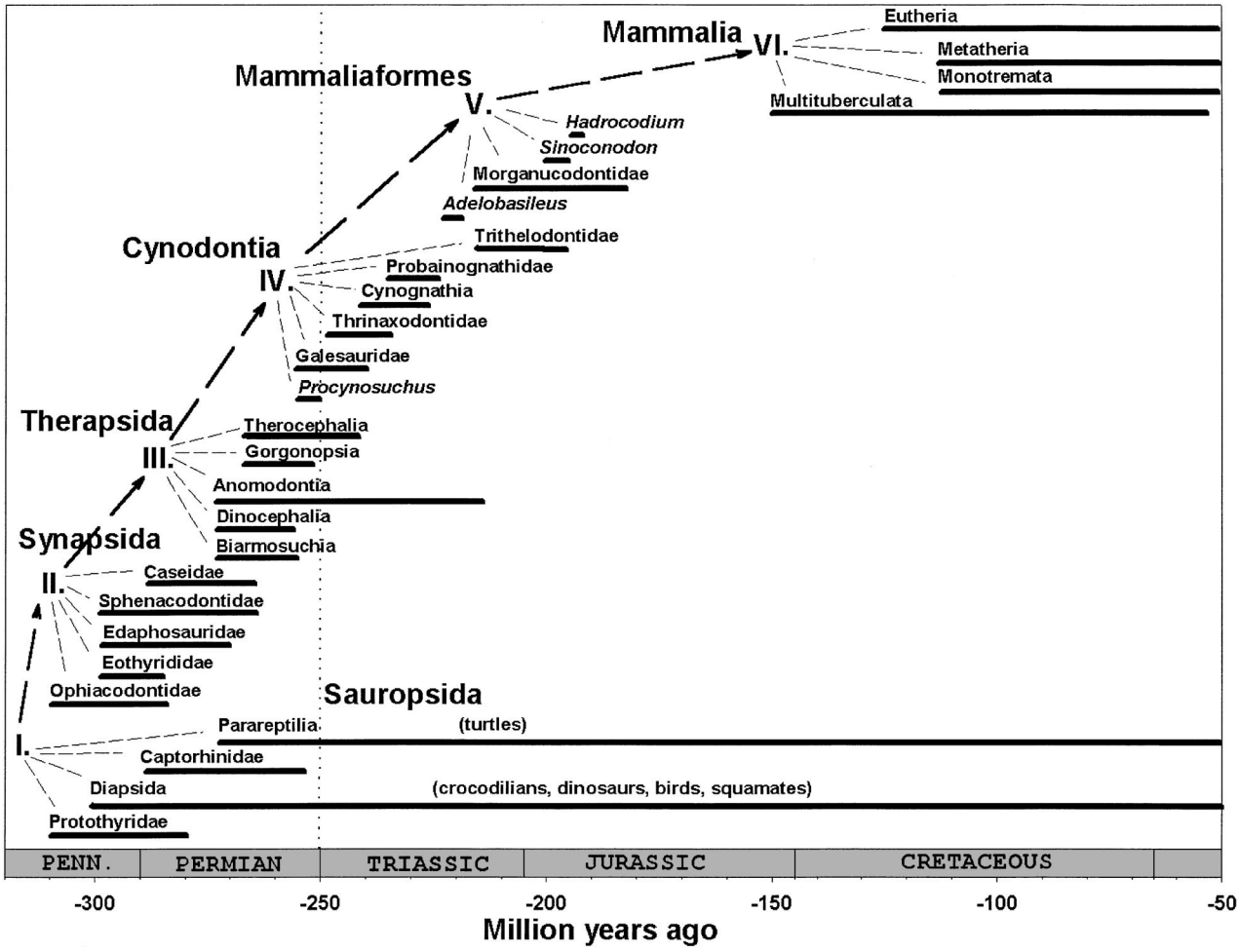

Fig. 2. A diagrammatic representation of sequential radiations beginning with Amniota (I) and concluding with Mammalia (VI). Note that each successive radiating clade derives from, and is a subset of, the preceding clade; both Synapsida and Sauropsida are subsets of Amniota (I). The figure illustrates some major and notable representatives of each radiation (as indicated by dashed radiating lines), but omits a number of taxa. The bold horizontal lines indicate the appearance and approximate duration of each taxon in the fossil record. Geologic ages and the end-Permian massive extinction (vertical dotted line) are indicated above the *x*-axis. The inclusion of turtles within Parareptilia is controversial. [Information primarily from Refs. 29–31 and 33–37]

recognition of the newly evolved amniotic egg. Although the eggs of prior tetrapods had been restricted by constraints of gas exchange and moisture loss to an aqueous or very wet environment, the amniotic egg had additional extraembyonic membranes and outer layers that facilitated gas exchange, nutrient utilization, waste storage, and/or water retention (32).

The synapsids are among the earliest amniotes to appear in the fossil record. Other early amniotes included creatures without any skull windows, that may have been ancestral to turtles, and some with dual temporal fenestrae, the diapsids, that were ancestral to extant squamates (lizards, snakes, amphisbaenians), rhynchocephalians (tuataras), crocodilians, and birds (Fig. 2). The split between the Synapsida and the remaining taxa (collectively termed the Sauropsida) is thus more than 310 million years old. To put this in perspective, the first evidence of bone (a vertebrate characteristic) is dated to only 200 million years earlier, in the late Cambrian (31). The synapsid clade has been separate from other vertebrate clades for about 60% of the time that vertebrates have been in existence.

It is important to recognize that mammals did not evolve from reptiles, but rather that both evolved from the earliest amniotes. We should not expect living reptiles to bear close resemblance in anatomy, physiology, or behavior to mammalian forebears.

Unfortunately, the nonmammalian synapsids were long called "mammal-like reptiles" (30,38), a holdover from a time when early amniotes (whether synapsid or sauropsid) were called "reptiles." This misleading term led many to assume that mammals and mammary glands evolved from a reptile-like creature with a scaly, mostly nonglandular epidermis and calcified eggshells. I believe both of these assumptions are incorrect.

Transformation of the Synapsids

In the 300+ million years since their first appearance, the synapsids have undergone repeated radiations and extinctions (Fig. 2), producing the dominant fauna in the Permian and Triassic until displaced by the dinosaurs in the late Triassic. This time also saw the emergence of the first mammal-like or mammaliaform synapsids at about 225 mya. True mammals initially radiated during the Jurassic and Cretaceous, while dinosaurs were in their heydey, and finally blossomed in the Tertiary, after the dinosaurs had been wiped out by the ecological catastrophe at the end of the Cretaceous.

Lactation evolved at some point during this long period of separation, presumably in response to selective pressures that favored increased parental investment in the young (39). Yet what were those pressures, and why would they favor transformation of skin gland secretions?

It is important to realize that a large number of transformations were occurring over the long evolutionary history from basal synapsid to early mammal. Those evident in the fossil record are of course best studied (30,34,36,37,40,41). Many of these reflect changes in diet (especially predatory specialization), locomotor ability, and energetic demands. For example:

1. The number, size, and sutural contact between skull bones changed to accommodate increased mass and power of the jaw musculature.
2. The jaws themselves changed greatly, with enlargement of the dentary bone in both posterior and dorsal directions to accommodate increased musculature.
3. The teeth became increasingly diversified and complex as they accommodated dietary specialization.
4. A change in posture, from a lizard-like lateral sprawl to a more upright stance, is evident in the structure of vertebrae, pectoral and pelvic girdles, and limb bones. By dorsoventral flexure of the spine and fore-and-aft limb movements in a vertical plane, mammalian ancestors achieved greater running ability, whether in pursuit of prey or in flight from predators.
5. Coupled with improved locomotor skills came an apparent improvement in aerobic respiration. The ribs became restricted to the thoracic region, presumably in support of diaphragmatic breathing.
6. A secondary hard palate evolved, separating nasal air flow from the oral cavity, and allowing animals to breathe while holding food in the mouth (and, in combination with fleshy cheeks, allowing neonates to suckle).
7. A network of cartilage and bone, termed the respiratory turbinals, developed in the nasal cavity to permit moisture and heat exchange, which may have been important for maintenance of homeothermy as metabolic rates increased.
8. The pattern of mineral deposition in long bones changed from a banded pattern, reflecting seasonal growth arrest, to a more uniform, highly vascularized structure that indicates more rapid and sustained growth.

These transformations did not occur at once. The fossil record of the synapsids is remarkably good, and reveals repeated radiations of taxa that incorporate an increasing number of mammalian characters (30,34,36). The gradual appearance of these characters in the fossils leading to mammals is often touted as a prime example of gradual, continuous evolution (31,42), and suggests correlated progression, whereby a host of characters are gradually transformed in an interdependent fashion. It is likely that such a major innovation as lactation also evolved gradually, rather than by a saltational jump, and that its transformation into an intensive period of nutrient transfer was correlated to the evolution of other features that now typify mammals, such as an elevated metabolic rate, high aerobic capacity, rapid processing of nutrients, and rapid rates of growth.

Premammalian Radiations

As indicated in Fig. 2, extant mammals are the end result of a series of evolutionary radiations that occurred more or less sequentially. The initial

amniote radiation (I) separated the synapsids from the sauropsids. At about the same time—the late Pennsylvanian (also known as the Upper Carboniferous) and the succeeding Permian—the synapsids radiated into a variety of basal groups (II), ultimately producing the therapsids. The primary therapsid radiation (III) occurred in the late Permian. At the end of the Permian an unprecedented mass extinction affected virtually all taxonomic groups, including therapsids (43). A few therapsid taxa survived the end-Permian crisis, including the cynodonts which radiated in the subsequent Triassic (IV). By the end of the Triassic, cynodonts included forms that were very mammal-like in dental, cranial and skeletal features. These mammaliaforms radiated in the late Triassic and Jurrasic (V), ultimately producing true mammals by the late Jurassic or Cretaceous (VI). In fact, many authors have considered all mammaliaforms as early mammals, as they had a functional dentary-squamosal jaw joint (see below). The three taxonomic clades (monotremes, metatherians [= marsupials], and eutherians [= placental mammals]) that are still extant, and thus constitute the crown-group Mammalia, first appear in the fossil record in the Cretaceous, but may have diverged earlier, in the Jurassic (Fig. 2). The many radiations of marsupials and eutherians that occurred in the Cretaceous and thereafter (29,44) are beyond the scope of this review, as lactation was well established before this time.

To set the stage for a discussion of the origin of lactation, I will briefly review what has been inferred from structural remains about reproductive, physiologic, and ecologic traits within each radiation. Descriptions and illustrations of these taxa may be found in references 30,31 and 33,34, and their temporal distribution is indicated in Fig. 2.

The initial amniote radiation began over 310 mya in the middle of the Pennsylvanian, a time of vast swamps and riverine forests containing giant sphenophytes (e.g., horsetails), huge lycopodophytes (e.g., clubmosses), and diverse pteridophytes (e.g., ferns and seed ferns). Early amniote fossils have been found in North America and Europe, which at that time were close to the equator and were warm, humid, and tropical (45). The amniotes derived from tetrapods that were already adapted to terrestrial locomotion, but amniotes are generally thought to have been less moisture-dependent, and thus able to occupy habitats further from water. However, fossils of both groups are often found together. On the basis of jaw structure, the amniotes mostly fed on small prey, such as insects and other invertebrates; they presumably had low metabolic rates, relied on behavioral thermoregulation, and grew slowly, like modern ectotherms (38). The presumption that amniotes laid amniotic eggs is not based on fossil evidence, but on the fact that all extant amniote groups (turtles, squamates, rhynchocephalians, crocodiles, birds, and mammals) include taxa that produce eggs of similar structure (8).

The basal (i.e. nontherapsid) synapsids have traditionally been called "pelycosaurs." The earliest representatives, such as *Eothyris* (Fig. 3(A)) and ophiacodontids, were relatively small and differed little in anatomy or habitat from other basal amniotes, but the synapsid radiation in the Permian produced a variety of large carnivores and herbivores, including a group of herbivorous caseids that appear to have occupied drier, upland habitat (30). At body masses of 200–300 kg or more, the larger "pelycosaurs" were dominant members of terrestrial ecosystems (33). Large sail-backed forms developed in both the Edaphosauridae and Sphenacodontidae; the sails apparently served as heat collectors or dissipators. In general, "pelycosaurs" retained primitive features in posture, locomotion, metabolism and growth (30,46), and apparently produced parchment-shelled eggs (8). Most of these basal synapsids became extinct by the end of the early Permian, although more advanced forms, such as sphenacodontid carnivores and caseid herbivores lingered into the lowest part of the late Permian (Fig. 2).

The therapsids first appear at the outset of the late Permian as a variety of carnivorous and herbivorous forms (Fig. 2). Even the early therapsids such as *Biarmosuchus* (Fig. 3(B)) exhibit strengthening of the skull, advances in dentition and skeletal changes consistent with increased dietary specialization and locomotor improvements; these become more pronounced in later taxa (30). The one supercontinent, Pangea, moved northward towards the equator during the Permian causing the climate to become increasingly hot and arid. Deserts became widespread in the late Permian, and the global flora underwent a profound transformation as moisture-loving plants such as tree clubmosses and peat-forming plants were replaced by newly evolved taxa, such as conifers (43,47). Some early therapsid taxa, such as dinocephalians and primitive anomodonts, became extinct during the late Permian but were replaced by new radiations of carnivorous gorgonopsids and therocephalians, as well as herbivorous advanced anomodonts (dicyonodonts; not shown in Fig. 2). The most mammal-like of the

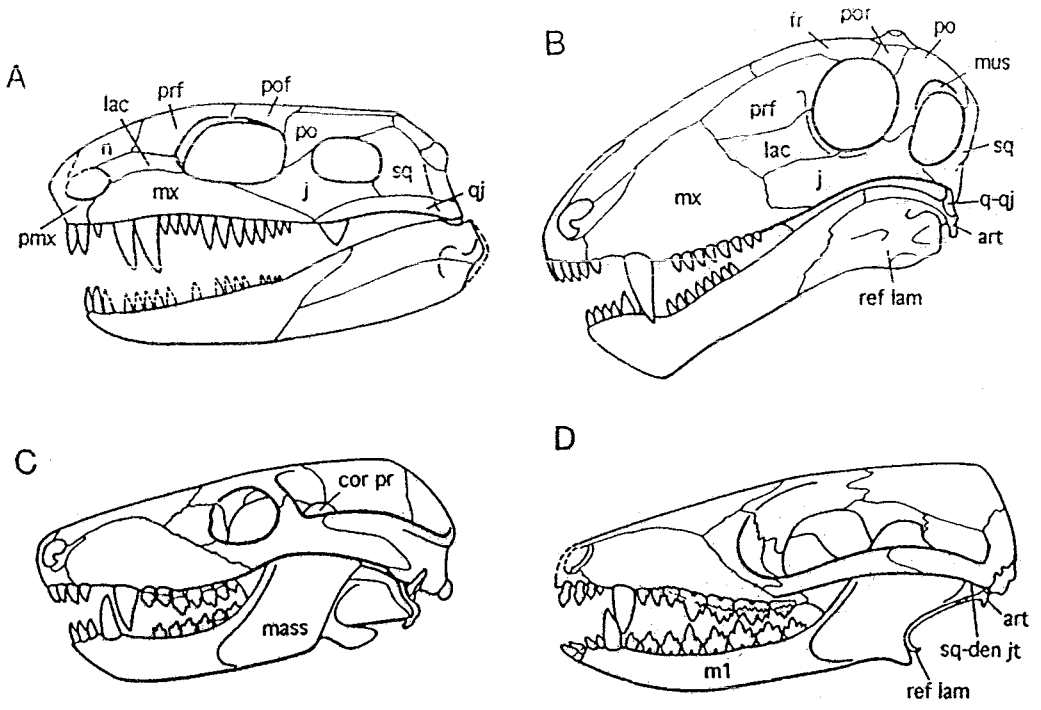

Fig. 3. Skulls representing successive synapsid radiations. (A) A basal synapsid, *Eothyris*, of the early Permian. Note the temporal fenestra (window or opening) behind the orbit. (B) A biarmosuchid therapsid, *Biarmosuchus*, of the late Permian. Note the increased size of the anterior bone (dentary) in the lower jaw. (C) A thrinaxodontid cynodont, *Thrinaxodon*, of the early Triassic. Note the large posterio-dorsal projection of the dentary as a coronoid process (cor pr) for muscle attachment. (D) A mammaliaform, *Morganucodon*, of the early Jurassic. Note the dentary-squamosal jaw articulation (sq-den jt) and the complex dentition. Skulls not to scale. *Abbreviations*: art, articular; cor pr, coronoid process of dentary; fr, frontal; j, jugal; lac, lacrimal; mass, fosseter for masseter muscle attachment; m1, first lowar molar; mus, facet for adductor muscle attachment; mx, maxillary; n, nasal; pmx, premaxillary; po, postorbital; pof, postfrontal; prf, prefrontal; q, quadrate, qj, quadratojugal; ref lam, reflected lamina; sq, squamosal; sq-den jt, squamosal-dentary jaw joint. [Modified from Hopson (34)]

therapsids, the carnivorous cynodonts, are first found toward the end of the late Permian.

The end of the Permian was marked by a massive extinction; 70% of all known genera of both marine and terrestrial organisms disappeared (43). Most therapsids were similarly eliminated, but representatives of three groups survived. Therocephalians endured past the end of the Permian, but became extinct in the Lower Triassic. Advanced anomodonts (dicynodonts) and cynodonts survived to become abundant and diverse through most of the Triassic (30,48). These three groups of advanced therapsids are characterized by a large number of mammal-like traits, including several associated with endothermy and elevated energy expenditure. In some dicyonodonts and cyonodonts the presence of well-vascularized fibrolamellar bone suggests high rates of bone growth and remodeling, but many specimens also contain rings of slower-growing lamellar bone (49,50). The development of a bony secondary palate in some therocephalians, in dicyonodonts and in cynodonts provides both strengthening of the skull and the opportunity to breath while the mouth is full (51). Respiratory turbinals appear in the anterior nasal cavity of therocephalians and cynodonts (52); the only known function of these structures is water conservation in animals with high respiratory rates (53). All three of these taxa evolve profound modifications of tooth, jaw, skull, and skeletal structure that appear to reflect dietary specialization and improved feeding efficiency, which would be important if these animals had to maintain high levels of food intake to support an elevated metabolic rate. As discussed in an accompanying paper (8), the development of elevated body temperature and endothermy may have been linked to the evolution of lactation.

The cynodont radiation in the Triassic (Fig. 2) is of particular interest due to the progressive changes in the jaw, skull, and skeleton that produced increasingly mammal-like forms (30,34,36). In the lower jaw

the dentary bone expands dorsally as a coronoid process and provides the site of attachment for a powerful new adductor muscle, the masseter (as in *Thrinaxodon*, Fig. 3(C)). It also expands in a caudal direction, displacing postdentary bones which shrink in size, loosen and assume a function in hearing. Remarkably, the dentary comes to make contact with a skull bone, the squamosal, and this contact evolves into a functional jaw joint (as in *Morganucodon*, Fig. 3(D)), replacing in the process the prior jaw joint between the articular bone of the lower jaw and the quadrate bone of the skull. Some early mammaliaforms, such as *Morganucodon* and *Sinoconodon*, represent transitional forms that had both jaw joints. In *Hadrocodium* and subsequent mammaliaforms the newly independent articular-quadrate joint is incorporated into the middle ear as the malleus and incus (31,37). Thus the mammalian middle ear derives from an earlier cynodont jaw joint!

Paleontologists have traditionally regarded the establishment of the mammalian-type jaw joint as the event which defines the advent of mammals (30,31,34). However, it is now common to follow cladistic practice and restrict the use of Mammalia to the "last common ancestor and its descendents" of the three living mammalian groups: the monotremes, marsupials, and eutherians (37). This is largely a semantic issue with little bearing on the origin of lactation, as lactation appears to predate not only the Mammalia as so restricted, but also the Mammaliaformes (see below).

Mammaliaforms and the Evidence for Lactation

The first mammaliaforms of the Triassic and Jurassic were very small insectivores, ranging in size from that of the smallest of living shrews (2–3 g, *Hadrocodium*), through that of mice or small hamsters (30–90 g, morganucodontids), to that of large rats (up to 500 g, *Sinoconodon*) (37). They were undoubtedly active endotherms, probably nocturnal, and apparently agile climbers (29,37). Small size and elusive habits may have been important to survival in a world that came to be ruled by a plethora of dinosaurs, beginning in the late Triassic. In metabolic rate these mammaliaforms may have resembled modern tenrecs that sustain metabolic rates well above reptilian, but below that of many modern mammals (54). As small endotherms they would, by necessity, have been well-insulated by dense fur. Dense fur has now been observed in exceptional fossils of small eutherian mammals from the early Cretaceous (29).

It is generally agreed that oviparity (egg-laying) can evolve into viviparity (live-bearing), at least among lines with relatively permeable, parchment-shelled eggs, via a sequential increase in the duration of egg-retention, as is seen in lizards and snakes (3). However, once committed to viviparity and its requisite reduction in thickness of the eggshell membrane, amniotes do not appear to be able to revert to oviparity. Thus the common ancestor of monotremes (which lay eggs) and therians (marsupials plus eutherians, which do not lay eggs) was presumably egg-laying, a feature of great importance to the evolution of lactation (8).

The biochemical, ultrastructural, developmental, and histological similarities of the mammary glands and mammary secretions of extant monotremes, marsupials, and eutherians provide convincing evidence that lactation had a common origin which predated the divergence of these groups (14,55,56). The first monotremes, marsupials, and eutherians appear in the early Cretaceous (Fig. 2) and we can assume their common ancestor, presumably a Jurassic form, lactated.

A dependence on lactation may be indicated by an osteological character, the epipubic bones. The paired epipubic bones articulate with the pubic bones and project forward and ventrally into the abdominal cavity. According to a modified "marsupium support hypothesis" these mobile bones provide support within the abdomen for the mass of developing young in a pouch (in pouched forms) or for the mass of suckling young attached to nipples (in pouchless marsupials) (57). They may also function in locomotion. Among living mammals, epipubic bones are only found in monotremes and marsupials, and they tend to be longer and/or wider in females than in males (57,58). In extant eutherians these bones have been lost, or, by one theory, have survived in altered form as the os bacculum and os clitoris (59). However, epipubic bones were present in some advanced cynodonts, such as tritylodonts (not illustrated), as well as a diverse array of Mesozoic mammaliaforms, multituberculates, and the earliest eutherians (29,58,60,61). If the purported function of epipubic bones is correct, these taxa must have transported eggs and/or dependent offspring in a pouch or attached to nipples. The importance of a pouch-like structure to prevent egg desiccation, and the hypothesized incompatibility of nipples and fluid provision to eggs (8), suggests that epipubic bones initially evolved to support eggs and/or suckling hatchlings in a pouch, much as in echidnas. The support of suckling young attached to nipples

(with or without a pouch) was presumably a later therian development. However, in eutherians the increase in fetal size consequent to placental evolution would rob epipubic bones of their function in supporting external young, and they may even have interfered with growth of the gravid uterus. Their loss in later eutherians is thus consistent with their purported function.

The transport of dependent young in a pouch implies parental feeding, but does not prove that the food was milk. Hopson (23) argued that as early mammaliaforms were both very small and endothermic, they would have been compelled to produce altricial (immature) young, as do small birds. To be capable of endothermy, newly hatched precocial young would need to be of such large size, relative to the small mother, that egg size would have been prohibitive. Hopson (23) therefore proposed that early mammaliaforms must have laid small-yolked eggs that produced small altricial hatchlings, and that these would have been incubated in a warm, humid environment. The young could survive only if given supplemental food, either directly (as in birds) or by means of special secretions (milk).

The pattern of tooth replacement also suggests prolonged dependence on parents during ontogeny. In basal synapsids and in most nonmammaliaform cynodonts, teeth were replaced continuously, usually in alternate waves (30,62). Any age class would have a fully functional dentition. Although individual teeth might be missing due to ongoing replacement, animals of all ages could presumably have fed independently (30). The Early Jurassic mammaliaform *Sinoconodon* also had multiple replacements of incisors and canines and apparent replacement of cheek teeth, but other known mammaliaforms in the early Jurassic had a single replacement of anterior teeth and developed permanent molars (34,63). The stability of a limited replacement pattern allowed greater interdependence among individual teeth. Thus early mammaliaforms evolved interlocking arrangements between neighboring teeth and precise occlusion and wear between opposing teeth, while in subsequent taxa the cheek teeth became highly specialized, with precise matching of complex cusp and basin structures (30,34,44). Yet diphyodonty (twofold teeth) entailed a delay in the eruption of deciduous or "milk teeth" until the juvenile jaw was a substantial proportion of adult jaw size. Calcium, phosphorus, and other nutrients would have had to be provided in considerable amount and over a prolonged period to support skeletal growth prior to self-feeding. Diphyodonty would have required well-developed lactation or a comparable method of nutrient provision (23,64).

I argue elsewhere, on physiologic grounds, that the endothermic therapsids that gave rise to mammaliaforms in the Triassic were egg-laying, pouched and provided moisture to eggs via cutaneous secretions (8). The small body size, epipubic bones, and diphyodonty of mammaliaforms imply that the young were hatched in an immature state, and dependent on parental provision of nutrients for a prolonged period. Milk production, complete with α-lactalbumin, casein, lipids, lactose, and perhaps oligosaccharides had arisen prior to the last common ancestor of monotremes, marsupials and eutherians, i.e., no later than the Jurassic. It takes no great leap of faith to conclude that it was nutrient transfer via lactation that allowed the young of early mammaliaforms to become altricial.

If lactation was already well developed as a means of nutrient transfer by the late Triassic, when mammaliaforms first appear, it is probable that milk had been progressively evolving as a nutrient-enriched fluid among early Triassic cynodonts. This is consistent with the view that mammary secretions must have been providing moisture to eggs before endothermic incubation could evolve (8). All three groups of therapsids that persisted beyond the end-Permian mass extinction, the dicynodonts (Anomodontia), the therocephalians, and the cynodonts (Fig. 2), have features suggestive of partial or complete endothermy (8) and hence all three may have produced skin secretions for their eggs.

The opportunity to recruit a skin secretion being produced for eggs into a new functional role as hatchling food may explain why mammals, unlike birds, never developed specialized secretions from hypertrophied pregastric glands for feeding their young. Altricial bird hatchlings are often fed from the mouth of attending parents, either by regurgitation or by direct prey transfer. To supplement such food, a few birds have developed nutritious secretions for feeding of chicks, such as the crop milk produced by holocrine secretion by doves and pigeons, by flamingos, and by emperor penguins (65–67). The advantages to these birds are similar to those for mammals: the hatchlings can be very altricial and receive easily digested food (e.g. pigeons and doves), they can attain larger size prior to developing the morphological features needed for a specialized diet (e.g. filter-feeding flamingos), and they can be fed at a distance from a remote food supply by mobilizing stored body reserves (e.g., emperor penguins). Yet each of these secretions

is delivered via mouth-to-mouth transfer, reflecting the type of parental-young interaction that predated crop milk secretion. I suggest that natural selection might have favored the evolution of specialized lingual, esophogeal, or gastric glandular secretions for feeding mammalian young if therapsids had not already had a functional secretory system based on cutaneous glands.

EVOLUTION OF GLANDULAR SECRETION FROM THE SKIN

The mammary glands of living mammals are large, intricate glandular systems capable of producing large volumes of nutrient-rich, complex secretions that vary greatly among taxa and, in some taxa, over the course of lactation (68,69). Yet they must have evolved from simple cutaneous glands in early synapsids or therapsids. To examine how this could be possible, I explore the likely primitive condition of synapsid skin and glands, contrast this to sauropsid skin and glands, and compare the major cutaneous glands in mammals. Lastly, I review the early embryological development of mammary glands in monotremes, marsupials, and eutherians seeking evidence of parallels to other skin glands.

Tetrapod Predecessors of Synapsids

Synapsids inherited an integument from tetrapods that was presumably modified to permit greater independence from water. The integument of the earliest tetrapods may not have been particularly resistant to transcutaneous water loss (70). Extant amphibians have a very thin stratum corneum, consisting of only one or a few keratinized cell layers, but this may be a derived rather than primitive condition, as it facilitates transcutaneous respiration (71). In contrast to fish integument, which is populated by unicellular epidermal glands, amphibian skin contains an abundance of multicellular flask-shaped or alveolar glands that form by the downgrowth of ectoderm into the underlying dermis (72,73). These glands are of two primary types: mucous glands, which are relatively small and enclosed within the upper dermal layer (the stratum spongiosum), and granular glands, which are somewhat larger and may project into the lower dermal layer (the stratum compactum) (73). Cells of both gland types secrete by exocytosis of vesicular contents into the gland lumen, but the granular glands may also release entire cellular contents by bulk discharge (holocrine secretion) upon the contraction of adjacent myoepithelial cells (74). Myoepithelial cells surround the secretory cells of amphibian granular glands, as well as the mucous glands in caecilians, a group of specialized burrowing, legless amphibians. Thus the primitive glands inherited by synapsids were likely multicellular, associated in some cases with myoepithelial contraction, and secreting by exocytosis and to a lesser extent by holocrine mechanisms.

In extant amphibians the primary secretory products of mucous glands are mucus-forming glycoproteins, while those of granular glands include an array of toxic compounds, including polypeptides, bufogenines, alkaloids, and aromatic amines (75). Granular glands are probably a primitive character among amphibians (73), but the toxins are complex and species-specific. Granular glands may have originally evolved for a purpose other than predator defense, such as antimicrobial activity to protect moist skin (76). Mucus discharge helps protect against abrasion, and by keeping the skin moist, facilitates transcutaneous respiratory gas exchange. However, some species, such as toads, produce less mucus and have relatively dry skin, while others, such as some tree frogs, may increase mucus discharge at high temperatures to facilitate evaporative cooling (77). A few arid-adapted frogs (Hylidae: *Phyllomedusa*) have a type of lipid-secreting gland in addition to mucus and granular glands. These alveolar lipid glands are profuse in many parts of the integument, and like the mucus and granular glands, have a distinct myoepithelium (78). The frogs spread the secreted triglycerides and wax esters over the epidermis by a wiping motion, and thereby greatly increase the resistance of the skin to moisture loss (73,77,78). At rest these frogs lose water at a rate that is only 5–10% of that typically seen in amphibians. Unfortunately, the mechanism of lipid secretion has not been reported.

Sauropsids: Extant Reptiles, Including Birds

If the Carboniferous ancestors of amniotes maintained moist glandular skin similar to that of extant amphibians, the transition to drier environments would have placed a premium on reducing moisture loss. The solutions adopted by the two major lineages, the sauropsids and synapsids, appear to have differed. In sauropsids the epidermis developed a thick stratum corneum that became folded into discrete keratinized

scales (72,79). The scales provide protection against abrasion, support against torque, and resistance to water flux. The latter is primarily due to lipid incorporated into intermediate layers rather than to the thickened stratum corneum per se, because keratin does not provide much resistance to moisture loss (71). An especially intricate cyclical pattern of maturation and shedding of the keratinocytes developed among squamates (lizards and snakes). Six discrete layers differentiate and are shed as a unit, including a lipid-rich mesos layer sandwiched between an overlying syncytial layer of β-keratin and an underlying layer of α-keratin (79). This is a far more complex keratinization process than that seen among extant synapsids (i.e., mammals), and is clearly a derived rather than primitive condition. Sauropsid skin is not an intermediate form between amphibians and mammals, but represents a separate evolutionary approach.

The relatively impervious integument of sauropsids is accompanied by a great reduction in cutaneous glands, which are restricted to specific areas, such as inguinal, femoral and chin surfaces. These glands typically secrete by the death and dissolution of cells lining the gland lumen (i.e., holocrine secretion), and the small amount of semisolid, lipid-rich product may be important for sociosexual communication (80,81). However, some sauropsids also have larger cloacal or musk glands of varied morphology and more voluminous output.

Birds resemble other sauropsids in having few integumentary glands. In the thickness and organization of the superficial horny layer birds resemble crocodilians (72), but they have multiple types of scales as well as feathers, which are highly derived scales (82,83). In most birds, a large holocrine gland, the uropygial or preen gland, secretes lipids that birds apply to their feathers by preening, thereby creating a water-repellant structure (84). Another special feature of avian skin is the amount of lipid that accumulates within keratinocytes during differentiation and maturation. These cells are classified as sebokeratinocytes due to the dual function of lipid and keratin accumulation. Stettenham (83) suggests that the entire skin of birds "acts as a sebaceous secretory organ, with the preen gland and ear glands as specialized parts."

Integument and Cutaneous Glands of Synapsids, Including Mammals

Many biologists have assumed that "mammal-like reptiles" (basal synapsids and nonmammalian therapsids) were endowed with keratinized scales; in fact Spearman (85) argued that since hair and hair follicles are not homologous to scales, they must have evolved in the hinge region between scales. As scales will form fossilized impressions under appropriate conditions, much information has been obtained on the scale patterns of extinct sauropsids, including a diverse variety of dinosaurs (86). Yet no such scale impressions have been found for either basal synapsids or therapsids, despite an extensive fossil record (30,34). One remarkable find of fossilized integument of a very early Permian therapsid, *Estemmosuchus* (Dinocephalidae), may explain why. Skin from the head of this dinocephalian reveals a dense pattern of concave lens-like structures. The detailed structure of these lenses is suggestive of multicellular, flask-shaped alveolar glands, such as in amphibian skin (87). Chudinov (87) concludes that therapsids had scaleless glandular skin, and argues that such integument is a primitive synapsid feature still evident in mammals.

The need to minimize cutaneous water loss would have favored lipid barriers both in differentiating keratinocyte layers (as in squamates and birds) and on the skin surface (as in lipid-secreting frogs). Thus the skin glands in early synapsids or nonmammalian therapsids may have been lipid-secreting as well as fluid-secreting. Although early synapsids probably developed considerable thickening of the stratum corneum, similar to the hairless regions of extant mammals (73), such keratinized tissue is rather permeable to moisture in the absence of lipids.

In living mammals cutaneous glands are often associated with hair follicles, and as such attain very high densities. During early development, as the ectodermal tissue that is to form a hair follicle penetrates downward into the dermis it develops lateral buds that grow out to form apocrine glands and sebaceous glands; hence the entire unit is termed an apo-pilo-sebaceous unit (88). The apocrine glands typically bud out closer to the surface than the sebaceous glands, but both gland types usually end up with ducts opening into or near the invagination about hair follicles. Thus their secretions, which are of modest volume and rich in a variety of organic constituents, end up both on the skin surface and as coatings on hair. Sebaceous glands contribute protective lipids to the skin surface, reducing transepithelial moisture loss, and to the hair, providing waterproofing and reducing brittleness (84). Large, specialized apocrine glands such as the Harderian gland can also provide lipids important to reduction

of cutaneous water loss and to waterproofing of hair, at least in some rodents (89). In some mammals (including humans) apocrine glands are absent from most mature hair follicles, but whether this is due to developmental regression of incipient apocrine buds or their complete suppression is not certain (88).

Apocrine and sebaceous glands may also develop into large glands that are important in scent-marking. These elaborate glands are found in specific anatomic locations, such as the perianal region in many insectivores, rodents, and carnivores (including skunks), the preorbital region in many ruminants, and the gular/sternal regions of various bats and primates (84). These glands may contain entirely sebaceous secretory cells, entirely apocrine secretory cells or a combination thereof, although in the latter case the two cell types secrete into separate lumina and the product is often transmitted to the surface via separate ducts (84). Hair is usually a component of these glands, although it may differ in structure from that of the general pelage. Thus these glands appear to be evolutionary modifications of more typical apopilo-sebaceous units. The same may be true of other specialized cutaneous glands in mammals, such as those of the external auditory meatus. Even though cetaceans have secondarily lost hair and cutaneous glands over most of the body surface as an adaptation to aquatic life, they retain glands in the ear canal (84).

A third gland type, the eccrine sweat gland, typically opens on to the epidermal surface independent of hair follicles. However, eccrine sweat glands are rarely profusely distributed in mammals. They are usually restricted to areas of skin that make regular surface contact, such as digital pads, palmar surfaces, the underside of a prehensile tail in some monkeys (Primates: Cebidae), and on the beak of the platypus (Monotremata: *Ornithorhynchus anatinus*) (90,91). Their simple dilute secretions have little organic material, and may leave less scent than would apocrine or sebaceous glands that are typically absent on such contact surfaces. Eccrine sweat glands are abundant over the general body surface only in humans and some primates, in which they assist in thermoregulation by providing dilute surface fluids for evaporative cooling. In some other mammals, such as horses and some ruminants, apocrine glands deliver dilute secretions to the skin surface for evaporative cooling (91).

These three gland types exhibit differences in their modes of secretion. Secretory cells in eccrine glands are similar to many other secretory cells (92) in packaging secretory products into vesicles that migrate from the Golgi apparatus to the apical plasma membrane. The vesicular membrane merges with the plasma membrane and vesicular contents are released into the gland lumen by exocytosis. Secretory cells in apocrine glands also produce secretory vesicles that release product by exocytosis. However, another characteristic of apocrine glands is the bulging out of large secretory vesicles through the apical membrane, such that these may appear stalked or even dissociated (with adhering cytoplasm) from the cell. The secretory mechanism whereby vesicles bulge out, are surrounded by apical membrane, and then break loose, carrying cytoplasmic fragments, has been termed apocrine secretion (88), and it is from this that apocrine glands derive their name. However, the mere bulging out of vesicles is not definitive evidence of apocrine secretion: the microscopic appearance of apocrine secretion may arise from artifacts of tissue preparation and fixation. Lipid globules that bulge out across the apical cell membrane of the Harderian gland (a derived apocrine gland) of the rabbit are released by exocystosis (93). Thus it is not certain that all apocrine glands are capable of apocrine secretion (91).

In contrast to both eccrine and apocrine glands, sebaceous glands rely on holocrine secretion in which secretory cells themselves die and disintegrate, thereby releasing secretory contents into the gland lumen. In sebaceous glands successive sets of secretory cells differentiate, mature, and undergo programmed cell death. Sebaceous secretory cells may swell a 100-fold or more in size as they accumulate product prior to lysis. Such secretion requires continual cell replacement, and the rate of secretion depends, in part, on rates of cell maturation (84).

The secretory tubules of both eccrine and apocrine glands are surrounded by a layer of myoepithelial cells, which by contraction may assist in conveying lumenal contents from secretory regions towards the surface. Given the great changes in volume of sebaceous cells and their transient nature, it is perhaps not surprising that sebaceous glands are devoid of myoepithelial cells.

A Comparison of Mammary Glands to Other Cutaneous Glands

Mammary glands are usually thought to have evolved directly from one or more of these gland types, or to be derived from an ancestral gland that was also ancestral to one or more of these glands (7,15–18,21,24,94). The notion that something

as complex as mammary glands could have arisen de novo by epithelial-stroma interactions seems implausible (94). Evolutionary novelties are normally derived by cooption of an existing developmental pattern or structure to a new function. The change in function brings new selective pressures to bear, which may result in rapid genetic and morphological transformation.

Yet the most recent attempt to investigate mammary gland origins came to a startling conclusion: although mammary glands share features with each of these gland types, none is particularly similar or suggestive of an ancestral type. Rather Blackburn (28) argued that mammary glands represent a novel mosaic, combining features of various gland types into a new structure without deriving from an ancestral form:

> In other words, the mammary gland may represent a neomorphic hybrid, a mosaic organ whose evolution involved the incorporation of characteristics coded for in the genome, but expressed differently by separate populations of skin glands. . . . The mammary gland may have evolved through recruitment of pre-existing developmental pathways and mechanisms, yielding a new organ that cannot be regarded as strictly having evolved from a single ancestral population of glands. (28)

Blackburn does not specify how this could occur in the absence of an ancestral gland population to which developmental pathways and mechanisms could be recruited. However, he suggests that the secret lies in potential dissociation of morphogenesis and cell function due to the inductive effects of mesenchyme on developing epithelial cells. While alterations in the timing and nature of reciprocal signalling patterns between epithelium and mesenchyme have certainly had great importance in mammary evolution (95), I see no need to abandon belief in "gradualistic modifications of the features of a single glandular precursor," as Blackburn (28) suggests.

Blackburn's theory arose out of his opinion that mammary glands could not be linked to any particular type of skin gland. In expanding upon the criteria used in his comparison (Table III), I come to a different conclusion. Sebaceous glands bear little resemblance to mammary glands: they are highly specialized for holocrine secretion, and virtually all aspects of their morphology and function reflect this. In many ways the secretory cells or sebocytes are more similar to keratinocytes in skin and hair follicles. Keratinocytes differentiate from a basal layer, accumulate keratin and membrane-coating materials, and then die. In fact the cells lining the ducts of sebaceous glands are intermediate in function, accumulating some lipid and some keratin, and have been termed sebokeratinocytes by analogy to bird epidermal cells (100). In contrast to the bilayered epithelium that lines secretory structures in mammary and other cutaneous glands, sebaceous glands accumulate multiple layers of basal, differentiating, and necrosing cells, and they lack myoepithelial cells. As individual lobules become exhausted, they are replaced by new lobules (84,91). Despite similarity in shape (rounded acini compared to rounded alveoli) and developmental origin (apopilo-sebaceous anlagen, like monotreme and marsupial mammary glands; see below), I cannot envision modifications of sebaceous glands that would produce mammary glands, nor are there aspects of secretion unique to sebaceous glands that seem to have been recruited into the mammary gland.

Eccrine sweat glands are more probable if still unlikely mammary ancestors, as they secrete by exocytosis, as do mammary glands in forming the aqueous phase of milk, have a single, if somewhat pseudostratified, layer of secretory cells, and contain myoepithelial cells between the secretory cells and the basement membrane (Table III). They produce a dilute solution of electrolytes and small organic molecules, but apparently lack capability for lipid, protein, or complex carbohydrate synthesis, and do not secrete by an apocrine process (91). Unlike other cutaneous glands, they bear no developmental association with hair follicles and thus may have evolved independently of them. Their restricted distribution to areas of contact or friction on the skin of most mammals, their lack of steroid responsiveness, and their early ontogenetic development independent of sexual maturity (Table III) also complicate any theory that would link these glands to reproduction and feeding of the young. Yet it is eccrine glands that people commonly think of when they envision mammary glands evolving from sweat glands. Eccrine sweating may have been important to hominid descent from the trees and occupation of open savanna habitat, as it enabled long-distance running without overheating (101), but it is doubtful if eccrine glands were involved in the origin of lactation so long ago.

In contrast to sebaceous and eccrine glands, apocrine glands bear many similarities to mammary glands (Table III). In both apocrine and mammary glands a single layer of secretory epithelial cells is underlain by a single layer of myoepithelial cells, and the volume of secretory cells changes little during secretion. Apocrine glands develop embryologically in association

Table III. Comparison of Features of Mammalian Skin Glands[a]

	Sebaceous gland	Eccrine gland	Apocrine gland	Mammary gland complex
Secretory unit	Single	Single	Single	Single to many
Embryonic origin	(Apo)-pilo-sebaceous anlagen	Separate anlagen	Apo-pilo-sebaceous anlagen	Mammo-pilo-sebaceous anlagen (Mo, M) or separate (E)
Depth of penetration	Dermis	Dermis and underlying fatty hypodermis	Dermis or deep into underlying fatty hypodermis	Very deep in subcutaneous mammary fat pad
Typical duct opening	Hair follicule infundibulum; some on skin surface (diverge from hair, some Pr)	Skin surface	Hair follicle infundibulum or adjacent skin surface	Hair follicle infundibulum (Mo) or nipple surface (M, E)
Duct	Short to long, unbranched or branched	Long, unbranched (rarely branched?, H)	Long, unbranched (rarely branched?)	Very long, extensively branched
Secretory structure	Cluster of acini, often as lobules	Simple convoluted tubule	Convoluted tubule, often w/branching shunts, diverticuli	Dense cluster of alveoli, in lobules
Secretory epithelium in active state	Multiple layers: undifferentiated, maturing, and necrotic sebocytes	Single but pseudostratified layer of pyramidal serous and mucous cells	Single layer of cuboidal or columnar epithelial cells	Single layer of cuboidal or columnar epithelial cells
Myoepithelial cell layer in secretory structure	Absent	Along longitudinal axis of secretory tubule	Along longitudinal axis of secretory tubule	Surrounding alveoli
Secretory cell volume	Increases 100-150-fold	Little increase	Little increase	Little increase
Secretory cell replacement	Frequent	Infrequent	Infrequent	Periodic; infrequent during secretory phase
Primary secretory mode	Holocrine	Exocytosis	Exocytosis + Apocrine	Exocytosis + Apocrine[b]
Fluid output	Minor	Minor to copious (H)	Minor to copious (Pe, A)	Very copious
Lipid output	Substantial	None	Insignificant to substantial	Substantial to very copious
Stage of secretory maturation	Puberty	Early: pre- (Pr) and postnatal	Puberty	Estrus (Mo, M, some E), Pregnancy (most E)
Steroidal stimulation of epithelial growth	Androgenic stimulation	No	Conflicting evidence	Estrogenic + progesterone stimulation
Topographical location	Most of skin, except contact surfaces (largely absent—C)	Contact surfaces such as palms, digits, prehensile tail (most of skin—some Pr, H)	Widespread, but may be restricted (inguinal, axillary, circumanal, etc.—some Pr, H)	Inguino-abdominal, thoracic, lateral trunk or axillary (species-specific)
Complex structures	Scent glands	No	Scent glands	Mammary gland complex

[a] Parenthetical statements refer to specific taxonomic groups: A = Artiodactyla, C = Cetacea, E = Eutheria, H = Hominidae, M = Marsupialia, Mo = Monotremata, Pe = Perissodactyla, Pr = Primates. Information primarily from the following Refs.: 14,18,84,88,91,94, 96–99.

[b] For present purposes, lipid secretion considered to be apocrine (93), but see text for discussion.

with hair follicles, as do the mammary glands of monotremes and marsupials; in fact the mammary duct openings in monotremes remain associated with hair follicles throughout life (see discussion of mammary gland ontogeny, below). During development a subset of apocrine glands penetrate into the hypodermis or subcutaneous fat layer, as does mammary epithelium in its critical expansion into the mammary fat pad (102). Although the convoluted tubular structure of apocrine glands may be a simple architecture compared to the dense lobular cluster of mammary alveoli, in monotremes the mammary gland begins

secretory function at a tubular grade of organization at the time of egg-laying and hatching (14). Apocrine glands first attain full secretory development in association with puberty, although the importance of steroidal stimulation has yet to be resolved.

At least some specialized apocrine glands resemble mammary glands in being able to synthesize and secrete lipids and other complex organic molecules (89,91). For example, in many mammals the Harderian gland secretes a variety of lipid constituents (including alkyldiacylglycerols) and porphyrins onto the external surface of the cornea (103,104). However, little is known about the secretory constituents of unspecialized apocrine glands that cover the skin surface of most mammals. Apocrine glands have limited importance in human medicine, apart from a role in certain carcinomas (99), and thus they are the least studied of the four gland types in Table III. Heterogeneity among mammals in apocrine gland structure and function has been reported, but is not well-studied (91). Most of our knowledge of apocrine glands relates to large specialized glands, such as human axillary glands, rodent Harderian glands, and rabbit scent glands, but these may not be representative of the nonspecialized apocrine glands on the general body surface of mammals (91). The molecular genetics and biochemistry of apocrine secretions warrant in depth study, as such information might shed light on the origin and evolution of milk constituents.

Apocrine glands release secretory product by exocytosis and, in at least some instances, by an apocrine process. In the mammary gland most milk components (including most proteins, lactose, oligosaccharides, and many aqueous phase constituents) are secreted into the gland lumen via exocytosis of secretory vesicles. By contrast, fat droplets bulge out through the apical plasma membrane and are released into the lumen clothed with an intact envelope of this membrane. Moreover, during this process cytoplasmic crescents may be incorporated into the milk fat droplet (105). Thus the process of mammary lipid secretion bears similarities to apocrine secretion, and has been considered homologous to apocrine secretion by Wooding (93). The amount of cytoplasm so lost from mammary secretory cells is minor, and the frequency of crescent inclusion quite variable from species to species (106). It is possible that upregulation of lipid secretion during mammary evolution has made it necessary that cytoplasmic loss be minimized to protect the integrity of secretory cells. Unfortunately, there is not sufficient information on the molecular controls of apocrine secretion in apocrine glands, or of fat globule secretion by mammary glands, to allow rigorous comparison. It is plausible, but not certain, that milk lipid secretion is a specialized, derived form of apocrine secretion reflecting the evolutionary origin of mammary glands from apocrine glands.

In arguing against the evolution of mammary glands from apocrine glands, Blackburn (28) stated that this would have required loss of androgen responsiveness and motor innervation. Yet the apocrine glands that have been shown to respond to androgens are mostly large, specialized scent glands, some of which share androgen-responsive sebaceous elements, and may not be typical. Montagna and Parakkal (91) concluded that gonadal hormones may play a role in development of human apocrine glands but are relatively unimportant to developed glands. Motor innervation has mostly been studied in large specialized human glands, such as axillary glands, the ceruminous glands in the external auditory meatus, and the glands of Moll in the eyelids. In primates, apocrine glands of the general body surface typically lack cholinesterase-containing nerves, whereas those in specialized skin areas are rich in them (91). Thus the features which were purported to be "lost" (28) may not be universally associated with apocrine glands in living mammals, let alone in mammalian ancestors. Until more comprehensive data become available, it is hazardous to generalize about this gland type.

It would be surprising if apocrine glands had remained unchanged since milk secretion first appeared among synapsids, given the many radiations that have produced such diversity in form and function among mammals (Fig. 2). The most likely scenario is that mammary glands evolved from an apocrine-like skin gland that was ancestral to both mammary glands and contemporary apocrine glands. Much comparative research is needed on a wide taxonomic and morphologic range of apocrine glands, and on potentially homologous glands in amphibians, to determine which gland traits are likely to be primitive, and thus characteristic of the glands from which mammary glands apparently evolved.

EARLY ONTOGENETIC DEVELOPMENT OF MAMMARY GLANDS

Somewhat overlooked in more recent arguments about mammary gland evolution is the fascinating early literature on early ontogenetic development of

mammary glands in monotremes and marsupials. Although the notion that "ontogeny recapitulates phylogeny," in the sense that embryonic stages reflect the phylogenetic sequence of ancestral adult organisms, was discarded early in the twentieth century (107), developmental sequences often retain genetically-determined "somatic programs" by which organ development was directed in ancestral forms (108). It is now clear that evolution tends to incorporate and retain modular units in development (109). The apparent retention of ancestral developmental sequences in living species can lead to greater understanding of how specific morphologies evolved. For example, the evolutionary sequence whereby postdentary jaw elements detached from the mandible and became incorporated into the middle ear of mammals (see above) was discovered as a developmental process in embryonic mammals before it was documented in the therapsid/early mammal fossil record.

Mammary Gland Development in Monotremes

Early ontogenetic development of the mammary glands of monotremes and marsupials was described by German anatomists in the late nineteenth and early twentieth century, and summarized by Bresslau (16–18). In the absence of subsequent descriptive work, more recent descriptions are all derived from this literature (14,94,97,110), but sometimes inaccurately so.

In the short-nosed echidna (Monotremata: *Tachyglossus aculeatus*), evidence of epidermal thickening in the area destined to become mammary glands occurs very early in embryonic life, when the egg is still being incubated and the embryo is less than 1 cm in length. There is also condensation of underlying mesenchyme (embryonic stroma) (16,18), indicating the probable role of mesenchyme in directing epithelial development, as in mice (95). This area, termed the mammary primary-primordium by Bresslau (18), becomes lens-shaped before flattening out by the time of hatching. In newly hatched young (ca. 380 mg body mass) the primary-primordia are well vascularized by capillaries, but initially remain bare while hair follicle primordia begin to develop in the surrounding epidermis. Shortly thereafter hair primordia appear in the mammary area, and develop "with a vigor never shown elsewhere in the skin" (18). The hair follicle buds grow downward into the stroma and develop gland buds very similar to the "sweat gland" (i.e., apocrine gland) buds in the surrounding areas, but these mammary buds elongate more rapidly into the stroma and ultimately become the lobules of the mammary gland. The hair follicles in the mammary area develop principal hairs as well as sebaceous glands. Thus the ultimate structure that develops consists of a mammary lobule, a mammary hair, and one or more sebaceous glands. This can be termed a mammo-pilo-sebaceous unit, by analogy to the apo-pilo-sebaceous unit. The duct of the mammary lobule opens into the invagination surrounding the mammary hair, as does the duct of the sebaceous gland (14).

In the echidna each mammary area, termed an areola, contains about 100–150 of these units clustered in a small oval area in a depressed incubatorium (18,111,112). Thus secreted milk exudes at the base of the hairs; there are no nipples. Beyond the periphery of the areola large apocrine glands develop in apo-pilo-sebacaceous units; otherwise apocrine glands are rare in the pelage of echidnas (14). The situation in the platypus (Monotremata: *Ornithorhynchus anatinus*) is comparable: the areola contains 100–200 mammo-pilo-sebaceous units in an oval area about 0.6 cm in length in the nonlactating animal stretching to 2 cm in length in the lactating animal (12,55). Enlarged apocrine glands are present in a ring of apo-pilo-sebaceous units surrounding the areola, but in contrast to echidnas smaller apocrine glands are common throughout the pelage.

Thus there are remarkable parallels in development and functional organization between mammo-pilo-sebaceous units in the monotreme areola and apo-pilo-sebaceous units in the surrounding skin. The most parsimonious explanation is that they are sister structures derived from a common ancestral pattern. Raynaud (94) suggested an alternative explanation, that mammary primordia became secondarily associated with pilosebaceous units in monotremes. Given that monotremes diverged from therians (marsupials and eutherians) prior to the divergence of marsupials from eutherians (14,29,37), any secondary association with hair follicles would have occurred twice, in both monotremes and marsupials. This would require parallel evolution that is improbable, especially as the association in marsupials appears to be vestigial.

Mammary Gland Development in Marsupials

A similar pattern of mammary development is seen in marsupials, although the final outcome is a glandular structure opening to the surface through nipples rather than a mammary areola. According to Bresslau (17,18) an oval mammary

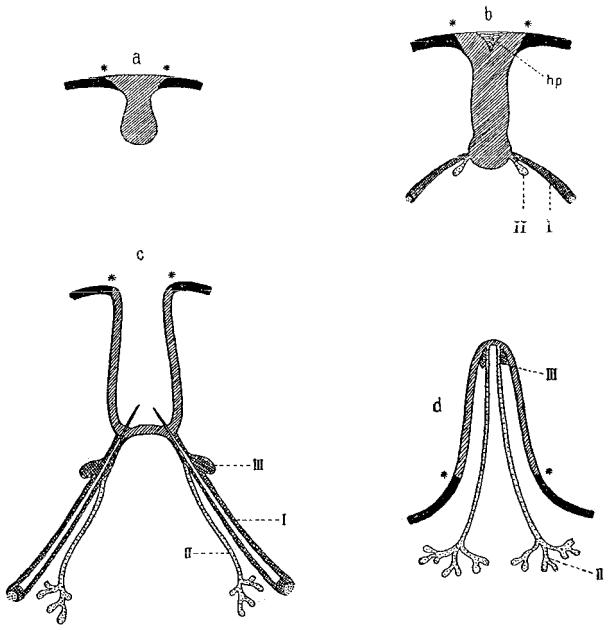

Fig. 4. A schematic view of early mammary development in marsupials that undergo nipple eversion, as interpreted by Bresslau (17,18). (A) Nipple primordium, prior to sprouting. (B) Elongation of the nipple primordium, with emergence of primary sprouts (I) that will become hair follicles, and secondary buds (II) that will become mammary lobules; note development of cornified horny plug (hp). (C) Hollowed-out "nipple pouch" with mammary hairs emerging from hair follicles (I), growth of mammary glands (II), and appearance of tertiary buds (III) that represent sebaceous glands. (D) Everted nipple, after regression of the hair follicle and shedding of the mammary hair; note that the illustrated galactophores in the nipple derive one-to-one from mammo-pilo-sebaceous units, and that sebaceous glands (III) may still be present. [From Bresslau (17)]

primary-primordium develops on the lateral abdominal surface of embryonic marsupials at a comparable developmental stage as in monotremes, but the marsupial embryo is developing in utero rather than in an incubated egg. By the time of birth, which occurs at a similar or somewhat less developed stage as that at hatching in monotremes (14,107,108), the primary-primordium has split into small separate epidermal thickenings. These subsequently deepen into knob-shaped nipple primordia (Fig. 4(A)) whose numbers vary from species to species (18). In opossums (Didelphidae: *Didelphis* sp.) eight primary epithelial buds sprout from each nipple primordium, and each of these in turn develops one secondary and a pair of tertiary outgrowths. The primary sprouts are those of hair follicles and the tertiary outgrowths are buds of sebaceous glands; these develop transiently and then regress. However, the secondary outgrowths produce mammary ducts, which subsequently ramify into mammary lobules (17,18). Thus opossums have eight lobules per nipple, each communicating to the surface via a separate duct. As opossums may have a dozen or more nipples, the total number of lobules can approach the number seen in monotremes, 100–200, but separated among a set of nipples rather than confined to a mammary areola. The glands appear to be derived from former mammo-pilo-sebaceous units.

A schematic representation of the development and fate of primary (I), secondary (II), and tertiary (III) buds is presented in Fig. 4. In most marsupials the knob-shaped nipple primordia elongate (Fig. 4(B)) and by cornification become hollowed out (Fig. 4(C)). The primary buds develop into follicles that project mammary hairs into these hollowed out "nipple pouches" and the tertiary sebaceous buds develop close to the base of the pouches (Fig. 4(C)). Subsequently, the hairs are shed, the hair follicles regress and the "nipple pouch" everts producing a nipple (Fig. 4(D)). The number of ducts penetrating each nipple depends on the initial number of primary (hair follicle) buds, which may be as few as three in mouse opossums (Didelphidae: *Marmosa robinsoni*), six in tiger quolls (Dasyuridae: *Dasyurus viverrinus*), 15–20 in tammar wallabies (Macropodidae: *Macropus eugenii)*, 24–26 in red kangaroos (*Macropus rufus*), and 20–33 in agile wallabies (*Macropus agilis*) (97). Remarkably, in the koala (Phascolarctidae: *Phascolarctos cinereus*) the mammary hairs that develop from the 24 primary buds per nipple persist through nipple eversion, forming a tuft of hairs projecting from the apex of the nipple (18). Obviously, these must be shed before a neonate can attach to the nipple.

In marsupials it is not clear that all secondary outgrowths of the nipple primordia develop into mammary lobules, at least in macropods with a large number of presumptive mammo-pilo-sebaceous units. In a young virgin red kangaroo (Macropodidae: *Macropus rufus*), Griffiths *et al.* (113) observed only a few large ducts lined with cuboidal epithelium embedded in the underlying fat pad, but they interpreted smaller and shallower glandular structures to be "Knäueldrüsen" or apocrine glands. If so, some of the "galactophores" in the nipple may be apocrine ducts. Is it possible that some secondary epithelial buds in presumptive mammo-pilo-sebaceous units differentiate into mammary ducts and lobules while others differentiate into apocrine glands? Alternatively, are these apocrine-like structures retained vestiges of former mammary glands or have apocrine glands from surrounding areas somehow become captured by mammary tissue

during development? These questions clearly warrant further study, and may help clarify the relationship of apocrine and mammary glands. It would be particularly interesting to know if these "apocrine glands" express gross cystic disease fluid protein-15, a protein considered diagnostic of apocrine differentiation (114).

Mammary Gland Development in Eutherians

Mammary gland development in eutherians presents many similarities, but also striking differences, from that seen in monotremes and marsupials. Early mammary development is best understood in laboratory mice (Muridae: *Mus musculus*) (115,116), the species that has become the standard model for experimental work, but much is known about other taxa, including the laboratory rat, the domestic pig, domestic ruminants and humans (18,94,117,118).

The first evidence of mammary development is enlargement of a single layer of ectoderm along an area between the front and rear limb buds, which has been termed the mammary band or streak depending on observed width. Central thickening of this band produces an externally visible mammary line. Bresslau (18) regarded this initial epidermal thickening with underlying mesenchymal condensation to be equivalent to the oval areas of primary-primordia in monotremes and marsupials, and speculated that it represents in all three groups the vestige of a vascularized brood patch that predated lactation. The milk line subsequently becomes thickened into mammary buds at the locations where nipples will develop, which varies among species, being axillary in manatees, pectoral in elephants and anthropoid primates, and abdominal or inguinal in ruminants, cetaceans, and equids (horses and zebras). At other locations the milk line rapidly disappears. It was initially thought that the accumulation of epidermal cells at the mammary buds was due to enhanced proliferation at these sites, but at least in rabbits and mice the development of mammary buds appears to be due to immigration of epidermal cells from surrounding areas (115). Some investigators have failed to find a clearly defined milk line, and suggest that some or all mammary buds develop independently in some species (94,95,117). However, if the buds are formed by immigration of epidermal cells from the surrounding area, clearly the buds are forming out of a more widely spread anlage regardless of whether a distinct band or line can be histologically defined.

In mice, the mammary streak is first seen at 10–11 days postconception, five pairs of mammary gland anlage are apparent at 12 days, and these gradually deepen into bulb-shaped rudiments by 14 days (115). The slow proliferation of the mammary strank and bud from 11 to 16 days is termed the resting phase, after which rapid downward proliferation produces a mammary sprout that invades the underlying precursor tissue of the mammary fat pad, and begins to branch within the pad and to form proliferative terminal end buds. This progressively branching duct system will eventually fill the entire fat pad. Thus the entire mammary gland structure derives from the single sprout and opens to the exterior via a single galactophore passing through the nipple. In species with multiple galactaphores per nipple, such as humans, a cleft forms early on in the mammary bud, and secondary buds then grow out, canalize, and branch within the mammary fat pad, forming separate ducts and lobules (94,117).

Thus in eutherians there is no direct evidence of a necessary and intimate association between the anlagen of mammary glands and those of hair follicles. In fact, there appears to be lateral inhibition of hair follicle formation in the immediate vicinity of the mammary anlagen (117). Given 1) that monotremes, marsupials, and eutherians are derived from a common ancestor endowed with mammary glands, 2) that mammary glands originate as buds from hair follicle primordia in the first two groups, and 3) that marsupials and eutherians have diverged more recently than did monotremes and marsupials, I propose that mammary association with hair follicles was the primitive condition in the earliest eutherians. However, as protruding mammary hairs are nonfunctional in the presence of a nipple, and would interfere with suckling, mammary gland evolution in the Eutheria apparently incorporated direct inhibition of follicle formation so that, in contrast to marsupials, rudimentary follicles never form at the sites (or even in the immediate vicinity) of nipple formation. If this hypothesis is correct, blocking the expression of the presumptive inhibiting compound(s) at the earliest stages of mammary development might allow hair follicles to grow in association with the mammary buds, recreating the primitive condition.

Many years ago Bresslau (17,18) reported a phenomenon in the European squirrel (Sciuridae: *Sciurus vulgaris*) that appears consistent with this interpretation. As in many other mammals, an initial single milk line per side degenerates to produce a series of nipple primordia. However, the two anteriormost primordia

on each side split in two, and a medial epithelial outgrowth separates and migrates towards the midline as a small lens-like structure. Although the larger "parent" primordia become knob-like and proceed to develop as mammary glands, the smaller medial primordia elongate into cylinders that then invade the dermis as hair follicles. The large "abdominal vibrissae" produced by these follicles are retained in adult life in this and other squirrel species (18). Could the different cell fates (hair follicle vs. mammary gland) be a consequence of inadequate or blocked inhibition in the smaller primordia? It might be particularly fruitful to look at epithelial-mesenchyme interactions and cytodifferentiation in the mammary glands of these squirrels. It has long been known that undifferentiated epidermal cells have a remarkable "equipotential" capability in generating different differentiated cell types, especially in response to burns and other trauma (119).

A REVISED SCENARIO FOR THE EVOLUTION OF LACTATION

Glandular Secretion and Eggs

The sequential synapsid radiations in the Carboniferous, Permian, Triassic, and Jurassic/Cretaceous demonstrate a gradual acquisition of mammalian characters over time. It is likely that lactation developed in a similar stepwise fashion.

Permian synapsids appear to have had a glandular skin, which they inherited from tetrapod forebears. Yet they produced parchment-shelled eggs that were susceptible to desiccation (8). The combination of potentially moist skin and moisture-needing eggs would have put an evolutionary premium on the use of glandular secretions in egg-tending. A similar situation arises among caecilians, frogs, and salamanders that nest in terrestrial habitats where egg desiccation poses a risk. Parental attendance of the eggs is the norm, and direct contact with parental skin may allow transcutaneous water flux into the eggs, at least in some species (73,122,123). The role of skin gland secretions in amphibian egg-tending has not been carefully studied, other than attempts to isolate antibiotic compounds that might protect eggs from fungal or bacterial infection (76). However, unpublished results by several investigators indicate that hatchlings of some caecilian species feed upon maternal skin and/or skin secretions, apparently using specialized hatchling teeth to grasp maternal skin in which skin glands are well developed (122). If verified, this remarkable discovery might provide a direct analogy for a transition from skin secretions as egg supplements to skin secretions as hatchling food. A comparative study of cutaneous gland secretions among amphibians with parental care of eggs, larvae, or hatchlings might provide extremely valuable insight into the selective factors influencing the early stages of skin gland evolution among synapsids.

In mammals, both mammary glands and apocrine glands appear to be derived from a common ancestral gland type, and it was presumably this ancestral gland that was involved in providing moisture to eggs. These ancestral glands probably were capable of producing both fluid constituents (by exocytosis) and lipids (by exocytosis or apocrine secretion), and it is possible that both were beneficial to eggs. The lipids may have served to increase resistance to water flux of both parental skin and incubated eggs, assuming a proportion of the eggshell pores would avoid occlusion by applied lipids (8). Evaluation of this hypothesis will require detailed study of the effect of surface lipids on parchment-shelled eggs, preferably to include monotreme eggs.

At some point in their evolution these ancestral glands became associated with hair follicles, as both apocrine and noneutherian mammary glands retain this association. This association may seem curious, especially if skin gland secretions are ancient, having evolved in early synapsids, and hair is more recently evolved, appearing in advanced therapsids that developed endothermy. However, hair may not have initially evolved for thermoregulatory purposes. Spearman's hypothesis (85) that hair evolved in the hinge regions of scales presupposes complex epidermal scales, which are a sauropsid rather than synapsid trait; the scales on rodent tails are apparently secondarily evolved rather than homologues of sauropsid scales. The hypothesis that hair evolved as tactile organs (as in whiskers) does not explain the association with skin glands.

I propose that hair may have initially evolved to facilitate the spreading of a thin film of fluid onto egg surfaces and to reduce air circulation around incubated eggs. In this scenario, hair follicles evolved at the sites of existing gland duct openings, rather than evolving elsewhere and secondarily associating with cutaneous glands. The implication is that hair follicles first evolved at abdominal or thoracic glandular patches, and were subsequently coopted into a thermoregulatory role of providing thermal insulation on the general body surface. If skin glands are a primitive

synapsid feature, and if modification of these glands to support the water balance of eggs predates endothermy (8), hair may have evolved well before the appearance of mammaliaform cynodonts in the late Triassic.

The primitive apocrine-like glands associated with egg incubation must have increased in complexity, ductal branching, and depth of penetration into underlying stromal tissues, becoming increasingly mammary-like. The divergence of primitive apocrine-like glands into mammo-pilo-sebaceous units on the one hand, and apo-pilo-sebaceous units on the other, must have become pronounced in therapsids. This divergence would have involved major changes in hormonal regulation, including an increased role for prolactin in stimulation of epithelial growth and secretion by evolving mammary glands. A similar role was played by prolactin in the evolution of crop milk secretion in pigeons and doves (67).

An Unstudied Role for Lactation in Monotremes?

The purported functional association of glandular secretion, hair, and eggs leads to an obvious if surprising prediction: that mammary secretion and mammary hair may continue to serve the same role in living monotremes. Unfortunately it is not known if monotremes secrete milk during egg incubation, or if they apply milk secretions to their eggs. However, fragmentary indirect data are consistent with this hypothesis (8):

1. Both platypus and echidna eggs take up fluid in utero, expanding many times in size, indicating fluid permeability of the eggshell (14). However, most of this uptake appears to occur prior to deposition of the thick outer layer of the eggshell (123).
2. Laid platypus and echidna eggs are coated with an outermost organic layer containing foreign particles (123), indicating either drying and hardening of a uterine secretion after birth, or postlaying deposition of organic matter (8). Platypus eggs are stuck together during incubation by a sticky material (124). The echidna egg is incubated in a pouch and is "retained there by hairs plastered across it" (112).
3. In the echidna, mammary glands develop a tubular grade of organization late in the breeding season, even before eggs have been laid. These tubules are secretory, producing a milk-like fluid containing 12% dry matter (112). The glands are in a similar grade of organization after the eggs hatch, and only gradually develop full alveolar proliferation (14).

Demonstration of milk secretion during egg incubation by monotremes, and of egg uptake of water and/or other milk consitutuents, would provide strong support for the hypothesis of mammary origins developed herein. It might also explain why monotreme mammae secrete via hairy areolar patches rather than nipples: nipples would not allow application of a thin fluid layer to eggs. I suggest that mammary patches in monotremes might be adaptive, and not just vestigial. It may be that nipples could not evolve in synapsids so long as eggs were incubated, and only secondarily arose in therian lineages in which egg-retention resulted in liveborn neonates with no need for a surface-applied fluid.

The regression of hair follicles associated with marsupial mammary glands suggests that mammary hair is a vestigial feature from a time when marsupials were egg-laying. For example, neonates of some marsupials, such as the brush-tailed possum (*Trichosurus vulpecula*) and the koala (*Phascolarctos cinereus*) still develop a vestigial egg tooth but it is no longer functional (125). The koala also retains mammary hairs for a greater period than other marsupials, with hairs projecting through the everted nipples (17,18).

The Evolution of "True" Milk

This review has focused primarily on the origin of mammary glands, and space does not permit detailed consideration of the many transformations required to convert an egg-bathing fluid to the primary source of nutrients for rapidly growing altricial young. The reduction in size of the amniote yolk required that these nutrients be provided by milk. Some of these transformations may have begun rather early in the evolution of glandular secretions.

The provision of fluids to eggs would have created a favorable environment for microbial attack, and hence the incorporation into glandular secretions of antimicrobial agents, such as lysozyme, iron-binding proteins, and some oligosaccharides, may have been favored by natural selection (7). In this regard, early mammary secretions may have functioned in a capacity similar to that of the large albumen fraction in some sauropsid eggs, which provides water to the developing embryo but is also important in defending the egg against microbial and fungal

attack. In bird eggs, the albumen contains high levels of lysozyme, iron-binding proteins (especially ovotransferrin), and vitamin-binding proteins, all of which may interfere with microbial growth (126). Oligosaccharides in mammary secretions are believed to serve both a prebiotic function, favoring benign microorganisms, and an inhibitory function by acting as receptor analogues for pathogens (127,128). Both functions might have been beneficial to synapsid eggs. Some antimicrobial compounds may already have been present in skin secretions to protect the epidermis, just as antimicrobials are found in amphibian skin secretions (75,76). Herein may lie an ancient origin for some milk constituents.

The role of the disaccharide lactose (Galactose β1-4 Glucose) in the evolution of lactation is of particular interest. Lactose is a component of virtually all milks (excluding those of a few marine mammals), either in free form or as the reducing end of oligosaccharides (128,129). Its synthesis in mammary glands depends upon the presence of both a mammary-specific protein (α-lactalbumin) and a β1,4-galactosyl transferase, the two together forming the heterodimer lactose synthetase (130,131). The α-lactalbumin apparently derives from the ubiquitous enzyme lysozyme, an enzyme that cleaves glycosidic linkages in bacterial cell walls and was likely present in the secretions of ancestral apocrine-like glands. The divergence of the genes that now produce lysozyme and α-lactalbumin is believed from molecular data to be an ancient event, predating the origin of synapsids, while the α-lactalbumin binding domain of β1,4 galactosyl transferase is found in birds and thus also predates the separation of synapsids and sauropsids (133,134).

Does this imply that free lactose was present in the skin secretions of early synapsids? Messer and Urashima (129) hypothesize that rates of α-lactalbumin synthesis were initially low in ancestral mammary glands, while a plethora of glycosyl transferases (possibly including galactosyl-, fucosyl-, sialyl-, and *N*-actetylglucosaminyl-transferases) would have been available to convert lactose to oligosaccharides, as occurs in extant monotremes and marsupials. If so, early secretions may have contained a preponderance of antimicrobial oligosaccharides that included galactose β1-4 glucose at the reducing end (128,129). A subsequent upregulation of α-lactalbumin synthesis could generate free lactose by increasing lactose synthesis beyond the capacity of glycosyl transferases to generate oligosaccharides. By this scenario, free lactose appeared later in the evolution of gland secretions, and only then became involved in the regulation of aqueous phase secretion via its osmotic effects within secretory vesicles (133).

However, Messer and Urashima (129) had difficulty envisioning why α-lactalbumin would have evolved more than 100 million years prior to the appearance of late Triassic "mammals" (= mammaliaforms in Fig. 2), as molecular data suggests. This difficulty disappears if early synapsid eggs benefited from applications of oligosaccharide-containing gland secretions.

A major redirection of mammary evolution occurred when the secretions came to provide not just water and protective agents, but also nutrients. This is a novel use of skin secretions, which may have begun at the egg-incubation stage, because egg uptake and metabolism of small carbohydrates would facilitate fluid uptake (8). Did lactase originally evolve in the yolk sac membrane of synapsid eggs to take advantage of lactose in applied glandular secretions? If so, lactose may have been important both to regulation of fluid secretion by ancestral glands and to fluid uptake in synapsid eggs. It would be interesting to know if lactase is expressed in the yolk sac membrane of monotreme eggs, as this membrane is an extraembryonic precursor of the digestive tract.

The fact that synapsid eggs do not have a calcified eggshell (8) means that the calcium required for embryonic development must have been invested in the egg yolk, as in squamates with parchment-shelled eggs (134). However, the amount of calcium so supplied appears to barely meet needs, leaving newly hatched squamates in need of a dietary calcium source upon hatching (134). This situation contrasts with the apparent surfeit of calcium available to birds, turtles, and crocodilians with calcified eggs. Is it possible that glandular secretions provided ionic calcium to eggs? Retained eggs in the uterus of squamates may take up calcium through the eggshell, at least in some taxa (135).

Caseins probably evolved as a source of amino acids, phosphate, and calcium for hatchlings, not for embryos, as the large size of casein micelles would block passage through eggshell pores. The origins of casein genes remain uncertain, although α_{S1}-, α_{S2}-, and β-casein genes may represent duplication and divergence of one initial gene, and κ-casein gene could be derived from the γ-fibrinogen gene (136). Casein(s) must predate the Triassic origin of mammaliaforms, since the limited tooth replacement of mammaliaforms implies substantial calcium intake for bone development prior to independent feeding.

Egg-supplementation probably favored secretion of relatively small amounts of a dilute fluid, in

which only small amounts of lipids were included. Yet hatchlings would benefit from the increased energy provided by milk lipids, and the secretion of lipids could have been upregulated after hatching. Herein lies the beginning of an effect of lactation stage on milk composition, which has become so pronounced in monotremes and marsupials, and may have been equally great in early eutherians if they had altricial young. In this view, placental development subsequently supplanted the early stages of lactation so eutherians now lack these early stages.

In both monotremes and some marsupials, the first milk produced is quite dilute (ca. 10–12% dry matter) and contains modest levels of both lipids and simple sugars (monosaccharides and lactose) (106,137–141). The glycosyltransferases required for oligosaccharide synthesis are not expressed initially (142,143). This early secretion may be the closest to a "primitive" milk to be found among extant mammals, and is quite different from the high oligosaccharide and high lipid milks that are secreted in subsequent lactation stages (14,69,128,144).

Milk must have attained a more-or-less modern composition before the end of the Triassic, and must have been central to the reproductive success of both advanced cynodonts and early mammaliaforms and mammals (64). Lactation had to become a dominant feature before cynodonts could become progressively smaller, and more endothermic, two of the requisite steps that preceded the appearance of miniscule mammaliaforms in the late Triassic and early Jurassic.

In conclusion, I have proposed that milk underwent an evolutionary transformation from egg-supplement to hatchling food. Yet it probably filled both roles in advanced egg-laying synapsids, and may still play both roles in extant monotremes. If future research demonstrates the transfer of mammary secretions from the monotreme mammary areolar patch to eggs, and water and/or nutrient uptake by these eggs, it will lend credence to the evolutionary scenario envisioned herein and thereby clarify our understanding of how mammary glands evolved.

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REFERENCES

1. W. K. Gregory (1910). The orders of mammals. *Bull. Am. Mus. Nat. Hist.* **27:**1–524.
2. D. G. Blackburn, L. J. Vit, and C. A. Beuchat (1984). Eutherian-like reproductive specializations in a viviparous reptile. *Proc. Natl. Acad. Sci. U.S.A.* **81:**4860–4863.
3. D. G. Blackburn (1992). Convergent evolution of viviparity, matrotrophy, and specializations for fetal nutrition in reptiles and other vertebrates. *Am. Zool.* **32:**313–321.
4. L. Fishelson and A. Baranes (1998). Observations on the Oman shark, *Iago omanensis* (Triakidae), with emphasis on the morphological and cytological changes of the oviduct and yolk sac during gestation. *J. Morphol.* **236:**151–165.
5. M. H. Wake (1993). Evolution of oviductal gestation in amphibians. *J. Exp. Zool.* **266:**394–413.
6. J. P. Wourms (1981). Viviparity: The maternal-fetal relationship in fishes. *Am. Zool.* **21:**473–515.
7. D. G. Blackburn, V. Hayssen, and C. J. Murphy (1989). The origin of lactation and the evolution of milk: A review with new hypotheses. *Mammal Rev.* **19:**1–26.
8. O. T. Oftedal (2002). The origin of lactation as a water source for parchment-shelled eggs. *J. Mammary Gland Biol. Neoplasia* **7:**253–266.
9. C. Darwin (1859). *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle of Life*, John Murray, London.
10. St. G. Mivart (1871). *On the Genesis of Species*, D. Appleton, New York.
11. C. Darwin (1872). *On the Origin of Species by Means of Natural Selection, 6th edn.*, Appleton-Century-Crofts, New York.
12. R. Owen (1832). On the mammary glands of the *Ornithorhynchus paradoxus. Philos. Trans. R. Soc. Lond.* **1832:**517–538.
13. A. Moyal (2001). *Platypus: The Extraordinary Story of how a Curious Creature Baffled the World*, Smithsonian Institution Press, Washington, DC.
14. M. Griffiths (1978). *Biology of the Monotremes*, Academic Press, New York.
15. C. Gegenbauer (1886). *Zur Kenntniss der Mammarorgane der Monotremen*, W. Engelmann, Leipzig, Germany.
16. E. Bresslau (1907). Die Entwickelung des Mammarapparates der Monotremen, Marsupialier und einiger Placentalier. I. Entwickelung und Ursprung des Mammarapparates von Echidna. *Jenaische Denkschr.* **7:**459–518, Plates 28–30. [Undated, but cited as 1907 in Ref. 18]

17. E. Bresslau (1912). Die Entwickelung des Mammarapparates der Monotremen, Marsupialer und einiger Placentalier. III. Entwickelung des Mammarapparates der Marsupialer, Insectivoren, Nagathiere, Carnivoren und Wiederkaeuer. *Jenaische Denkschr.* **7:**647–874, Plates 37–46. [Undated, but cited as 1912 in introductory note to Ref. 18]
18. E. Bresslau (1920). *The Mammary Apparatus of the Mammalia in Light of Ontogenesis and Phylogenesis*, Methuen, London.
19. J. B. S. Haldane (1964). The origin of lactation. *Rationalist Ann*. **1964:**19–30.
20. J. B. S. Haldane (1965). The possible evolution of lactation. *Zool. Jb. Syst.* **92:**41–48.
21. C. A. Long (1969). The origin and evolution of mammary glands. *Bioscience* **19:**519–523.
22. C. A. Long (1972). Two hypotheses on the origin of lactation. *Am. Nat*. **106:**141–144.
23. J. A. Hopson (1973). Endothermy, small size, and the origin of mammalian reproduction. *Am. Nat*. **107:**446–452.
24. B. M. Graves and D. Duvall (1983). A role of aggregation pheromones in the evolution of mammallike reptile lactation. *Am. Nat*. **122:**835–839.
25. D. Duvall (1986). A new question of pheromones: Aspects of chemical signaling and reception in the mammal-like reptiles. In N. Hotton, P. D. MacLean, J. J. Roth, and E. C. Roth (eds.), *The Ecology and Biology of Mammal-Like Reptiles*, Smithsonian Institution Press, Washington, DC, pp. 219–238.
26. V. Hayssen and D. G. Blackburn (1985). α-lactalbumin and the origins of lactation. *Evolution* **39:**1147–1149.
27. L. J. Guillette and N. Hotton (1986). The evolution of mammalian reproductive characteristics in therapsid reptiles. In N. Hotton, P. D. MacLean, J. J. Roth, and E. C. Roth (eds.), *The Ecology and Biology of Mammal-Like Reptiles*, Smithsonian Institution Press, Washington, DC, pp. 239–250.
28. D. G. Blackburn (1991). Evolutionary origins of the mammary gland. *Mammal Rev*. **21:**81–98.
29. Q. Ji, Z.-X. Luo, C.-X. Yuan, J. R. Wible, J.-P. Zhang, and J. A. Georgi (2002). The earliest known eutherian mammal. *Nature* **416:**816–822.
30. T. S. Kemp (1982). *Mammal-Like Reptiles and the Origin of Mammals*, Academic Press, London.
31. M. J. Benton (1997). *Vertebrate Paleontology*, 2nd edn., Chapman & Hall, London.
32. M. J. Packard and R. S. Seymour (1997). Evolution of the amniote egg. In S. S. Sumida and K. L. M. Martin (eds.), *Amniote Origins: Completing the Transition to Land*, Academic Press, San Diego, CA, pp. 265–290.
33. A. S. Romer and L. W. E. Price (1940). Review of the Pelycosauria. *Geol. Soc. Am. Spec. Pap.* **28:**1–538.
34. J. A. Hopson (1994). Synapsid evolution and the radiation of non-eutherian mammals. In D. B. Prothero and R. M. Schoch (eds.), *Major Features of Vertebrate Evolution*, Paleontological Society, Knoxville, TN, pp. 190–219.
35. F. S. Szalay (1994). *Evolutionary History of the Marsupials and an Analysis of Osteological Characters*, Cambridge University Press, Cambridge, UK.
36. C. A. Sidor and J. A. Hopson (1998). Ghost lineages and "mammalness": Assessing the temporal pattern of character acquisition in the Synapsida. *Paleobiology* **24:**254–273.
37. Z.-X. Luo, A. W. Crompton, and A.-L. Sun (2001). A new mammaliaform from the early Jurassic and evolution of mammalian characteristics. *Science* **292:**1535–1540.
38. N. Hotton, P. D. MacLean, J. J. Roth, and E. C. Roth (eds.) (1986). *The Ecology and Biology of Mammal-Like Reptiles*, Smithsonian Institution Press, Washington, DC.
39. C. G. Farmer (2000). Parental care: The key to understanding endothermy and other convergent features in birds and mammals. *Am. Nat*. **155:**326–334.
40. T. S. Kemp (1983). The relationships of mammals. *Zool. J. Linn. Soc.* **77:**353–384.
41. B. S. Rubidge and C. A. Sidor (2001). Evolutionary patterns among Permo-Triassic therapsids. *Ann. Rev. Ecol. Syst.* **32:**449–480.
42. E. Mayr (2001). *What Evolution Is*, Basic Books, New York.
43. D. H. Erwin (1993). *The Great Paleozoic Crisis: Life and Death in the Permian*, Columbian University Press, New York.
44. Z.-X. Luo, R. L. Cifelli, and Z. Kielan-Jaworowska (2001). Dual origin of tribosphenic mammals. *Nature* **409:**53–57.
45. D. S. Berman, S. S. Sumida, and R. E. Lombard (1997). Biogeography of primitive amniotes. In S. S. Sumida and K. L. M. Martin (eds.), *Amniote Origins: Completing the Transition to Land*, Academic Press, San Diego, CA, pp. 85–139.
46. R. L. Carroll (1988). *Vertebrate Paleontology and Evolution*, W. H. Freeman, New York.
47. P. Michaelson (2002). Mass extinction of peat-forming plants and the effect on fluvial styles across the Permian–Triassic boundary, northern Bowen Basin, Australia. *Palaeogeogr. Palaeoclimatol. Palaeoecol*. **179:**173–188.
48. G. King (1990). *The Dicynodonts: A Study in Palaeobiology*, Chapman and Hall, London.
49. A. Chinsamy and B. S. Rubidge (1993). Dicynodont (Therapsida) bone histology: Phylogenetic and physiological implications. *Palaeont. Afr*. **30:**97–102.
50. J. Botha and A. Chinsamy (2000). Growth patterns deduced from the bone histology of the cynodonts *Diademodon* and *Cynognathus*. *J. Vert. Paleont*. **20:**705–711.
51. W. Maier, J. van den Heever, and F. Durand (1996). New therapsid specimens and the origin of the secondary hard and soft palate of mammals. *J. Zool. Syst. Evol. Res.* **34:**9–19.
52. W. J. Hillenius (1994). Turbinates in therapsids: Evidence for late Permian origins of mammalian endothermy. *Evolution* **48:**207–229.
53. J. Ruben (1995). The evolution of endothermy in mammals and birds: From physiology to fossils. *Ann. Rev. Physiol*. **57:**69–95.
54. A. W. Crompton, C. R. Taylor, and J. A. Jagger (1978). Evolution of homeothermy in mammals. *Nature* **272:**333–336.
55. M. Griffiths, M. A. Elliott, R. M. C. Leckie, and G. I. Schoefl (1973). Observations of the comparative anatomy and ultrastructure of mammary glands and on the fatty acids of the triglycerides in platypus and echidna milk fats. *J. Zool. Lond*. **169:**255–279.
56. M. Griffiths (1983). Lactation in Monotremata and speculations concerning the nature of lactation in Cretaceous Multituberculata. *Palaeont. Polonica* **28:**93–102.
57. T. D. White (1989). An analysis of epipubic bone function in mammals using scaling theory. *J. Theor. Biol*. **138:**343–357.
58. M. J. Novacek, G. W. Rougier, J. R. Wible, M. C. McKenna, D. Dashzeveg, and I. Horovitz (1997). Epipubic bones in eutherian mammals from the Late Cretaceous of Mongolia. *Nature* **389:**483–486.
59. J. A. Lillegraven (1979). Reproduction in Mesozoic mammals. In J. A. Lillegraven, Z. Kielan-Jaworowska, and W. A. Clemens (eds.), *Mesozoic Mammals: The First Two-Thirds of*

Mammalian History, University of California Press, Berkeley, CA, pp. 259–276.
60. Y. Hu, Y. Wang, Z. Luo, and C. Li (1997). A new symmetrodont mammal from China and its implications for mammalian evolution. *Nature* **390:**137–142.
61. J. Qiang, L. Zhexi, and J. Shu-an (1999). A Chinese triconodont mammal and mosaic evolution of the mammalian skeleton. *Nature* **398:**326–330.
62. K. A. Kermack (1956). Tooth replacement in mammal-like reptiles of the suborders Gorgonopsia and Therocephalia. *Philos. Trans. R. Soc. Lond. B* **670:**95–133.
63. A. W. Crompton and Z. Luo (1993). Relationships of the Liassic mammals *Sinoconodon*, *Morganucodon oehleri*, and *Dinnetherium*. In F. S. Szalay, M. J. Novacek, and M. C. McKenna (eds.), *Mammal Phylogeny: Mesozoic Differentiation, Multituberculates, Monotremes, Early Therians, and Marsupials*, Springer-Verlag, New York, pp. 30–44.
64. C. M. Pond (1977). The significance of lactation in the evolution of mammals. *Evolution* **31:**177–199.
65. J. Kear and N. Duplaix-Hall (eds.) (1975). *Flamingos*, T & AD Poyser, Berkhamstead, England.
66. S. Shetty, L. Bharathi, K. B. Shenoy, and S. N. Hegde (1992). Biochemical properties of pigeon milk and its effect on growth. *J. Comp. Physiol. B* **162:**632–636.
67. N. D. Horseman and J. D. Buntin (1995). Regulation of pigeon cropmilk secretion and parental behaviors by prolactin. *Ann. Rev. Nutr.* **15:**213–238.
68. O. T. Oftedal (1984). Milk composition, milk yield and energy output at peak lactation: A comparative review. *Symp. Zool. Soc. Lond.* **51:**33–85.
69. B. Green and J. C. Merchant (1988). The composition of marsupial milk. In C. H. Tyndale-Biscoe and P. A. Janssens (eds.), *The Developing Marsupial. Models for Biomedical Research*, Springer-Verlag, Berlin, pp. 41–54.
70. L. M. Frolich (1997). The role of the skin in the origin of amniotes: Permeability barrier, protective covering and mechanical support. In S. S. Sumida and K. L. M. Martin (eds.), *Amniote Origins: Completing the Transition to Land*, Academic Press, San Diego, CA, pp. 327–352.
71. H. B. Lillywhite and P. F. A. Maderson (1982). Skin structure and permeability. In C. Gans and F. H. Pough (eds.), *Biology of the Reptilia, Vol. 12: Physiology C: Physiological Ecology*, Academic Press, New York, pp. 397–442.
72. R. I. C. Spearman (1973). *The Integument: A Textbook of Skin Biology*, Cambridge University Press, Cambridge, England.
73. W. E. Duellman and L. Trueb (1994). *Biology of Amphibians*, Johns Hopkins University Press, Baltimore.
74. G. Delfino, R. Brizzi, R. Kracke-Berndorff, and B. Alvarez (1998). Serous gland dimorphism in the skin of *Melanophryniscus stelzneri* (Anura: Bufonidae). *J. Morphol.* **237:**19–32.
75. B. T. Clarke (1997). The natural history of amphibian skin secretions, their normal functioning and potential medical applications. *Biol. Rev*. **72:**365–379.
76. L. P. Fredericks and J. R. Dankert (2000). Antibacterial and hemolytic activity of the skin of the terrestrial salamander, *Plethodon cinereus. J. Exp. Zool*. **287:**340–345.
77. W. A. Buttemer (1990). Effect of temperature on evaporative water loss of the Australian tree frogs *Litoria caerula* and *Litoria chloris*. *Physiol. Zool*. **63:**1043–1057.
78. L. A. Blaylock, R. Ruibal, and K. Platt-Aloia (1976). Skin structure and wiping behavior of Phyllomedusine frogs. *Copeia* **1976:**283–295.
79. P. F. A. Madderson, T. Rabinowitz, B. Tandler, and L. Alibardi (1998). Ultrastructural contributions to an understanding of the cellular mechanisms involved in lizard skin shedding with comments on the function and evolution of a unique lepidosaurian phenomenon. *J. Morphol*. **236:**1–24.
80. P. D. Oldak (1976). Comparison of the scent gland secretion lipids of twenty-five snakes: Implications for biochemical systematics. *Copeia* **1976:**320–326.
81. W. B. Quay (1986). The skin of reptiles: Glands. In J. Bereiter-Hahn, A. G. Matoltsy, and K. S. Richards (eds.), *Biology of the Integument, Vol. 2: Vertebrates*, Springer-Verlag, Berlin, pp. 188–193.
82. R. O. Prum (1999). Development and evolutionary origin of feathers.*J. Exp. Zool*. **285:**291–306.
83. P. R. Stettenheim (2000). The integumentary morphology of modern birds—An overview. *Am. Zool*. **40:**461–477.
84. V. R. Wheatley (1986). *The Physiology and Pathophysiology of the Skin, Vol. 9: The Sebaceous Glands*, Academic Press, London.
85. R. I. C. Spearman (1977). Hair follicle development, cyclical changes and hair form. In A. Jarrett (ed.), *The Physiology and Pathophysiology of the Skin, Vol. 4: The Hair Follicle*, Academic Press, London, pp. 1255–1292.
86. S. A. Czerkas (1997). Skin. In P. J. Currie and K. Padian (eds.), *Encyclopedia of Dinosaurs*, Academic Press, San Diego, CA, pp. 669–675.
87. P. K. Chudinov (1968). Structure of the integuments of theromorphs. *Dokl. Acad. Sci. U.S.S.R. Earth Sci. Sect.* **179:**226–229.
88. M. B. L. Craigmyle (1984). *The Apocrine Glands and the Breast*, Wiley, Chichester, England.
89. H. J. Harlow (1984). The influence of Hardarian gland removal and fur lipid removal on heat loss and water flux to and from the skin of muskrats, *Ondatra zibethicus*. *Physiol. Zool*. **57:**349–356.
90. W. Montagna and R. A. Ellis (1960). Sweat glands in the skin of *Ornithorhynchus paradoxus*. *Anat. Rec*. **137:**271–277.
91. W. Montagna and P. F. Parakkal (1974). *The Structure and Function of Skin*, 3rd edn., Academic Press, New York.
92. B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter (2002). *Molecular Biology of the Cell*, 4th edn., Garland Science, New York.
93. F. B. P. Wooding (1980). Lipid droplet secretion by the rabbit Harderian gland. *J. Ultrastruct. Res*. **71:**68–78.
94. A. Raynaud (1961). Morphogenesis of the mammary gland. In S. K. Kon and A. T. Cowie (eds.), *Milk: The Mammary Gland and Its Secretion, Vol. 1*, Academic Press, New York, pp. 3–46.
95. G. W. Robinson, A. B. C. Karpf, and K. Kratochwil (1999). Regulation of mammary gland development by tissue interaction. *J. Mammary Gland Biol. Neoplasia* **4:**9–19.
96. A. T. Cowie and J. S. Tyndal (1971). *The Physiology of Lactation*, Edward Arnold London.
97. H. Tyndale-Biscoe and M. Renfree (1987). *Reproductive Physiology of Marsupials*, Cambridge University Press, Cambridge, UK.
98. A. J. Thody and S. Shuster (1989). Control and function of sebaceous glands. *Physiol. Rev*. **69:**383–416.
99. L. Requena, H. Kiryu, and A. B. Ackerman (1998). *Neoplasms With Apocrine Differentiation*, Lippincott-Raven, Philadelphia.
100. R. Wrench, J. A. Hardy, and R. I. C. Spearman (1980). Sebokeratocytes of avian epidermis—with mammalian comparisons. In R. I. C. Spearman and P. A. Riley (eds.), *The Skin of Vertebrates*, Academic Press, London, pp. 47–55.

101. A. M. W. Porter (2001). Why do we have apocrine and sebaceous glands? *J. R. Soc. Med.* **94:**236–237.
102. M. C. Neville, D. Medina, J. Monks, and R. C. Hovey (1998). Editorial commentary: The mammary fat pad. *J. Mammary Gland Biol. Neoplasia* **3:**109–116.
103. G. R. Buzell (1996). The Harderian gland: Perspectives. *Microsc. Res. Tech.* **34:**2–5.
104. Y. Seyama, H. Otsuka, K. Ohashi, B. Vivien-Roels, and P. Pevet (1996). Sexual diversity of the lipid metabolism in the Harderian gland of the golden hamster. *Microsc. Res. Tech.* **34:**71–76.
105. I. H. Mather and T. W. Keenan (1998). Origin and secretion of milk lipids. *J. Mammary Gland Biol. Neoplasia* **3:** 259–273.
106. M. M. T. Janssen and P. Walstra (1982). Cytoplasmic remnants in milk of certain species. *Neth. Milk Dairy J*. **36:**365–368.
107. S. J. Gould (1977). *Ontogeny and Phylogeny*, Harvard University Press, Cambridge, MA.
108. E. Mayr (1994). Recapitulation reinterpreted: The somatic program. *Q. Rev. Biol.* **64:**223–232.
109. S. F. Gilbert and J. A. Bolker (2001). Homologies of process and modular elements of embryonic construction. *J. Exp. Zool*. **291:**1–12.
110. G. Mayer and M. Klein (1961). Histology and cytology of the mammary gland. In S. K. Kon and A. C. Cowie (eds.), *Milk: The Mammary Gland and Its Secretion*, Academic Press, New York, pp. 47–126.
111. M. Griffiths (1968). *Echidnas*, Pergamon Press, Oxford, England.
112. M. Griffiths, D. L. McIntosh, and R. E. A. Coles (1969). The mammary gland of the echidna, *Tachyglossus aculeatus*, with observations on the incubation of the egg and on the newly-hatched young. *J. Zool. Lond*. **158:**371–386.
113. M. Griffiths, D. L. McIntosh, and R. M. C. Leckie (1972). The mammary glands of the red kangaroo with observations on the fatty acid components of the milk triglycerides. *J. Zool. Lond*. **166:**265–275.
114. P. Viacava, A. G. Naccarato, and G. Bevilacqua (1997). Apocrine epithelium of the breast: Does it result from metaplasia? *Virchows Arch.* **431:**205–209.
115. T. Sakakura (1987). Mammary embryogenesis. In M. C. Neville and C. W. Daniel (eds.), *The Mammary Gland: Development, Regulation, and Function*, Plenum, New York, pp. 36–66.
116. M. M. Richert, K. L. Schwertfeger, J. W. Ryder, and S. M. Anderson (2000). An atlas of mouse mammary gland development. *J. Mammary Gland Biol. Neoplasia* **5:**227–241.
117. B. A. Howard and B. A. Gusterson (2000). Human breast development. *J. Mammary Gland Biol. Neoplasia* **5:**119–137.
118. P. A. Masso-Welch, K. M. Darcy, N. C. Stangle-Castor, and M. M. Ip (2000). A developmental atlas of rat mammary gland histology. *J. Mammary Gland Biol. Neoplasia* **5:**165–185.
119. W. Montagna (1962*). The Structure and Function of Skin*, 2nd edn., Academic Press, New York.
120. T. L. Taigen, F. H. Pough, and M. M. Stewart (1984). Water balance of terrestrial anuran (*Eleutherodactylus coqui*) eggs: Importance of parental care. *Ecology* **65:**248–255.
121. R. A. Nussbaum (1985). The evolution of parental care in salamanders. *Misc. Publ. Mus. Zool. Univ. Mich.* (No. 169), pp. 1–50.
122. Anonymous (1999). Legless amphibians nurse their young. *Science* **283:**623.
123. J. P. Hill (1933). The development of the Monotremata. Part II. The structure of the eggshell. *Trans. Zool. Soc. Lond.* **21:**443–477.
124. H. Burrell (1974). *The Platypus*, Rigby, Adelaide, Australia.
125. J. P. Hill (1949). Development of the Monotremata. Part VII. The development and structure of the egg-tooth and the caruncle in the monotremes and on the occurrence of vestiges of the egg-tooth and caruncle in marsupials. *Trans. Zool. Soc. Lond.* **26:**503–544.
126. H. B. White (1991). Maternal diet, maternal proteins and egg quality. In D. C. Deeming and M. W. J. Ferguson (eds.), *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*, Cambridge University Press, Cambridge, England, pp. 1–15.
127. D. S. Newburg (1996). Oligosaccharides and glycoconjugates in human milk: Their role in host defense. *J. Mammary Gland Biol. Neoplasia* **1:**271–283.
128. T. Urashima, T. Saito, T. Nakamura, and M. Messer (2001). Oligosaccharides of milk and colostrum in non-human mammals. *Glycoconj. J.* **18:**357–371.
129. M. Messer and T. Urashima (2002). Evolution of milk oligosaccharides and lactose. *Trends Glycosci. Glycotechnol.* **14:**153–176.
130. M. Charron, J. H. Shaper, and N. L. Shaper (1998). The increased level of β1,4-galactosyltransferase required for lactose biosynthesis is achieved in part by translational control. *Proc. Natl. Acad. Sci. U.S.A.* **95:**14805–14810.
131. N. L. Shaper, M. Charron, N.-W. Lo, and J. H. Shaper (1998). β1,4-Galactosyltransferase and lactose biosynthesis: Recruitment of a housekeeping gene from the nonmammalian vertebrate gene pool for a mammary gland specific function. *J. Mammary Gland Biol. Neoplasia* **3:**315–324.
132. C. P. Piotte, C. J. Marshall, M. J. Hubbard, C. Collet, and M. R. Grigor (1997). Lysozyme and α-lactalbumin from the milk of a marsupial, the common brush-tailed possum (*Trichosurus vulpecula*). *Biochim. Biophys. Acta* **1336:**235–242.
133. J. L. Linzell and M. Peaker (1971). Mechanism of milk secretion. *Physiol. Rev*. **51:**564–597.
134. M. J. Packard and N. B. Clark (1996). Aspects of calcium regulation in embryonic lepidosaurians and chelonians and a review of calcium regulation in embryonic archosaurians. *Physiol. Zool*. **69:**435–466.
135. J. R. Stewart and M. B. Thompson (2000). Evolution of placentation among squamate reptiles: Recent research and future directions. *Comp. Biochem. Physiol. A* **127:**411–431.
136. M. R. Ginger and M. R. Grigor (1999). Comparative aspects of milk caseins. *Comp. Biochem. Physiol. B* **124:**133–145.
137. B. Green, K. Newgrain, and J. Merchant (1980). Changes in milk composition during lactation in the tammar wallaby (*Macropus eugenii*). *Aust. J. Biol. Sci*. **33:**35–42.
138. E. A. Crisp, P. E. Cowan, and M. Messer (1989). Changes in milk carbohydrates during lactation in the common brushtail possum, *Trichosurus vulpecula* (Marsupialia: Phalangeridae). *Reprod. Dom. Anim.* **1:**309–314.
139. E. A. Crisp, M. Messer, and J. L. VandeBerg (1989). Changes in milk carbohydrates during lactation in a didelphid marsupial, *Monodelphis domestica*. *Physiol. Zool*. **62:**1117–1125.
140. B. Green, J. L. VandeBerg, and K. Newgrain (1991). Milk composition in an American marsupial (*Monodelphis domestica*). *Comp. Biochem. Physiol. B* **99:**663–665.
141. B. Green, W. J. Krause, and K. Newgrain (1996). Milk composition in the North American opossum (*Didelphis virginiana*). *Comp. Biochem. Physiol. B* **113:**619–623.

142. M. Messer and K. R. Nicholas (1991). Biosynthesis of marsupial milk oligosaccharides: Characterization and developmental changes of two galactosyltransferases in lactating mammary glands of the tammar wallaby, *Macropus eugenii*. *Biochim. Biophys. Acta* **1077:** 79–85.
143. T. Urashima, M. Messer, and W. A. Bubb (1992). Biosynthesis of marsupial milk oligosaccharides II: Characterization of a β6-N-acetylglucosaminyltransferase in lactating mammary glands of the tammar wallaby, *Macropus eugenii*. *Biochim. Biophys. Acta* **1117:**223–231.
144. O. T. Oftedal (1980). Milk and mammalian evolution. In K. Schmidt-Nielsen, L. Bolis, and C. R. Taylor (eds.), *Comparative Physiology: Primitive Mammals*, Cambridge University Press, Cambridge, pp. 31–42.
145. R. L. Hughes and L. S. Hall (1988). Structural adaptations of the newborn marsupial. In C. H. Tyndale-Biscoe and P. A. Janssens (eds.), *The Developing Marsupial: Models for Biomedical Research*, Springer-Verlag, Berlin, pp. 8–27.
146. R. L. Hughes (1993). Monotreme development with particular reference to the extraembryonic membranes. *J. Exp. Zool.* **266:**480–494.