SYMPOSIUM: LIMITS TO LACTATION: LESSONS FROM COMPARATIVE BIOLOGY

Lactation: Historical Patterns and Potential for Manipulation

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ABSTRACT

The advent of biotechnology has made data on undomesticated mammals relevant to dairy science. Such data indicate the potential of lactation for modification, reveal genetic material available for use through bioengineering, help distinguish adaptive features from historical artifacts, and clarify limits on lactational diversity that date from early evolution. Evolutionary analysis indicates that a complex degree of lactation preceded divergence of the extant mammalian lineages during the Mesozoic Era. Although aspects of monotreme lactation appear to be ancestral for extant mammals, the marsupials and eutherians exhibit divergent specializations. Evidence is consistent with the idea that protolacteal glands evolved by combining features of skin gland populations into a new functional complex. Secretions of these ancestral glands may have had antimicrobial properties that protected the eggs or hatchlings and organic components that supplemented offspring nutrition. Following development of highly nutritious milks, evolution produced diversity in milk composition and function, milk output, length of lactation, mammary gland anatomy, and contributions of lactation to offspring nutrition. Certain marsupials are specialized in terms of functional independence and temporal plasticity of mammary tissues. Mammalian diversity indicates that artificial selection and physiological manipulation of domestic artiodactyls has only modestly exploited the potential of mammary glands as a nutritional source for humans.

(**Key words**: evolution of lactation, mammary gland, mammal reproduction, biotechnology)

INTRODUCTION

Over 120 million yr ago, when dinosaurs dominated terrestrial ecosystems, some small, quadrupedal animals underwent a series of unprecedented evolutionary experiments in feeding their offspring. These early evolutionary experiments culminated in the development of the most efficient, effective, and adaptable means of postnatal nutrient provision that has ever arisen among vertebrates—lactation. Lactation had important consequences for the evolutionary success of the descendants of these animals; after dinosaurs and other large reptiles finally disappeared at the end of the Mesozoic Era, lactation played an important role in the worldwide, adaptive radiation of mammals. As mammals defined and exploited niches in nearly all conceivable ecological circumstances, lactation was subject to further modification and proved to be enormously adaptable to different environments, diets, reproductive cycles, and breeding seasons. The current diversity of lactation characteristics among mammals is the result, therefore, of many millions of years of evolutionary experimentation.

Human use and control of lactation in other mammals for nutritional purposes may be nearly as old as civilization but, from a geologic perspective, has involved little time and only a few species. Most mammalian diversity has been outside human exploitation, and the results of the innumerable evolutionary experiments in lactation therefore have seemed to be of distant relevance to attempts to understand

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and to control lactation in cows, sheep, and goats. However, as milk output and quality continue to be improved in domesticated species, it is appropriate to consider what comparative biology can reveal about the unrealized potential for modification and the nature of the limitations on that potential. Moreover, as bioengineering opens the prospects and problems of human-directed evolution, information from comparative and evolutionary biology will become increasingly important in revealing potentialities and limitations. Such issues provide the rationale for the symposium from which this paper is derived.

This review examines lactation from an evolutionary perspective with the goal of exploring fundamental attributes of this phenomenon that were established early in mammalian history. Such attributes establish the boundaries within which mammalian diversity has been expressed and, consequently, may reveal limits and potentialities for future modification. The present discussion begins with a historical overview of human control of milk production as a means of showing the value of comparative and evolutionary data to current and future attempts at manipulation. Next, patterns of lactation and reproduction of the three major groups of living mammals are outlined, and their historical relationships are considered. The evolutionary origins of mammary glands and lactation are then reviewed in detail, and aspects of the current diversity of mammary glands and lactation parameters are summarized.

HUMAN CONTROL OF MILK PRODUCTION IN HISTORICAL PERSPECTIVE

Human attempts to control and to manipulate lactation in cows, sheep, and goats for nutritional purposes can be categorized in three overlapping, historical stages (Table 1). The first stage, which began over 5000 yr ago, was that of domestication and artificial selection. Human populations in parts of Europe, Africa, the Middle East, and Southeast Asia adopted a few species of the order Artiodactyla that had several features in common that facilitated their exploitation. These features included a relatively large size (hence, copious milk output), a long lactation period, and production of precocial offspring. Other features included a well-developed social organization (which, along with docility and a behavioral adaptability to humans, made possible the keeping of herds) and a digestive system specialized for a high cellulose diet of little nutritional value to humans, minimizing competition for food resources.

Throughout this first historical stage, the immediate raw material available for use was preexisting, heritable variation and spontaneous mutations arising in the breeding populations. Selective breeding led to increased milk output, increased docility and adaptability to human control, and, as some evidence indicates, smaller brain size. In the small populations that breeding herds constitute, evolution was probably accelerated under the influence of genetic drift, a factor that probably contributed to the eventual development of the domesticated breeds (92). Selection, which tends to act on all participants in a symbiotic relationship, also led, in various human populations, to the retention into adulthood of the ability to digest lactose (60). Development of lactase deficiency in mammals is a heritable trait that may help to increase reproductive output by regulating the time of weaning (51). Thus, the evolution of prolonged lactose tolerance in humans may have contributed to dual trends toward increased offspring survivability and decreased maternal reproductive output—trends for which acceleration in all human

TABLE 1. History of human attempts to control and to manipulate lactation in other mammals for nutritional purposes.

Historical stage	Raw material	Database
Selective breeding, domestication	Intraspecific variation; spontaneous mutations	Little initially; genetics; animal husbandry
Physiological manipulation	Innate physiological characteristics	Reproductive physiology, endocrinology
Bioengineering	Interspecific variation	Comparative and evolutionary biology

societies is vital to the future of biodiversity and the quality of human life.

For most of its history, artificial selection has been conducted, at best, under a rudimentary understanding of principles of inheritance and animal husbandry. However, selection has been used with increasing effectiveness through application of data on genetics and reproductive physiology and through the use of artificial insemination, embryo transfer, and other techniques made possible by technological advance.

In contrast, the second historical stage, that of physiological manipulation, has been empirically based since its inception. Drawing on basic research on reproductive physiology, endocrinology, development, and animal nutrition, this approach takes advantage of innate characteristics of the domestic mammals in manipulations of milk quality and in the timing and circumstances of milk production (Table 1). The effects of selective breeding and physiological manipulation on milk quantity, energetic quality, and mammary gland size have been documented in detail in the literature [for a comparative review of domestic mammals, see (33)]. Physiological manipulation has continued potential as a means of modifying milk production, as understanding of endocrine and paracrine regulation of the mammary gland and milk synthesis (18, 67) is refined and biotechnology increases hormone and growth factor availability.

VALUE OF COMPARATIVE AND EVOLUTIONARY BIOLOGY

Dairy science now may stand at the threshold of a third stage of development. Absolute limits are no longer imposed by the genetic variation available within existing breeding stocks and the capacity of individual animals to respond to physiological manipulation. On the contrary, techniques of genetic engineering will allow the indiscriminate crossing of species boundaries. Theoretically, at least, much of mammalian diversity is available for use—the product of evolutionary experiments involving over 100 million yr of geological history. If information on genetics and animal husbandry offered the best database for selective breeding and data on endocrinology and animal nutrition for the physiological manipulation of milk output, what sorts of data will be relevant to lactation biology in the age of bioengineering?

As dairy science enters the 21st century, data from comparative physiology and anatomy will be of importance for several reasons. First, comparative studies have revealed the range of variation possible within the limits imposed by historical constraints and aspects of mammalian biology (40, 41, 66). In other words, the extensive data on undomesticated species reveal some of the potential for manipulation under human-directed evolution. Second, natural populations of mammals contain genetic raw material that is potentially available to be sampled and incorporated into the genomes of domesticated stocks. Familiarity with phenotypic variation among mammalian species, as well as its genotypic basis, will be a prerequisite for attempts to draw upon that variation through genetic engineering. Third, comparative biology reveals the limitations on diversity imposed by functional constraints. For example, the trade-off between milk lipid and carbohydrate content (44, 69) became apparent through comparisons of a variety of mammalian species. Synthetic reviews comparing many species (33, 41, 65, 66) have revealed various other unexpected relationships between lactational and other reproductive parameters.

Equally important to comparative data is an understanding of the diversity of lactation in a broad functional context, i.e., in the context of a species' physiological ecology, developmental biology, neonatal and maternal physiology, and life history pattern. Lactation strategies represent different adaptive solutions to different problems. Mammals differ according to litter size and frequency, the degree of development of the offspring at birth, the types of nutrients supplied by the placenta, postnatal thermoregulatory and nutritional needs, and maternal resource availability. These features are reflected in such aspects of lactation as milk quantity, frequency and duration of suckling, time until weaning, and milk composition. Why do marine mammals provide milks that are high in lipid content? Why is milk relatively concentrated in desert rodents? In ruminants, how can one account for the specialized synthetic pathways for short- and medium-chain fatty acids of milks? Such questions can only be answered with reference to other aspects of a species' biology.

Evolutionary biology might seem at first to be of distant relevance to applied issues of dairy science. However, several reasons exist why an understanding of lactation from an evolutionary perspective is important to attempts to comprehend and to draw upon mammalian diversity. First, only by reference to phylogenetic relationships can the taxonomic distribution of diversity be interpreted, including, for example, milk composition. Milks of the domesticated goat and sheep are more similar to each other than to milk of the bottlenose dolphin, not simply because of nutritional needs of their offspring, but because of their common evolutionary heritage. To ignore phylogenetic relationships is to overlook the most powerful tools that biology has developed to explain and to categorize diversity. Second, a phylogenetic approach confers an extraordinary degree of predictability because of the conservative nature of evolutionary change. Comparative biologists can, with reasonable accuracy, extrapolate general characteristics of uninvestigated organisms from those of related forms, making unnecessary the detailed study of every species in a lineage.

Third, only by reference to evolution can the limitations imposed on diversity by the past be understood. The fundamental features of lactation that were established early in mammalian history pose limits within which all species operate; certain characteristics of lactation are conferred by the nature of the mammary gland, which in turn may reflect origins and early functions of the gland. Fourth, a functional and historical perspective is necessary if features that are currently adaptive are to be distinguished from evolutionary vestiges. If recent hypotheses (6, 34) are correct, features of the milks of monotremes and certain other mammals may reflect functional solutions to problems that have since disappeared. A naive approach would be to regard lactation in each species as optimally designed to that species' characteristics. Various factors may have limited optimization in particular lineages, including a lack of adaptive, heritable variation; insufficient evolutionary time; developmental constraints; conflicting selection on parents and offspring; and functional tradeoffs between incompatible needs. Only a multidisciplinary approach that integrates function and development in an evolutionary framework can distinguish adaptive from nonadaptive features.

Finally, one of the most important reasons why a historical approach may be useful is that bioengineering is human-directed evolution and likely to be subject to evolutionary principles. The similarities between natural and artificial selection offer a useful analogy. One example of a relevant principle is that of the functional compromise, such as the inverse relationship between milk lipid and carbohydrate already mentioned. As another example, contemporary models of evolutionary change acknowledge that small genetic changes can have large phenotypic consequences, as seems to have occurred in the evolution of the lactose synthetase system (6). In summary, comparative, functional, and evolutionary approaches can demonstrate the potential for manipulation of lactation by revealing diversity, functional and evolutionary causes of that diversity, and limitations within which any modifications of lactation must occur.

AN OVERVIEW OF MAMMALIAN REPRODUCTIVE DIVERSITY

Reproductive Patterns

Appreciation of the history and diversity of mammalian lactation requires familiarity with reproductive patterns in the major groups of mammals. Living mammals constitute three major groups: monotremes, marsupials, and eutherians (Table 2). As the only surviving members of the mammalian subclass Pro-

TABLE 2. Outline of the traditional classification of the major groups of extant mammals.[1]

Class Mammalia
Subclass Prototheria
Order Monotremata (i.e., echidna, platypus)
Subclass Theria
Infraclass Metatheria (marsupials)
Infraclass Eutheria (eutherians or placental mammals)

[1] As defined to include monotremes plus a wide variety of extinct forms, the taxon Prototheria is probably artificial. Some contemporary sources classify all living mammals as therians.

totheria, monotremes have elicited considerable interest, because aspects of their reproduction may shed light on ancestors to all living mammals. Monotremes constitute three genera confined to the Australian region: *Ornithorhynchus* (the duckbill platypus) and the echidna genera, *Tachyglossus* and *Zaglossus.* Aspects of monotreme reproduction recently have been summarized elsewhere (25, 38, 90). Monotremes lay eggs from which hatch tiny undeveloped young that are so altricial as to be termed "premature" (91). At ovulation, the egg is a mere 3 to 4 mm in diameter, but it swells considerably through absorption of oviductal fluids. The length of time that each egg develops in the oviduct is not precisely known but is thought to approximate 10 d in *Ornithorhynchus anatinus* and to be between 16 and 27 d in *Tachyglossus* (29). Following oviposition, eggs are incubated by the female platypus in a burrow system until hatching, whereas, in the echidnas, they are carried by the female in a pouchlike incubatorium. Monotreme offspring are totally dependent on milk as a source of nutrition. Contrary to a widespread misconception, monotremes exhibit true suckling behavior, despite the absence of a nipple (25). Milk is a vital source of nutrients, and the period of suckling is prolonged, ranging up to 200 d in *Tachyglossus aculeatus* (28).

Marsupials (metatherians) and "placentals" (eutherians) constitute the other subclass of living mammals, the Theria, within which they represent the infraclasses Metatheria and Eutheria (Table 2). The common names applied to these groups are misleading; many marsupials lack pouches, and placentas are universal in all therians. However, the groups are accepted as valid lineages, partly on the basis of reproductive features. Marsupials are viviparous, and give birth to highly altricial offspring after a short gestation (50, 78, 90). The ovum is microscopic, containing little or no yolk. The embryo obtains nourishment by means of uterine fluids and, following shell rupture and implantation, by means of a yolk sac placenta, which is supplemented in bandicoots and a few other species by a chorioallantoic placenta (58, 68). Gestation length varies from 12 to 38 d, and birth weight is less than .5 g and as small as 5 mg, depending on the species (11, 78, 90). The altricial newborn is approximately equivalent in developmental stage to monotreme hatchlings (26, 79, 90). Upon birth, the marsupial neonate makes its way to a teat (located in the pouch, if one is present), where it attaches semipermanently and begins to suckle. Lactation tends to be prolonged in marsupials; the time until weaning ranges among species from 50 to 550 d, during which the young are totally dependent on milk (90).

Eutherians represent the great majority of extant mammalian species. Like marsupials, eutherians are viviparous, but they exhibit considerably more interspecific reproductive diversity than either the marsupials or monotremes. A chorioallantoic placenta supplies nutrients for development and is supplemented by means of a yolk sac placenta in most species (58). Reported gestation lengths range from 20 d (a shrew genus, *Sorex*) to 660 d (the African elephant, *Loxodonta africana*) (15). At birth, neonates can be altricial, semialtricial, or precocial (86), ranging in weight from 7 to 91,000 g (15). Lactation length is also highly variable; values reported for nonhuman species range from 4 to 5 d (elephant shrew, fur seal) to over 900 d (orangutan, chimpanzee).

Lactation Strategies

Eutherians and marsupials are often viewed as exhibiting alternative reproductive strategies that are reflected in their differential reliance on lactation (78, 79, 90). Compared with marsupials, eutherians almost always have longer gestations and invariably give rise to offspring that are more well-developed at birth. A recent analysis indicated that gestation in representative eutherians averages over half of the time from conception to weaning versus only 12% in marsupials; virtually no overlap exists between the two groups (35). This analysis also showed that parental investment at birth (measured as litter mass divided by maternal body mass) is far smaller in marsupials, but total maternal effort at weaning is comparable in the two groups. Such data reflect the greater reliance of marsupials on lactation than gestation to provide nutrition to the offspring. Given the extreme altriciality of their hatchlings, monotremes are similar to marsupials in this regard. The heavy reliance of marsupials on lactation has been interpreted by some as part of a specialized strategy that allows them to reproduce optimally in harsh environments by modifying reproductive investment according to nutrient availability (48, 57, 70).

However, generalized comparisons between metatherians and eutherians ought not to be allowed to obscure the diversity of lactational and reproductive parameters expressed within each group (79). In addition, the relative length of lactation and gestation, a feature often used in comparisons of eutherians and marsupials, is not always an accurate indicator of the apportionment of maternal energetic investment. For example, female pinnipeds can provide large quantities of milk relative to offspring size, despite a very short lactation. The best indicator of the apportionment of maternal investment is offered by the information on offspring mass mentioned, given the paucity of data on organic content and metabolism of neonates and suckling young.

The dependence of marsupials on lactation is reflected in some unusual specializations. In marsupials, milk composition can be constantly altered to meet the changing nutritional needs of the suckling (12, 13, 61, 64). For example, the relative proportions of milk carbohydrate and lipid can change dramatically over lactation, and particular proteins and amino acids appear in the milk at particular stages of lactation (22, 23). In some species, milk during the first half of lactation is low in lipid and protein but high in carbohydrate, which is mainly oligosaccharides. Later in lactation, milk is high in lipid content, and the little remaining carbohydrate is mainly monosaccharides; lipid content rises by more than 400% (90). These shifts in milk production presumably reflect, at least in part, ontogenetic shifts in gene expression by the mammary epithelium. Although eutherians often exhibit ontogenetic shifts in milk composition (66), such shifts are neither as extensive nor as continuous as in marsupials (79, 90). Thus, the situation in marsupials may reflect the evolutionary accentuation of a functional property common to mammary epithelia. Among monotremes, milk composition changes throughout lactation in *Tachyglossus* (25) but perhaps not in *Ornithorhynchus* (62, 90).

Another unusual specialization of some marsupials is that of concurrent asynchronous lactation; adjacent teats in a female can deliver milks that differ markedly in quality (17, 55, 90). This specialization has been documented among macropodids, which can simultaneously suckle a tiny altricial neonate and a sibling that has recently emerged from the pouch. In certain kangaroos of the genus *Macropus*, two of the four glands can produce milks of entirely different quality, while the third gland undergoes postlactational regression, and the fourth, an unsucked gland, regresses from its prepartum preparation for birth (90). The remarkable ability of these marsupials to produce simultaneously milks that differ so markedly in composition, as well as to maintain cycling glands at different functional stages, is evidence of the functional and evolutionary plasticity of lactation mechanisms.

Other Aspects of the Diversity of Lactation

Mammalian species vary with respect to a variety of lactation parameters in addition to those just discussed. Interspecies differences include daily milk production and energy output (33, 66), milk caloric content (40, 66), maternal behavior during lactation (91), mammary gland development (18, 77), and endocrinological control (19, 49). Milk composition varies strikingly among species (40, 41, 44, 65, 66, 90), often reflecting the nutritional, immunological, and thermoregulatory needs of the young. Carbohydrates, for example, range among eutherians from 1 to 50% of milk calorific content (44) and in concentration from trace amounts to 100 g/L (40, 42). Lactose, the principle milk sugar in most mammals, is essentially absent in such distantly related mammals as the platypus (59) and the sea lion, *Zalophus californianus* (40). Milk lipid content ranges from trace amounts to 500 g/L and 75% of calorific content (40). Milks also vary in content of proteins, water, immunoglobulins, transferrin, minerals, lysozyme, and other factors (6, 10, 40, 41, 66, 88).

Variation is also evident in the glandular apparatus through which lactation is effected. Although mammary glands in all mammals share fundamental morphological and physiological attributes, glands vary in gross structure, size, and number (56). For example, glands of monotremes are paired structures consisting of discrete elongated lobules that fan out in oval clusters beneath the skin, where they lie surrounded by connective tissue.

Gland ducts in monotremes open on specialized patches of skin, the areolae, with a well-defined structure (26, 27). The massive glandular lobules of cetaceans may occupy an opposite extreme in gland form. Nipples or teats are present in all therian mammals but are lacking in monotremes. The number of mammae varies among mammals from 2 in humans, to 14 in pocket gophers (*Thomomys*) and a maximum of 24 in certain tenrec insectivores. The quantity bears some relationship (although not a precise one) to litter size (56). Mammae also vary in position. In many mammals, the nipples are located in two lines along the ventral region of the abdomen, but the glands also can be inguinal, as in bovids and cetaceans; pectoral as in bats, elephants, and humans; and even on the dorsal side of the trunk, as in the aquatic rodent, *Myocaster coypus* (56). In pouched marsupials, the nipples are concentrated in the marsupium.

EVOLUTION OF REPRODUCTIVE PATTERNS

Reconstructions of Evolutionary Change

Before consideration of the historical relationships between the reproductive patterns and lactational features just outlined, a review of some relevant concepts may be useful. It once was fashionable to order taxa in hierarchies that were intended simultaneously to represent ancestor-descendant relationships, patterns of unidirectional change, and abstract (and unquantified) notions of efficiency, degree of advancement, and specialization. Accordingly, the extant mammals sometimes were arranged with monotremes (as "primitive" mammals) at the base and with eutherians (as "highly evolved" mammals) at the pinnacle of a Scala Mammalia (7). Although long since abandoned by evolutionary biology, such hierarchical arrangements dominate the public imagination and are enshrined among biologists in the unfortunate use of the adjectives "higher" and "lower" in reference to particular primates, mammals, or vertebrates. Among the many problems with such arrangements in that living taxa do not necessarily represent direct ancestors and descendants of one another, as opposed to termini of divergent lineages that originated from a common distant ancestor—hence, the branching phylogenies of works dating back to Darwin's writings and before. Other problems with such arrangements include assumptions that characteristics of organisms change in concert and that change is unidirectional and invariably toward increased specialization.

The concept of mosaic evolution recognizes that evolutionary change in separate lineages can occur at different rates, in different directions, and with respect to different features. Accordingly, all living taxa are likely to combine relatively ancestral (i.e., primitive) and derived (i.e., advanced) characteristics. For example, compared with features of other mammals, the ability of bovids to synthesize milk fatty acids from acetate and β-hydroxybutyrate of ruminal origin (2) is a derived feature, whereas hair and mammary glands are features that are primitive (ancestral) for living mammals (6). "Primitive" and "derived" are relative terms that can only be applied in the context of particular lineages; thus, although lactation is primitive for extant mammals, having been present in their common ancestor, this feature is derived in the broad context of vertebrate evolution. Another point is that description of a living taxon as primitive is misleading and should be avoided; a taxon can be conservative, having changed little over a long evolutionary period, but, given the reality of mosaic evolution, the term "primitive" should be retained for particular features in particular phylogenetic contexts.

These points are relevant to the present discussion because, until recent decades, the Scala Mammalia and its attendant misconceptions implicitly dominated attempts to reconstruct the evolution of reproductive patterns in mammals. In contrast, the systematic approach of cladistics, which incorporates the concepts discussed herein, has provided objective and well-defined methods for determining the directionality of evolutionary change and the phylogenetic relationships among taxa (16). A cladistic approach seeks parsimonious (conservative) interpretations of evolutionary change that minimize convergences and reversals. In brief, clades (lineages) are defined and grouped according to shared derived characteristics, and characteristics of these clades are extrapolated to their common ancestor (14). For example, mammals are defined as a group on the basis of structure of the jaw articulation and other derived characteristics that distinguish them from their reptilian progenitors. Cladistic analysis has proved to be useful in quantifying the evolution of vertebrate reproductive patterns (5) and other specializations (58) and in clarifying phylogenetic relationships among mammals (46). This approach shows particular promise in revealing the phylogeny of milk composition in extant mammals.

Mammalian Phylogenetic Relationships

The phylogenetic relationships among the three groups of living mammals have received much attention, and the traditional concept is essentially that presented by Huxley (39). Accordingly, an ancestral mammalian stock has been considered to have split into a prototherian stock ancestral to the monotremes and a therian stock ancestral to the remaining living mammals (53, 54). Thus, the eutherian and marsupial clades would have derived from a therian ancestor that postdated divergence of the monotremes. This phylogeny allows for the likelihood that each of the groups of extant mammals contain derived specializations, such as the incubatorium of the monotreme echidnas (6, 36) and the lactational specializations of macropodid marsupials mentioned herein.

Fossil evidence now places the separation of eutherians and marsupials to sometime before the late Cretaceous period (54), about 100 million yr ago. However, the earliest mammalian fossils are considerably more ancient, dating back over 210 million yr to the late Triassic period. Thus, the first half of mammalian history can be viewed as a period of intense evolutionary experimentation that included production of several major lineages with no living representatives, including multituberculates, docodonts, symmetrodonts, tricondonts, and eupantotherians (53). Precise relationships between these extinct lineages have not been determined definitively, nor have their relationships to the extant monotremes. Although monotremes traditionally have been classified with some of these lineages as a prototherian group, the group is artificial; early mammalian evolution is far more complex than previously realized (53, 54). In addition, some recent evidence and analyses suggest a much closer relationship between monotremes and therians than is usually presumed (1, 46, 47). In any case, living monotremes can be considered to be specialized remnants of a stock that preceded derivation of eutherians and metatherians (50).

Evolutionary Patterns of Reproduction and Lactation

What can be reconstructed about reproductive evolution in the common ancestors to the extant mammalian lineages? The following summary is based implicitly on the cladistic approach (3) [see (14, 52, 53, 79, 84, 90)]. The presence of a complex degree of lactation in all living mammals (22, 25, 88) clearly indicates that it preceded divergence of the extant stocks (26, 84, 90). The only alternative, that lactation evolved convergently in two or more lineages, is not credible, given the detailed similarities in mammary gland structure, development, and function in all extant mammals (6, 27, 59, 77). A host of features associated with lactation must have evolved prior to divergence of monotreme ancestors, including mammary glands with the normal complement of histological and cytological features: the capacity to produce milk that is rich in carbohydrate, protein, and lipid; neurohormonal control of milk synthesis and secretion; and suckling behavior.

Oviparity occurs in most reptiles, and its presence in monotremes represents persistence of an ancestral characteristic. However, several aspects of the oviparity of monotremes are specialized beyond that of many reptiles, such as ovulation of small ova, the oviductal retention of eggs for the early part of development, absorption by the eggs of large quantities of oviductal fluids, maternal incubation of the eggs following oviposition, and production of altricial hatchlings. Thus, if reproduction in living monotremes is similar to that of the closest common ancestor of all living mammals, that ancestor already had begun evolving toward the viviparity and uterine nutrient provision of therians (5). Production of altricial young that rely heavily on milk, such as occurs in monotremes, marsupials, and generalized eutherians, conservatively can be interpreted as a feature of the common ancestor to these groups (53, 90). Indeed, strong evidence and theoretical arguments indicate that altriciality may have been a common feature among Mesozoic mammals (26, 36, 79, 90). A related possibility is that some degree of altriciality was present in the ancestors of extant mammals but that trends toward extreme altriciality occurred independently in monotremes and marsupials (36). The presence of lactation would have made such trends possible.

The common ancestor to the marsupials and eutherians must have retained the complex degree of lactation exhibited by ancestors to the monotremes but had refined it through delivery via a teat system. Some important characteristics of this ancestor are unclear and have been a source of controversy. Thus, some researchers (53, 54) consider the common ancestor to have reproduced much as do extant marsupials, implying a large maternal investment into lactation relative to gestation, whereas others (63, 84) view similarities between marsupials and eutherians to be the result of convergence. For example, viviparity with short gestations could have evolved once in mammals in some therian ancestor (53, 90) or could have arisen independently in eutherians and marsupials (63, 84). The first explanation is the more conservative, but either is plausible, especially given the frequency with which viviparity and placentation have evolved in other vertebrates (5). Differences between the placentas of marsupials and eutherians (58), as well as the derived nature of the female reproductive tract in marsupials (79, 90), may be evidence of independent origins of viviparity in the two groups.

In any case, several specialized aspects of marsupial lactation and reproduction appear to be specializations that postdate divergence of the two therian lineages, including pouches (6, 36, 82, 84), striated mammary musculature (30), concurrent asynchronous lactation (17, 55, 90), the sternal swelling of dasyurids that is thought to uncurl the neonate and help it attach to the teat (11), and features of the female urogenital system (90). Likewise, assuming altriciality to be primitive for eutherians, such features as prolonged gestation lengths, production of precocial offspring, and secondary development of prolonged lactational periods among large-bodied species represent eutherian specializations that postdate their divergence from marsupials. Ancestry of the extreme temporal plasticity of mammary gland secretion exhibited by marsupials remains to be explicated. Perhaps an ontogenetic shift in milk composition was an ancestral mammalian feature that has been accentuated in marsupials and in *Tachyglossus* in association with the production of altricial young. Much of the variation occurring within each therian infraclass, such as in milk composition, teat number and position, and mammary gland structure and development, reflects evolution that occurred subsequent to the divergence of marsupial and eutherian stocks. This diversity is, therefore, not directly relevant to the broad outlines of early mammalian evolution, the focus of this paper, but offers fertile ground for comparative biologists interested in analyzing diversity in functional and evolutionary terms.

The common ancestor of the extant mammals appears likely to have been an oviparous mammal with well-developed lactation and maternal care and one that gave rise to altricial hatchlings that relied heavily on milk as a nutritional source (54, 79, 90). The common therian ancestor of marsupials and eutherians retained a complex degree of lactation and may also have produced altricial young, but whether that ancestor reproduced viviparously is uncertain. Convergent trends possibly have occurred toward viviparity in marsupials and eutherians and production of highly altricial offspring in monotremes and marsupials. Subsequent to divergence of eutherians and marsupials, representatives of the latter group evolved specializations associated with lactation, some of which enhanced survival of the altricial young, and others that maximized female reproductive effort. During their radiation in the Cenozoic period, eutherians evolved enormous diversity in gestation lengths, lactation lengths, and degree of development of the offspring at birth.

ORIGINS OF THE MAMMARY GLAND

After the summary of lactational diversity and its evolution in the three major clades of living mammals, it is now appropriate to consider the specializations for lactation that lie in the more distant past and that may date to the origins of mammals. One of these specializations is the mammary gland, the functional complex to which mammals owe much of their success. Evolutionary origins of the mammary gland not only are relevant to an understanding of lactational characteristics of living mammals, but to the original functions of protolacteal secretions and the potential of the gland complex to evolutionary modification.

Unfortunately, the mammary gland, along with the vertebrate eye and the amniotic egg, is one of a few functional complexes for which evolution has resisted explanation. Even the most conservative of living mammals have fully functional, complex mammary glands (26, 27, 65) but reveal little information about the origins of this remarkably organ. No homologue to the gland is found among extant reptiles, which are, in any case, only distantly related to the cynodont ancestors of mammals, and the fossil record has yielded little useful information. In the absence of living or extinct species revealing early and intermediate stages in the evolution of the mammary gland, biologists have great latitude for speculation, and a variety of hypotheses have been advanced (4).

Most attempts to reconstruct mammary gland origins have theorized one of the cutaneous gland populations of living mammals as a likely precursor, notably eccrine glands, sebaceous glands, and apocrine glands. For example, several investigators (56, 77, 85) have considered the mammary gland to have arisen from one of these three populations, whereas others have argued for an origin from unspecified "sweat glands" or an ancestral population of generalized cutaneous glands (7, 56, 76). Others have supported a diphyletic origin, concluding that mammary glands originated from sweat glads in monotremes and from sebaceous glands in therians [for historical reviews, see (4, 7)].

How can such hypotheses be evaluated? Like most postulates about past evolutionary history, they cannot be tested directly because they deal with singular, nonrepeatable sets of events that have never been observed. Following a contemporary approach, predictions would be derived from such evolutionary hypotheses and formally tested. Such an explicit approach has been used effectively in a variety of evolutionary analyses. However, this approach would mistakenly be considered to be a marked departure from those that have dominated evolutionary biology over the past century. The plausibility (and parsimony) of evolutionary hypotheses traditionally have been evaluated by consideration of their congruence with available information, an approach that offers implicit tests of these hypotheses. The diphyletic theory for mammary gland origins can be used to compare the explicit and implicit approaches. One could predict from the diphyletic theory that therian mammary glands would be similar to sebaceous glands in morphology, physiology, and development, whereas monotreme mammary glands would be more similar to sweat glands (7). A variety of derivative predictions explicitly could be formulated and tested, and the theory could be supported, refuted, or modified accordingly. However, the numerous studies of mammary glands and skin glands represent implicit tests of the theory that, arguably, are no less rigorous than formal explicit tests of predictions. The diphyletic theory has been abandoned because it is inconsistent with the information revealed in such studies (7), and explicit verbal tests of the hypothesis would seem to offer no additional insight.

Detailed studies of gland anatomy, physiology, and embryology dating back to the mid-1800s have provided a wealth of data available for evaluation of the hypotheses on mammary gland origins. The simplest and most common approach is to compare mammary glands with each of the populations of integumentary glands in mammals; the expectation is that evolutionary heritage will be reflected in similarities between gland populations. A possible criticism of such a comparative approach is that it assumes a conservative evolutionary process—that evolutionary change has not unrecognizably obscured the phylogenetic relationships among the extant gland populations as natural selection has led to modifications of gland function. However, gland evolution clearly has been conservative; despite the extensive radiation of mammals into a variety of habitats and the specialization of glands in particular lineages, three populations of skin glands are still recognizable in most species (80, 81, 86). Moreover, mammary glands all share fundamental attributes of structure and function (4). Thus, basic features of gland populations have been retained since the divergence of the ancestors of extant mammals. More serious criticism can be leveled against the assumption that similarities in gland characteristics indicate the degree of relatedness in a phylogenetic sense. This assumption is questionable, and its widespread acceptance may have done more to obscure than to reveal mammary gland origins.

Integumentary Glands as Ancestors of the Mammary Gland

Characteristics of the three populations of mammalian integumentary glands are summarized in Table 3. As the table indicates, the mammary gland bears similarities to each of the gland populations. As in eccrine and apocrine glands, myoepithelial cells are present, and secretion is, at least in part, merocrine. However, like sebaceous glands, the mammary gland is branched, tends toward an alveolar structure, and lacks a functional motor innervation. Moreover, the topographic distribution of sebaceous glands is what would be expected of a population that gave rise to a gland of the thoracoabdominal region.

The mammary gland sometimes is compared with eccrine glands of the type occurring in anthropoid primates; both have a copious output of aqueous secretions. The similarity is only superficial, and primate eccrine glands are atypical by virtue of their abundant distribution and copious secretions. However, the mammary gland shares several characteristics with typical apocrine and sebaceous glands, including a developmental association with hair follicles (in some species) and extensive synthetic abilities. Although mammary glands differ markedly from typical apocrine and sebaceous glands in the composition and quantity of their secretions, each of these gland populations exhibit specializations for synthesis and secretion of organic substances, as well as seasonal changes in size and activity (43) and a maturation that accompanies the onset of puberty (87). Effects of hormones on the various gland populations differ, and the precise neurohormonal control of mammary function must be presumed to postdate evolutionary differentiation of the gland (6). However, seasonal and maturational cyclicity of the three glands in various mammals, as well as the responsiveness of sebaceous glands to prolactin in some species (Table 1), may reflect some form of evolutionary relationship between the glands, if not a measure of endocrinological control that preceded mammary gland origins.

These comparisons indicate that the mammary gland is more similar to sebaceous glands and apocrine glands than to eccrine glands. However, a postulated derivation of the mammary complex from any extant population of glands raises some problematic issues. First, the mammary gland is a compound, glandular complex that is less like one of the unitary glands than like a large glandular aggregation. This factor does not preclude an origin of the mammary gland from one of the simpler gland populations; both apocrine and sebaceous glands have been modified evolutionarily into complex gland aggregates (43, 75), structures that may lie in the ancestry of the mammary gland. Another issue is that each of the extant populations of skin glands exhibit specializa-

TABLE 3. Comparison of the lactating mammary gland with the cutaneous gland populations of mammals (5).

	Gland type			
Characteristic	Mammary	Eccrine	Apocrine	Sebaceous
Histological type	Compound, branched tubulo-alveolar	Simple, coiled tubular	Simple, coiled tubular	Simple, branched alveolar
Myoepithelial cells	Present	Present	Present	Absent
Motor innervation	Absent	Present[1]	Present[1]	Absent
Mode of secretion	Merocrine, apocrine	Merocrine	Merocrine, apocrine, holocrine	Holocrine
Output	Very copious	Variable[2]	Variable[2]	Small
Anlagen from hair follicle	Yes	No	Yes	Yes
Distribution[2]	Ventral thorax and abdomen	Friction surfaces[3]	Variable	Widespread
Synthesis	Extensive	Limited	Extensive	Extensive
Prolactin response	Present	Absent	Absent	Present[2]
Androgen response	Absent	Absent	Present[2]	Present

[1] Indirect innervation in some.

[2] Species-dependent.

[3] Abundant on the general body surface in humans and other anthropoid primates.

tions that seem unlikely to have been present in a mammary gland ancestor. Among these specializations are the holocrine secretory mode and androgen responsiveness among sebaceous and apocrine glands, the motor innervation of apocrine and eccrine glands, and the functional attributes and restricted distributions of typical eccrine glands. Consequently, derivation of the mammary gland from these populations would have required evolutionary reversals or convergences (4).

Another possibility is that lacteal glands predate full differentiation of the extant gland populations, for example, having originated from a precursor to apocrine and sebaceous glands. Such an origin would require loss of myoepithelial cells in sebaceous glands or their independent expression in apocrine and mammary glands (4). Nevertheless, although evolutionary biology seeks parsimonious explanations that minimize convergences and reversals, none of the extant gland populations or their hypothetical precursors can be eliminated at present as possible mammary gland ancestors. Evolution is, after all, a process that has produced jaws from gill arches, legs from fins, and the ability to comprehend nature (and to compose symphonies!) by means of an organ originally adapted for olfactory processing.

Neomorphic Mosaicism and Mammary Gland Origins

No particular reason exists to indicate that the mammary gland had to evolve through modification of features of a single population of integumentary glands. On the contrary, mammary glands could have arisen through the association of characteristics of several gland populations into a new morphological and physiological combination—a neomorphic structure representing a mosaic of preexisting features. Despite its functional complexity, the mammary gland shares most of its component parts (and generally, its physiological attributes) with other, less specialized gland populations. An organism with the normal complement of skin glands contains genetic information for constructing every component of these glands and the ability to express those genes under appropriate developmental conditions. Consequently, that organism should have a finite potential for recombining those components into a new morphological assemblage through modifications in the timing or site of gene expression (4). Such an origin for the mammary gland does not preclude transformation of a specific ancestral population of abdominal glands. However, such transformation could have involved loss of existing features from those glands and incorporation of features from other exocrine glands through modifications in gene expression during development; thus, in a broad sense, no single population of glands necessary constitutes the sole ancestor to the mammary gland.

Several lines of evidence are consistent with the idea that the mammary gland evolved as a neomorphic mosaic or hybrid of preexisting characteristics into a new functional complex. For example, the mammary gland exhibits characteristics of each of the existing populations of skin glands. In addition, the mammary gland shares synthetic pathways and enzymes with a variety of tissues that cannot themselves be considered to be directly ancestral to the gland (6, 76). Furthermore, the mammary gland produces several antimicrobial substances secreted by nonintegumentary glands, such as lacrimal and salivary glands (34), indicating that genes expressed in such glands have been coopted for expression by the mammary gland. Moreover, a wealth of data from experimental morphology and developmental genetics has revealed an important role for differential gene expression and modification of developmental pathways in the evolution of form (76).

Experimental studies on mammary gland development have yielded data relevant to gland evolution. Modifying a technique that previously had helped elucidate the mesenchymal-epithelial interactions in the control of mammary gland development (18), Sakakura et al. (83) combined mammary epithelium and salivary mesenchyme from neonatal mice and transplanted them to the kidney capsule of adult females. The grafted glands underwent morphogenesis as does the salivary gland, but their epithelia exhibited functional characteristics of the mammary gland, secreting α-lactalbumin upon pregnancy. Gland morphogenesis apparently is directed by the underlying mesenchyme (76, 83), as in several other gland populations. The fact that morphogenesis and cytodifferentiation are functionally disengaged indicates that evolutionary modification of these developmental processes need not have occurred in concert. Rather, the two processes could have been modified at different times under somewhat different selective pressures. Thus, modification of epithelial cell synthesis and secretion in protolacteal glands, though cooptation of synthetic pathways and secretory mechanisms of other gland populations, could have either preceded or followed development of a gland with a copious output and large storage capacity.

A mosaic or hybrid origin for the mammary gland may have implications for functional properties of the early lacteal glands. Scenarios for the evolution of lactation often assumed that these lacteal glands supplied a copious watery secretion; subsequent evolution gradually led to production of secretions with significant organic content (32). However, if early lacteal glands coopted features of apocrine and sebaceous glands (or related glandular precursors), the lacteal glands may already have had the ability to secrete lipids and proteins. Thus, production of secretions with some nutritional value may have occurred early rather than late in the evolution of lactation and may have set the stage for the selection of females that were able to supply more nutritious secretions.

ORIGINS OF LACTATION

Although lactation clearly evolved prior to divergence of the extant lineages of mammals, how widespread it was among the early mammals or their reptilian cynodont ancestors remains unknown. Inferences of lactation in fossil species from tooth replacement patterns and other osteological features are tenuous (6). As a consequence, suggestions that lactation was present in various groups of Mesozoic mammals (26, 53, 73) or even in the cynodonts (9, 31) lack definitive corroboration. Based on fossil evidence and extrapolations from extant lineages, a recent reconstruction concluded that lactation arose in an endothermic, oviparous amniote with a glandular, hairy integument and maternal care (6). Most, and perhaps all, of these features were shared by the early mammals and their cynodont ancestors, and identity of the particular group within which lactation originated remains elusive. If, however, altriciality was widespread among the Mesozoic mammals, as suggested herein, lactation seems likely to date to the early mammals of the Triassic period (53, 73).

Given the complexities of milk synthesis, secretion, delivery, and uptake, lactation could hardly have arisen in its present form. Consequently, evolutionary scenarios typically begin with the assumption that lactation arose as a much simpler process involving integumentary glands that secreted substances with different properties and functions than the milks of modern mammals. Thus, scientists have postulated that protolacteal secretions functioned to warm the eggs (7), to adhere the eggs to the maternal abdomen (24), to provide water to the offspring (32), and to bond the offspring to the mother by olfactory means (21). All but the last of these hypothetical functions are based on inferences that have been seriously challenged (6), and none specifically accounts for the transition from secretions with low nutrient content to milk.

Protolacteal Secretions as Microbial Inhibitors

The importance of milk to nutrition of the offspring tends to obscure a vital function that may account for the origins and early evolution of lacteal secretions. The following summary is based on two recent reviews (6, 34). Milk shares with the secretions of mucous membranes and certain integumentary glands a variety of substances with antimicrobial properties, including lysozyme, transferrin, peroxidase, xanthine oxidase, and immunoglobulins. In milk, these substances act against bacteria, fungi, and protozoa by several mechanisms, protecting the offspring (and the mammary gland itself) from microbial infection. Some of these microbial inhibitors are similar to substances that protect eggs and other structures from microbial attack. For example, lysozyme protects avian eggs from bacterial infection, and lactoferrin of exocrine secretions and ovotransferrin of avian albumen control bacterial concentrations by chelating iron.

Microbes are significant predators on eggs and hatchlings, and vertebrates have evolved a variety of adaptations that protect eggs against microbial attack. If the antimicrobial components that occur in milk were also present in the secretions of protolacteal glands, they could have protected eggs by controlling microbial populations on the eggshell or in the nest environment. The inferred presence of maternal care and endothermy in mammalian ancestors raises the likelihood that females remained with and incubated their developing eggs, as do extant monotremes. A recent scenario (6) suggested that secretions of integumentary glands of the ventral abdomen controlled potential pathogens in the nest environment. Likewise, incidental ingestion of such secretions by the hatchlings would have enhanced offspring survival by controlling flora of the digestive tract and pharynx and, possibly, by conferring a degree of immunological protection. Accordingly, protolacteal secretions originally may have enhanced offspring survival more because of their antimicrobial properties than because of their nutritive content (6).

Evolution of α-Lactalbumin

Functional and evolutionary links between the α-lactalbumin of the mammary gland and the lysozyme of milk and other glandular secretions provides striking evidence that the ancestral lacteal secretions had antimicrobial properties. Lysozyme is a ubiquitous component of vertebrate exocrine secretions that protects mucous membranes, other surfaces, and eggs from bacterial and fungal attack (6). α-Lactalbumin is a component of the lactose synthetase system that complexes with the enzyme galactosyltransferase to catalyze production of the milk sugar lactose. The evolutionary relationship between lysozyme and α-lactalbumin is unequivocal (8, 20), and genes for α-lactalbumin clearly evolved from a duplicated gene for lysozyme (34, 89).

The time frame of this evolutionary transformation relative to monotreme divergence is still under investigation. In the echidna, *Tachyglossus aculeatus,* milk contains abundant quantities of lysozyme (89) that weakly stimulate lactose synthesis (37, 45), raising the possibility that monotremes exhibit proteins that are functionally and evolutionarily intermediate between lysozyme and α-lactalbumin (6). Recent studies on the echidna have not yet verified the role of lysozymes in lactose synthesis and have provided indirect evidence for a protein that is similar to α-lactalbumin (88). In addition, a recent attempt to isolate lysozyme from milk of the platypus revealed only very low concentrations, despite lactose synthase activity (88); thus, further study is needed. Other than lysozyme, milk from echidna contains large quantities of another bacteriocidal substance, transferrin, that functions in delivery of iron to the suckling (45). The abundance of these substances in milks of a monotreme may reflect the original attributes of protolacteal secretions as well as the functional needs of the hatchlings in extant species. These substances seem to have been coopted for nutritional purposes in particular lineages, rather than having functioned in nutrition from their inception.

The evolution of α-lactalbumin from lysozyme and its incorporation into the lactose synthetase system appears to have had important functional consequences (6), for example, synthesis of milk carbohydrates from common precursor molecules and a mechanism for controlling lactose synthesis. In addition, lactose synthesis contributed carbohydrates in a form suitable for lumenal storage and yielded osmotically active substances that facilitated storage of a secretion with a high water content (71). Other functional consequences include promotion of calcium absorption and establishment of intestinal flora in the young.

Early Evolution of Lactation

If the protolacteal glands produced antimicrobial secretions that enhanced offspring survival, natural selection could have led to production of larger quantities of such secretions, with attendant changes in gland size and structure. Likewise, any nutritional components of these secretions would have served as a facultative dietary supplement to the hatchlings. Such secretions would have allowed the offspring to grow and to develop at little energetic cost to themselves. Consequently, an evolutionary trend would have been produced toward production of secretions with higher organic content.

Direct advantages would accrue to the adult female as lacteal secretions became increasingly nutritious and provided a major portion of maternal investment into the young. By making extreme altriciality possible, lactation would have allowed females to minimize egg size and weight, which, in the small mammals of the Mesozoic Era (90) could have had significant advantages in reducing the burden imposed by the clutch prior to deposition. The trend toward substantial altriciality in turn would have required a parallel shift from facultative to obligative ingestion of secretions, the latter of which characterizes living mammals. Another advantage of providing nutrients through lacteal secretions is that females could buffer the young from seasonally restricted nutrients, allowing occupation of environments that lacked suitable food for the young (72, 73, 74). In addition, females could time their reproductive investment to minimize energy drain and to diminish the costs of offspring loss (6). Other functional implications of lactation are discussed elsewhere (35, 70, 72, 73, 90).

The trend toward development of increasingly nutritious secretions probably accompanied increases in ingestion efficiency and hormonal control of synthesis and secretion. Effective synchronization of gland hypertrophy with reproduction would have required establishment of some degree of hormonal control. Extensive studies on marsupials (90) have revealed many similarities with eutherians in the control of milk synthesis and secretion. Thus, fundamental features of the endocrinological control of mammary gland function appear to have evolved prior to divergence of marsupials and eutherians, if not prior to divergence of monotremes and therians. Further work on monotremes (25, 27) may reveal additional information about the evolutionary history of the neurohormonal control of lactation.

SOME IMPLICATIONS FOR EVOLUTIONARY BIOLOGY AND DAIRY SCIENCE

Evolutionary Implications

The evolution of lactation has always been difficult to explain because of the magnitude of the inferred genetic changes and the difficulty of envisioning stages in its evolution. However, the evolution of α-lactalbumin illustrates how a small genotypic modification can have large phenotypic effects, producing a cascade of functional and evolutionary changes with important implications for the biology of the species.

Origins of the mammary gland may also have occurred without the large-scale genetic changes implied by the complexity and size of the organ. If lacteal glands evolved through reassembly of existing components into a new combination, then modifications of regulatory genes (as opposed to evolution of new structural genes) may have played a major role by altering the timing and site of gene expression, tissue interactions, and epigenetic factors. Moreover, the fact that epithelial cytodifferentiation and gland morphogenesis are not closely linked indicates a degree of plasticity that could have enhanced gland evolution, by allowing temporally separate modifications on the two processes. Contrary to most previous scenarios, functional specializations of ancestral lacteal glands could have preceded morphological evolution, eventually leading to a large specialized gland complex with copious output. Consequently, past difficulties in construction of plausible evolutionary explanations may reflect a lack of appreciation of possible mechanisms of evolutionary change, as well as the phenotypic consequences of small genotypic modifications of developmental pathways, rather than the inherent improbability of the historical transformations.

Implications for Dairy Science

Comparative, functional, and evolutionary data have several implications for dairy science. Interspecific diversity in mammary gland size, location, and output (40, 56, 66, 78) that has resulted from natural selection suggests a great potential for modification under genetic control by humans. Moreover, attempts to manipulate gland form and function may well be enhanced by the plasticity of the gland and its ability to incorporate and to integrate new structural components into functioning units. Given the functional independence of cytodifferentiation and morphogenesis, experimental modifications of gland size and form are likely to be independent of the modification of secretory function. Moreover, mammary glands have an evolutionary potential for developing extraordinary functional flexibility, as revealed by such marsupial specializations as concurrent asynchronous lactation and ontogenetic changes in milk quality. Interestingly, if the mammary gland is a mosaic neomorphic organ, any future attempts to produce mammary glands with new characteristics may not be so very different from the natural processes that originally gave rise to the gland. In a sense, mammary gland bioengineering may bear much the same relationship to the natural processes of gland evolution as human-directed breeding does to natural selection.

The diversity of extant mammals with respect to milk composition and output also suggests a large potential for human manipulation. The fact that milks of all living species are descended evolutionarily from an ancestral milk with carbohydrates, lipid, and protein indicates that, with appropriate genetic modification, milks of the domesticated artiodactyls can probably be modified in a variety of directions. Moreover, mammalian diversity offers a wealth of genetic material that is potentially available for responsible incorporation.

Nevertheless, human attempts to manipulate lactation do not have unlimited potential. Limits may be imposed, for example, by functional characteristics of the mammary epithelium or other mammary features that were established early in mammalian history, the finite life span of secretory epithelia, functional trade-offs between milk components (40, 69), the need to maintain milks that satisfy the nutritional needs of the offspring, the requirement for an osmotically active secretion (71), the need to produce carbohydrates in a form suitable for storage (71), maternal nutritional requirements, and the need to maintain antimicrobial properties that protect both the mammary gland and the offspring.

One final limit to human attempts to direct the evolution of lactation is imposed by the very factors that help to make increased milk production desirable. The genetic diversity represented among the living mammals represents an untapped resource that is now threatened by human overpopulation and habitat destruction. The current decimation of such diversity not only represents a loss to the planet of incalculable proportions but imposes constraints on human potential by irrevocably limiting attempts to understand and to control lactation, the very process that has contributed so importantly to mammalian success.

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