TRIENNIAL LACTATION SYMPOSIUM/BOLFA: Historical perspectives of lactation biology in the late 20th and early 21st centuries[1]

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ABSTRACT: The latter half of the 20th century and the early portion of the 21st century will be recognized as the "Golden Age" of lactation biology. This period corresponded with the rise of systemic, metabolomic, molecular, and genomic biology. It includes the discovery of the structure of DNA and ends with the sequencing of the complete genomes of humans and all major domestic animal species including the dairy cow. This included the ability to identify polymorphisms in the nucleic acid sequence, which can be tied to specific differences in cellular, tissue, and animal performance. Before this period, classical work using endocrine ablation and replacement studies identified the mammary gland as an endocrine-dependent organ. In the early 1960s, the development of RIA and radioreceptor assays permitted the study of the relationship between endocrine patterns and mammary function. The ability to measure nucleic acid content of tissues opened the door to study of the factors regulating mammary growth. The development of high-speed centrifugation in the 1960s allowed separation of specific cell organelles and their membranes. The development of transmission and scanning electron microscopy permitted the study of the relationship between structure and function in the mammary secretory cell. The availability of radiolabeled metabolites provided the opportunity to investigate the metabolic pathways and their regulation. The development of concepts regarding the coordination of metabolism to support lactation integrated our understanding of nutrient partitioning and homeostasis. The ability to produce recombinant molecules and organisms permitted enhancement of lactation in farm animal species and the production of milk containing proteins of value to human medicine. These discoveries and others contributed to vastly increased dairy farm productivity in the United States and worldwide. This review will include the discussion of the centers of excellence and scientists who labored in these fields to produce the harvest of knowledge we enjoy today.

Key words: evolution, historical, mammary biology

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INTRODUCTION

In his fifth edition of *Dairy Cattle and Milk Production*, Dr. Clarence Eckles (1939, p. 4) reported that the "ratio between the human population and the number of cows in the United States had remained essentially the same since 1850." As shown in Fig. 1, that number dramatically changed over the last half of the 20th century and first half of the 21st century. This was due to the rapid increase in milk yield per cow and, despite a concomitant expanding human population, made it possible to meet an increased

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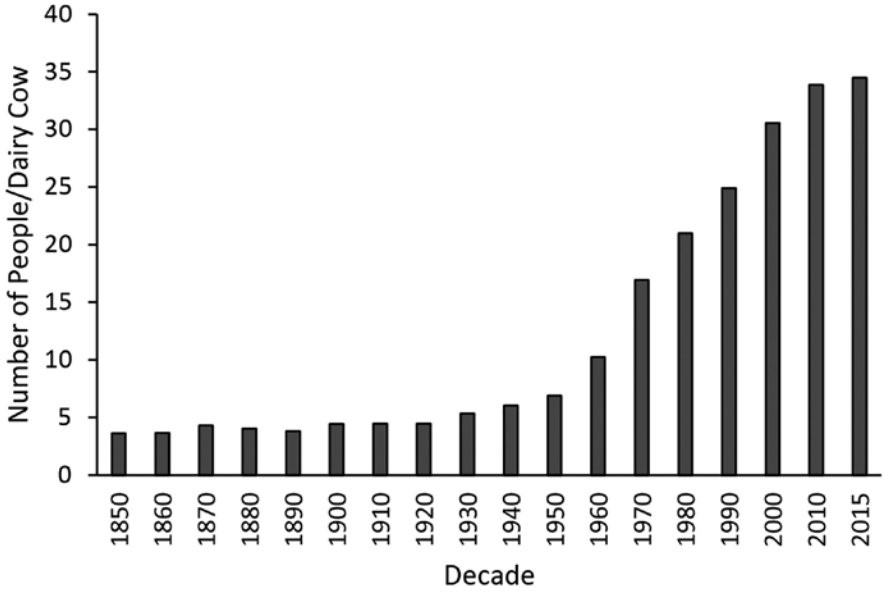

Figure 1. Historical ratio of the number of people per dairy cow in the United States from 1850 to 2015.

milk demand with a smaller number of cows. This improvement in milk yield per cow occurred during a rapid expansion of knowledge in biology including the fields of lactation biology, nutrition, genetics, and reproduction, which were quickly translated to commercial farm conditions via the land-grant system in the United States. The objective of this review is to highlight some of the contributions lactation biology made to this progress.

The latter part of the 20th century and the first half of the 21st century are generally referred to by many as the "Golden Age of Biology" (Smith et al., 2014). Lactation biology is nested within this period, and because of the strong association between structure and function in the mammary epithelial cell, it has provided spectacular examples of the relationship among metabolic pathways and specific structures within the mammary epithelial cell (Wellings et al., 1960). The age of biology may be further divided into the early stage of discovery biology, the development of computational biology, and the current rapidly expanding field of cell-free synthetic biology. The explosion of discoveries during this period rose from the availability of an array of new tools to study biology from the molecular to the whole-animal level and the concomitant rapid growth of biotechnology and molecular biology (Smith et al., 2014). In the field of lactation biology, these discoveries have been applied to address 2 major societal issues, the increasing need of high-quality protein for a rapidly expanding human population and the identification and treatment of mammary cancer in the human population.

Since J.D. Watson and F.H. Crick published the helical structure of DNA (Watson and Crick, 1953), we have steadily increased our knowledge about the genes on which life is based. The identification of restriction enzymes by Werner Arber, Daniel Nathans, and Hamilton O. Smith in the late 1960s and their subsequent application to the problems of molecular genetics (Roberts, 2005) led to the ability to engineer bacteria to produce high-value proteins, such as bovine ST and vaccines, the genetic mapping of a variety of life forms including the human and bovine, and the identification of polymorphisms in the genome, which can be used as markers for genetic selection and for cloning domestic animals

The development of recombinant DNA tools; the advent of rapid, inexpensive sequencing technology; and most recently, the development and stockpiling of genetic pathways referred to as BioBricks (Church et al., 2014) allows biotechnologists unprecedented speed and power to master the genetic framework of an organism (Rebatchouk et al., 1996; Knight, 2003). In particular, researchers can make site-specific, rationally designed genome modifications or replace genomes in only a few days (Shetty et al., 2008). This capability has led to the expanding field of synthetic biology. Synthetic biology is considered the engineering discipline of biology, which seeks to manipulate biology toward a desired product. Modern day synthetic biology has its roots in the development of recombinant DNA tools and has exponentially grown in the 21st century. Currently, products of genetically modified cells comprise 2% of the U.S. economy (US$350 billion per year; Church et al., 2014).

Therefore, the "Golden Age of Lactation Biology" has largely completed its discovery phase and is rapidly moving through its computational phase to accel-

erate genetic progress in production, reproduction, and health of dairy animals. In the future, we will see more use of the mammary gland as a bioreactor and the cell-free synthesis of commercially important milk proteins.

EVOLUTION OF LACTATION

The origin of lactation has remained a major unresolved issue in evolutionary biology since the time of Darwin (Blackburn et al., 1989). The mammary gland is a soft tissue, and as a consequence, there is no fossil record to assist in the evolutionary development of the mammary gland. Frazzetta (1975, p. 45) stated, "The highly integrated nature of the morphological, physiological and behavioural features of milk production, ingestion and digestion renders lactation a complex adaptation that challenges gradualistic explanations for its evolution." In particular, the path from the beginning stages in the evolution of milk production and the adaptive value of early proto-lacteal secretions has been difficult to map out. Additional unresolved issues include embryonic origins of the mammary gland and the evolutionary transformation from production of proto-lacteal fluids to the complexities of milk synthesis add to this complexity.

Blackburn et al. (1989) reviewed the extant literature and proposed that evolution of egg-incubation behavior in a mammal-like reptiles followed development of hair, endothermy, and cutaneous glands in synapsids and therapsids during the Permian period about 250 million yr ago. They hypothesized that this was followed by evolution of a well-vascularized incubation patch on the ventral abdomen with subsequent enhancement of egg survival via antimicrobial properties of secretions of cutaneous glands of the incubation patch in the Cynodontia and Mammaliaformes during the Triassic and early Jurassic periods followed by appearance of the Mammalia in the late Jurassic and early Cretaceous periods from 200 to 150 million yr ago. A major protein in these secretions was lysozyme C, which would have provided antibacterial protection and later evolved to become α-lactalbumin (Irwin et al., 2011). Offspring survival may also have been enhanced by ingestion or absorption through the egg surface of the secretions. Further development included hypertrophy of these cutaneous glands of the incubation patch, with production of a somewhat more copious secretion, which may have been under hormonal control. The gradual transition of this maternal secretion to a more nutritious product, associated first with a supplementation and then with a replacement of yolk nutrient provision, gradually occurred over time. Blackburn et al. (1989) further proposed that the mammary gland prototype evolved synthesis capabilities in part through co-optation and modification of existing synthetic pathways, enzymes, and end products. This path of evolution led to production of nutritious secretions, which shifted from facultative to obligatory provision of extravitelline nutrients. The similarities of monotreme and therian lactation, as demonstrated by M. Griffiths and his colleagues (Griffiths, 1965, 1968, 1978; Griffiths et al., 1969, 1973), indicate that these features evolved before divergence of monotremes from therians.

This framework for the evolution of lactation was further elaborated on by Oftedal (2002), who pointed out that synapsid vertebrates developed a glandular rather than a scaled integument some 300 million yr ago, setting up the opportunity for specialization of some of those glands for development into mammary glands. He suggested that 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 (Griffiths et al., 1973; Griffiths, 1983; Oftedal, 2002). The specific glands cited are the apocrine sweat glands associated with hair follicles (Oftedal, 2002). This association is still evident in monotremes and early stages of development in marsupials. Oftedal (2002, p. 245) proposed that "milk underwent an evolutionary transformation from egg supplement to hatchling food." Furthermore, in advanced egg-laying synapsids, milk participated in both roles, and this may still be in the case in some monotremes (Oftedal, 2002).

In both monotremes and some marsupials, the milk at the beginning of lactation has low nutritional value and is very dilute (10 to 12% DM). This milk is low in both lipids and simple sugars (monosaccharides and lactose; Janssen and Walstra, 1982). The galactosyltransferase required for lactose synthesis is not expressed at the beginning of lactation in marsupials (Messer and Nicholas, 1991; Urashima et al., 1992). This early secretion may be the closest to a "primitive" milk to be found among mammals and is quite different from the high-oligosaccharide and high-lipid milks that are secreted in subsequent lactation stages (Green and Merchant, 1988; Urashima et al., 2001). Therefore, the change in milk composition across lactation in monotremes and marsupials may reflect the evolution of milk composition within the class Mammalia.

The study of the evolution of milk proteins provides another perspective to examine the timeline of evolution of milk secretion. The appearance of α-lactalbumin by duplication and modification of the lysozyme C enzyme is estimated to have occurred at 260 million yr ago during the late Permian period (Shewale et al., 1984). Recently, Kawasaki et al. (2011) reported that casein genes arose from mutation

of tooth protein genes in synapsids approximately 300 million yr ago, before the appearance of mammals, as a component of skin secretions bathing incubating eggs to supply additional calcium for the developing embryo and to strengthen the shell. The appearance of milk protein genes and placentation in the mammalian genome was later coupled with the loss of egg yolk protein genes approximately 30 to 70 million yr ago (Brawand et al., 2008). Therefore, the evolutionary record of milk proteins indicates that their appearance preceded the development of mammals and mammary glands. Furthermore, as reported by Capuco and Akers (2009), the core milk protein genes are highly conserved in the class Mammalia, in particular those proteins associated with the process of secretion. A greater rate of mutation has occurred in those proteins associated with immune function of the mammary gland and the nutritional value of milk (Lemay et al., 2009). Interestingly, Lemay et al. (2009) also reported that the species-specific variation in milk composition was primarily due to mechanisms other than protein sequence variation. The taxonomic diversity of milk composition may be due to a difference in the number of copies of milk protein genes or differences in transcriptional and translational regulation of genes expressed in the mammary gland. Additionally, other organs involved in energy partitioning may also contribute to milk composition variation. They proposed that future studies of genomic regulation of milk composition should focus on the noncoding regions of the genome, particularly those with putative regulatory function, as potential sources of species-specific variation in milk composition.

THE ANATOMY AND COMPOSITION OF THE MAMMARY GLAND AND ITS SECRETIONS

Early studies on mammary biology focused on the gross and microscopic anatomy of the mammary gland, location and numbers of glands, the identification of cell types within the mammary glands, and the composition of mammary tissue and its secretions (Turner, 1952; Reece, 1956; Tucker, 1981; Jenness, 1986). The lactating mammary gland consists of secretory and ductal epithelial cells, referred to as parenchyma, embedded in a collagen matrix containing 4 other cell types that include adipocytes, fibroblasts, myoepithelial cells, and smooth muscle. These are collectively referred to as stroma. Other cell types that are also present in the mammary gland include those associated with the vascular, nervous, and immune systems. Early studies of mammary tissue required labor-intensive histometric procedures. The introduction of quantitative methods for measuring the DNA content of mammary tissue by Kirkham and Turner (1953) greatly increased the pace of studies on the regulation of growth of the mammary gland. The correlation between amount of secretory tissue (parenchyma) in a gland and the total volume of milk produced is quite high (Tucker, 1966, 1987; Hackett and Tucker, 1969; Nagai and Sarker, 1978), ranging from 0.58 to 0.85, depending on the endpoint measuring secretory tissue and stage of lactation. Additionally, it has been shown that the metabolic rate of mammary secretory tissue and milk energy output scales in agreement with Kleiber's law governing basal metabolic rate across all orders of the class Mammalia (Martin, 1984). These findings very clearly indicated that selection for milk yield in dairy cattle would, in part, require growth of additional mammary tissue to accommodate the increase in milk output.

Milk composition varies considerably among species and across stage of lactation (Jenness and Sloan, 1970; Jenness, 1986; Oftedal, 1984). Factors that have been identified as causative for this variation include body mass, diet, length of lactation, growth rate of the neonate, environment, and evolutionary relationships (Oftedal, 1980; Skibiel et al., 2013). A recent review by Skibiel et al. (2013) was among the first to examine in detail the evolutionary relationships in the evolution of milk composition and they found that major factors in selective pressures acting on milk composition include the shared evolutionary history among species, the diet consumed, and the relative length of the lactation period.

An additional aspect of the studies on milk composition included the fact that many components of milk are not found elsewhere, such as the milk proteins casein, α-lactalbumin, and β-lactoglobulin and the milk sugar lactose. Gowen and Tobey (1928) determined that there was sufficient lactose present in the mammary gland at milking to account for all of the lactose in milk removed from the gland. They surmised from this that lactose was synthesized de novo in the mammary gland from carbohydrate precursors. Later investigators would focus on these molecules and their role in the milk synthesis and secretory process.

MAMMOGENESIS

The determination that peak milk yield was highly correlated with the quantity of secretory tissue in the mammary gland spurred considerable work on factors regulating development of the mammary gland. Several excellent reviews are available for a thorough study of the topic (Tucker, 1981; Neville, 2009; Berryhill et al., 2016). Therefore, milk production potential is a function of the number of mammary epithelial cells in the gland as well as the secretory activity of

Table 1. Factors affecting mammary development in cattle

Stage of development	Factor	Impact	Reference
Before weaning	High protein and energy	Increased growth	Bar-Peled et al., 1995
Prepubertal	Excess energy	Decreased growth	Sejrsen et al., 1982
Peripubertal	ST	Increased growth	Sejrsen et al., 1986
Pregnancy	Conceptus mass	Increased growth	Kensinger et al., 1986
Pregnancy	Sire of fetus	Variable	Adkinson et al., 1977
Pregnancy	Fetal weight	Positive correlation	Thatcher et al., 1980
Late gestation	Heat stress	Reduced growth	Tao et al., 2011
Late gestation	Long-day photoperiod	Decreased growth	Dahl and Thompson, 2012
Late gestation	Short-day photoperiod	Increased growth	Dahl et al, 2012
Dry period	Dry period < 40 d	Increased senescence	Annen et al., 2004
Early lactation	Milking frequency	Increased growth	Hale et al., 2003
Mid to late lactation	Growth hormone	Increased growth	Sejrsen et al., 1999
Mid to late lactation	Long-day photoperiod	Increased growth	Dahl and Thompson, 2012

those cells (Knight and Peaker, 1984; Capuco et al., 2003; Boutinaud et al., 2004).

Mammary development has been separated into 5 phases: 1) fetal, 2) prepubertal, 3) postpubertal, 4) pregnancy, and 5) lactation (Anderson, 1978). During some of these growth stages (prepubertal and pregnancy), mammary tissue undergoes allometric growth (growing at rates 2 to 4 times faster than the rest of the body; Sinha and Tucker, 1969; Sejrsen et al., 1986). The mammary gland, unlike other branched organs, undergoes most of its branching during adolescent rather than fetal development (Sternlicht, 2006). Mammary branching may therefore be separated into embryonic, adolescent, and adult phases, each of which is differentially regulated (Sternlicht, 2006). Mammary growth in the bovine is isometric (i.e., grows at the same rate as general body growth) for the first 2 to 3 mo after birth. There is then an increase in growth rate when the ductal tree grows into the mammary fat pad. The fat pad is essential to this process, and this allometric growth will not occur in the absence of the fat pad (Imagawa et al., 1994; Hovey et al., 1999). During pregnancy, the ducts will differentiate into milk secretory cells. Therefore, the formation of a duct network that occurs before puberty will determine the extent of lobulo-alveolar development during gestation.

Several studies have examined the impact of nutritional factors or hormonal manipulation on ductal development during the peripubertal period and subsequent milk yield in cattle (Table 1). Feeding programs with increased nutrient density are currently being used to enhance lactation performance at maturity in cattle (Drackley, 2008). Calves under this management program, fed at an increased rate and fed a diet with a greater protein-to-energy ratio, have faster growth rates and altered body composition with increased lean tissues and reduced fat deposition (Blome et al., 2003).

The majority of mammary growth takes place during gestation (Forsyth, 1986; Knight and Wilde, 1987). The endocrine regulation of this growth has been extensively reviewed (Tucker, 1981, 2000; Collier et al., 1989; Akers, 2006). Collectively, the laboratory of H. Allen Tucker and his academic trainees (graduate students, postdoctoral scholars, and scientists on sabbatical leave and their trainees) had the greatest influence in studying all aspects of the endocrine regulation of mammary growth.

Studies on factors impacting mammary growth during pregnancy indicate that the biggest impacts occur during the last trimester (Table 1). Collier et al. (1982) demonstrated that heat stress during late gestation reduced birth weight and subsequent milk yield of cattle. These effects were confirmed by several studies performed by the Dahl lab at the University of Florida (Gainesville, FL), including the demonstration that heat stress during late gestation reduced mammary growth (Tao et al., 2011), which Collier et al. (1982)had speculated on but for which he did not provide evidence. Other factors impacting mammary growth include the sire of the fetus, gender, and fetal weight (Adkinson et al., 1977; Thatcher et al., 1980). The effect of gender is confounded with fetal weight because male fetuses are heavier than female fetuses (Thatcher et al., 1980). The availability of recombinant bovine placental lactogen in the 1990s permitted the identification of the clear role of this molecule in mammary growth during pregnancy in the bovine (Byatt et al., 1992, 1994, 1997).

During established lactation, mammary growth can be influenced by milking frequency, photoperiod, and bovine ST (**bST**; Table 1; Knight and Wilde, 1987). Increased milking frequency increases milk production

(Erdman and Varner, 1995), and in most cases, milk production remained elevated for a period after the treatment was terminated (Bar-Peled et al., 1995; Hale et al., 2003). Changes in milking frequency lead to changes in mammary cell turnover in cattle (Hillerton et al., 1990; Hale et al., 2003). The activity of key enzymes involved in milk synthesis also changes because of changes in milking frequency (Wilde et al., 1987).

The role of long-day photoperiod (**LDPP**) in increasing milk yield during established lactation was first reported by Peters et al. (1978; Table 1). Miller et al. (1999) demonstrated that the positive influence of LDPP on milk yield in established lactation was additive to recombinant bovine ST (**rbST**). Long-day photoperiods during the dry period were shown to be detrimental to milk yield in the next lactation by Velasco et al. (2008), who demonstrated that short-day photoperiods, combined with a targeted 42-d dry period, increases milk yield in the subsequent lactation, relative to a 42-d dry period combined with LDPP. For a complete review of the impact of photoperiod length on mammary growth and lactation, see reviews by Dahl et al. (2000, 2004).

Recombinant bovine ST is a recombinantly derived hormone that is virtually identical to naturally occurring bST. Exogenous administration of rbST has been shown to enhance galactopoietic performance of dairy cows in laboratory and field environments, with greater peak milk yield and increased persistency of yield over the lactation cycle (Bauman et al., 1988, 1989). Bovine ST increases milk yield per cow (10 to 15%; Bauman and Vernon, 1993), enhances persistency of lactation, changes the shape of the lactation curve in the dairy cow, and increases profit for the producer (Bauman, 1992). Somatotropin is secreted from the anterior pituitary and is found in greater concentrations in superior cows (selection lines; Kazmer et al., 1986). Indirect effects of bST are probably moderated by IGF, because direct infusion of IGF in mammary glands of goats caused an increase in milk yield (Prosser et al., 1994). There are limited data concerning the effect of bST on mammary growth during lactation. However, it appears that mammary growth is unaffected by rbST treatment in early lactation (Sejrsen et al., 1999). It has been shown to increase the amount of mammary parenchyma in mid lactation (Capuco et al., 2001). The mechanism of action of bST on mammary growth remains unknown but most likely involves IGF-I (Sejrsen et al., 1999).

MILK SYNTHESIS AND SECRETION

Mammary epithelial cell structure and function are tightly integrated, and the study of the onset and regulation of milk synthesis and secretion required tools that would allow researchers to visualize the structures and their makeup during this process. Therefore, the availability of transmission and electron microscopy beginning in the 1950s allowed researchers to delineate the changes in structure of mammary epithelial cells during onset and maintenance of milk secretion (Bargmann and Knoop, 1959; Hollmann, 1968; Kinura, 1969). This included the secretion mechanism for milk fat (Wooding, 1971; Elias et al., 1973) and milk protein secretion (Kurosumi et al., 1968) and early effects of prolactin on the mammary epithelial cell at onset of lactogenesis (Ollivier-Bousquet, 1978) as well as the changes in cell-to-cell contacts with onset of lactogenesis (Pitelka et al., 1973). These tools allowed the description of the effects of insulin, cortisol, and prolactin on ultrastructure of bovine mammary epithelial cells in culture at onset of lactogenesis in the bovine (Collier et al., 1977) as well as the impact of continued milking during a normal dry period on the ultrastructure and secretory capacity of mammary epithelial cells in the subsequent lactation (Annen et al., 2007)

Dr. Margaret Neville, in her article "Classic Studies of Mammary Development and Milk Secretion: 1945–1980" (Neville, 2009, p. 194), pointed out that "insight into the cellular structure of the mammary secretory cell gained from examination of electron micrographs had a huge influence on lactation physiologists." Before this, physiologists pictured the cell as composed of a limiting membrane holding in a saline solution containing soluble proteins (Neville, 2009). As shown in Fig. 2 (from Heald, 1974), the structure of a lactating mammary epithelial cell at the ultrastructural level demanded new models to study the process of milk synthesis and secretion. This challenge was answered by several investigators, most notably the team of Jim Linzell and Malcolm Peaker at the Institute of Animal Physiology, Babraham, Cambridge, UK. These investigators developed new models that included transplantation of the mammary gland to the neck in the goat to study close arterial infusion and uptake of various nutrients and metabolites across the mammary gland (Linzell and Peaker, 1971). They identified key locations of synthesis of milk components in the mammary epithelial cell as well as the process of milk secretion and its regulation and identified 5 routes of secretion (Linzell and Peaker, 1971). Recently reviewed by Shennan and Peaker (2000), these are 1) exocytosis, which includes water, lactose, calcium, and proteins; 2) lipid secretion, which includes the fat globule and its membrane and sometimes includes some cytoplasm associated with the membrane, which is known as a "signet"; 3)

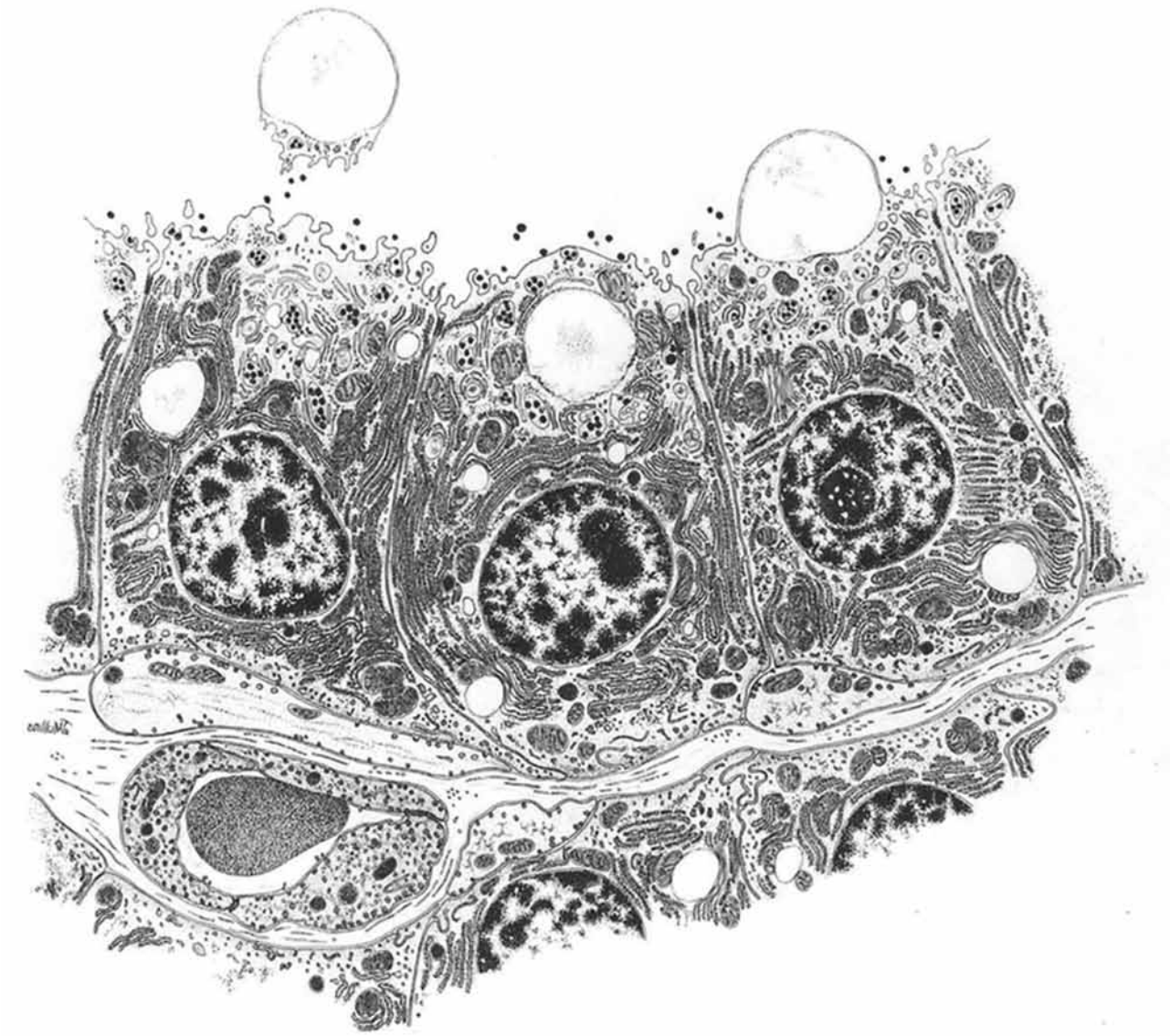

Figure 2. Graphic illustration of fully differentiated, lactating mammary epithelium representing the general space allocation of the organelles and the polarity of cells. From Heald (1974).

transmembrane secretion, whereby substances may traverse the apical cell membrane (and, for those directly derived from blood, the basolateral membrane); examples are water, urea, glucose, Na^+, K^+, and Cl^-; 4) transcytosis or vesicular transport, which involves various large molecules; examples are immunoglobulins during colostrum formation, transferrin, and prolactin; and 5) paracellular pathway, where there is direct passage from interstitial fluid to milk such as cells of the immune system during mastitis as well as serum albumin and electrolytes.

Studies by Stuart Smith on regulation of fatty acid chain length identified the enzyme fatty acid synthase (Smith and Dils, 1966). Not all lipids in milk are synthesized in the mammary gland. Several sources of preformed fatty acids are available from plasma; very-low-density lipoprotein particles synthesized by the liver or chylomicra-bearing triacylglycerols are delivered to the mammary gland. The role of lipoprotein lipase in the uptake of these nutrients was detailed by Scow et al. (1973). Although the pathways of lactose and milk protein synthesis were shown to be similar across species, major differences between ruminants and nonruminants were identified for milk fat synthesis: both carbon flow and generation of reducing equivalents and the absence of ATP citrate lyase in ruminant mammary tissue (Bauman et al., 1970, 1973).

Advances in the use of high-speed centrifugation and electrophoresis along with electron microscopy also permitted an additional line of investigation during this period involving the evaluation of the structure of the membranes of the mammary epithelial cell to include the cell membrane, milk fat globule membrane, golgi vescicles, endoplasmic reticulum, and the

secretory vesicles. Thought leaders in this area developed the concept of membrane flow, whereby the secretion of the milk fat globule membrane required loss of the apical plasma membrane of the lactating mammary epithelial cell that was replaced by the secretory vesicle, which fused with the apical plasma membrane (Keenan et al., 1971; Wooding, 1971; Mather and Keenan, 1975).

Milk serum components (lactose, water, and protein) are secreted by fusing the membranes of secretory vesicles that condense milk secretions with the apical regions of the plasma membrane. This occurs through the formation of a ball and socket configuration where the secretory vesicle forms a tubular-shaped projection into the apical membrane as well as by simple fusion. Intracellular lipid droplets are directly extruded from the mammary epithelial cells by progressive envelopment of the plasma membranes in the apical regions. The balance between the surface volume lost in enveloping lipid droplets and that provided by fusion of the secretory vesicle and other vesicles with the apical plasma membrane was termed "membrane flow," as shown in Fig. 2 (Keenan et al., 1971).

Local regulation of milk secretion and transport was given a new dimension when the laboratory of Nelson Horseman demonstrated the presence of serotonin receptors in the mammary epithelium of the mouse and that these receptors were involved in feedback regulation of milk secretion and alteration in tight junction competency between mammary epithelial cells (Matsuda et al., 2004). Subsequently, Hernandez et al. (2008) confirmed these results in cattle. It is also now apparent that regulation of calcium turnover during lactation is mediated by secretion of parathyroid hormone-related protein (PTHRP), which is, in turn, regulated by serotonin pathways in mammary epithelial cells (Hernandez et al., 2012; Laporta et al., 2014). This discovery opens new pathways for treating peripartum hypocalcemia in dairy cows.

METABOLISM

Metabolic requirements for lactation have traditionally taken 2 separate paths, the first being the study of the nutrient requirements of the animal, which are based on extant literature and on the NRC (2001) guidelines for feeding dairy cows. The second area has been the study of the use of specific nutrient requirements for synthesis and secretion of milk, which began in earnest after the Second World War and continues to the present. Several excellent reviews are published on the requirements for AA, carbohydrates, and lipids (Moe et al., 1971; Bickerstaffe et al., 1974; Miller et al., 1991). The study of mammary blood flow and the uptake of nutrients across the mammary gland began with the classic work of Linzell and coworkers (Linzell, 1960; Annison et al., 1967) in the goat and was continued by others in the cow (Davis and Collier, 1985; Prosser and Davis, 1992; Berger et al., 2016) and by Farmer et al. (2008) in the sow, to cite a few. This work provided evidence of both systemic and local control of mammary uptake of nutrients for milk synthesis.

Studies on the metabolic adaptations that occur at the end of pregnancy and onset of lactation by Mellenberger et al. (1973) set the stage for work on the endocrine changes that also occurred during this period (Hart et al., 1978; Tucker, 1981; Vernon, 1988; Flint, 1995; Boyd et al., 1995). Ultimately, these and other studies led the Bauman group at Cornell University (Ithaca, NY) to propose a new regulatory mechanism to describe this type of control system, which they termed "homeorhesis" or the orchestration of metabolism to support a physiological state (Bauman and Currie, 1980; Bauman and Elliot, 1983). This concept has been extended to many physiological states, as shown in Table 2, including the coordination of metabolism that occurs when lactating dairy cows are treated with rbST (Bauman and Vernon, 1993). Currently, studies are oriented toward understanding the gene networks that are tied to the homeorhetic regulation of lactation (Lemay et al., 2007; Vailati-Riboni et al., 2016).

MAMMARY IMMUNE SYSTEM

It has been known for some time that the mammary gland evolved from the innate immune system of mammals (Hayssen and Blackburn, 1985; Vorbach et al., 2006). Recently, it was discovered that the adaptive immune system participates in the remodeling process during pubertal and postpubertal mammary growth (Plaks et al., 2015). This process involves immune system cells known as antigen-presenting cells, which continually proliferate and survey the rapidly developing organoids, sending signals to nearby immune T cells called cluster of differentiation 4 (**CD4+**) cells. The CD4+ T cells, in turn, secrete a substance called interferon-γ, which signals epithelial cells that they should cease their advancement. These repeated interactions between antigen-presenting cells and CD4+ T cells accompany the sculpting of the lumen and the branching of the tissue (Plaks et al., 2015).

Both the adaptive and innate immune systems also provide protection for the mammary gland during pregnancy and lactation. The interest in the role of these systems in the defense of the mammary gland against mastitis has grown because mastitis is one of the most costly diseases in dairy cattle, with losses associated with reduced milk production, discarded milk, early culling,

Table 2. Partial list of physiological conditions where the general concept of homeorhetic regulation has been applied[1,2]

Lactation	Hibernation
Pregnancy	Premigration/migration
Growth	Egg laying
Puberty	Incubation anorexia
Aging	Seasonal cycles
Exercise	Environmental limitations
Chronic undernutrition	Management limitations
Chronic illness	Physiological limitations

[1]From Collier et al. (2004).

[2]References include Bauman and Currie (1980), Bauman et al. (1982), Dilman (1982), Nicolaïdis (1983), Mrosovsky (1990), Wade and Schneider (1992), Vernon (1998), Chilliard (1999), and Kuenzel et al. (1999).

veterinary services, and labor costs (Thompson-Crispi et al., 2014). Substantial work has gone into identifying the pathogens associated with the disease and the mammary defense system (Lascelles, 1979; Paape et al., 1979, 2002; Sordillo and Streicher, 2002). Currently, new genomic tools are being used to quickly identify pathogens and to develop treatment strategies (Mahmmod, 2013; Thompson-Crispi et al., 2014).

A final area of research in the bovine immune system has been the transfer of immunity from the dam to the neonate in colostrum (Butler, 1974; Hurley and Theil, 2011). The mechanism of immune transfer during pregnancy varies among species and has now been fairly well documented (Larson, 1992; McFadden et al., 1997; Wheeler et al., 2007). Research continues on the possible use of bovine colostrum and milk as a medium for the heterologous transfer of passive immunity and possible disease protection in a range of species (Hurley and Theil, 2011).

CENTERS OF EXCELLENCE

Over the last 100 yr of lactation research, several research centers dedicated to lactation biology have come and gone, and this process has been driven largely by societal changes in funding priorities for agriculture and gain and loss of major thought leaders in these centers. In the United States, these centers, with the exception of the USDA research lab at Beltsville, MD, have largely been located at land-grant universities, which are greatly influenced in a given state by the agriculture of that state. As the dairy industry in the United States has developed, the cow population distribution has shifted, altering research priorities in given states with time and, in many cases, the closing of research dairy herds. Additionally, the national commitment to agricultural research peaked in the immediate period following World War II to ensure that cheap food was available for a growing population. This focus has greatly diminished with the advent of surplus dairy supplies and a declining population growth rate. However, it is worthwhile to note the contributions these centers of excellence and thought leaders made to our food security. In many cases, the major research contributions are found within academic families. For instance, C.W. Turner at the University of Missouri (Columbia, MO) trained J. Meites, who went on to establish a major program at Michigan State University (Lansing, MI). Similarly, R.P. Reece at Rutgers University (Brunswick, NJ) trained H. Allen Tucker, who went on to establish a major program at Michigan State University. Dr. Tucker trained 32 postdoctoral scholars and PhD and Master of Science candidates and populated lactation programs across the United States and 16 different countries. A similar example can be found when examining the academic tree of Dale Bauman; the postdoctoral scholars, visiting scientists, Masters of Science, and PhDs who trained with him at the University of Illinois (Urbana, IL) or Cornell University have gone on to supervise 42 postdoctoral scholars, 204 Master of Science degrees, and 173 PhD degrees.

Other Centers of Excellence outside the United States have included the Hannah Research Institute in Ayr, Scotland; the Physiology Unit at Cambridge University (Cambridge, UK); and the lactation biology research group at Shinfield, Reading, England. The research group at Jouy-en-Josas, France, and the Swine and Dairy Research Center in Sherbrooke, Canada, as well as the lactation program at the University of Ghent, Belgium, have also made many notable contributions. Some of these units are no longer functioning, but they have made historical and lasting contributions to the field of lactation biology.

SUMMARY

The application of discoveries on the regulation of mammary growth and milk synthesis and secretion have resulted in substantial changes in the lactation curve of the dairy cow, as shown in Fig. 3. Adoption of the knowledge obtained from research centers such as photoperiod, milking frequency, rbST, and reducing negative environmental factors while inserting genetic gains and improved nutrition has greatly increased the average milk yield of dairy cows. Currently, the world record holder for the Holstein breed is a Wisconsin cow named Ever-Green-View My Gold-ET. “My Gold” had a 365-d milk production of 35,218 kg (905 kg fat and 934 kg true protein; Lancaster Farming, 2017). There is currently no evidence this is the metabolic limit of milk yield potential for the dairy cow. Use of new reproductive techniques will allow future dairy produc-

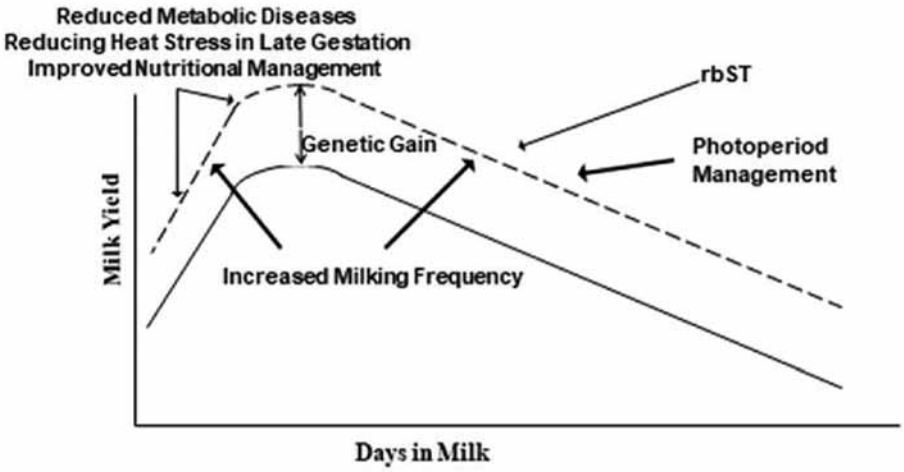

Figure 3. The current lactation curve compared with the lactation curve in 1960. rbST = recombinant bovine somatotropin. Adapted from Annen et al. (2004).

ers to insert new genetics at a more rapid pace, including the ability to control the gender of the calves to allow faster expansion of genetic gain in a herd. The potential for the cow to serve as a bioreactor of peptides of high value to the medical community remains a largely untapped resource.

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