ABSTRACT

Mammary involution is a gradual process that occurs following cessation of milking. Regression of mammary secretory tissue accompanies dramatic changes in secretion composition during the transition from lactation to involution. Conversely, rapid differentiation of secretory tissue and copious accumulation of colostrum occur as parturition approaches. The duration of the nonlactating period, mammary gland health, and secretory cell response to hormones influence subsequent lactational performance in most species. Manipulation of the bovine mammary gland in an attempt to hasten involution has been studied. The primary objective of these studies was to determine if hastened involution would decrease new intramammary infections during the early nonlactating period. Results of these studies have also led to a more fundamental understanding of events that occur during physiological transition of the mammary gland. Adequate regression, proliferation, and differentiation of mammary secretory epithelium during the nonlactating period of ruminants appear to be essential for maximal milk production during lactation. Factors that interfere with these mechanisms can adversely affect mammary function during the impending lactation. A greater understanding of these processes may provide new approaches for increasing milk production in dairy cattle.

INTRODUCTION

The mammary gland is a complex organ that has diverse physiological, immunological, and biochemical functions. Mammary glands undergo several physiological transitions within a lactation cycle. During successive lactations, mammary glands progress through three distinct functional transitions: 1) from involution to colostrogenesis, 2) from lactogenesis to lactation, and 3) from lactation to involution (123, 132). Marked changes in mammary gland size, structure, and function as well as mammary secretion composition occur during a lactation cycle. Progression from one functional state to the next involves transition either from or to a state of active milk synthesis.

In comparison with lactation, much less is known about the physiological, histological, ultrastructural, immunocytological, and biochemical changes that occur during mammary involution. Furthermore, the reasons for the importance of mammary involution with respect to subsequent lactational performance are not well understood. However, within the last 10 yr, reports have been published that have increased our understanding of mammary involution. In the context of this symposium, the following review focuses on: 1) changes that occur in the mammary gland and its secretion during physiological transitions of the bovine udder from lactation to involution and from involution to colostrogenesis, 2) the relationship of the nonlactating period with susceptibility to new intramammary infection, 3) manipulation of bovine mammary involution, and 4) modulation of mammary cell growth, differentiation, and milk biosynthesis near parturition.

Changes in the Mammary Gland and Its Secretion During Physiological Transitions of the Udder

Previous studies (25, 141, 142, 152) have shown that dairy cows require a nonlactating
period prior to lactation to achieve maximal milk production in the subsequent lactation. Dairy cows with 10- to 40-d nonlactating periods produced significantly less milk in the following lactation than cows with a 40- to 60-d nonlactating period (25). Adequate proliferation and differentiation of mammary secretory epithelium during the nonlactating period are essential for optimal synthetic and secretory function in the subsequent lactation, and the duration of the nonlactating interval is closely related to milk production (5, 80, 136, 137, 139). Although the need for a nonlactating period of adequate duration has been established, the physiological events necessitating a nonlactating period between lactations are poorly understood.

Dramatic changes in the mammary gland and its secretion occur during involution. Smith and Todhunter (132) suggested that mammary glands progressed through three distinct stages during the nonlactating period: 1) a period of active involution, which begins with cessation of milking and is most likely completed by 30 d into the nonlactating period, 2) a period of steady state involution that represents the time when mammary glands are fully involuted, 3) a period of colostrum formation and the initiation of lactation, which most likely begins about 2 wk before parturition. The early portion of the nonlactating period following abrupt cessation of milk removal is characterized by engorgement of cisternal spaces, ducts, and alveoli with milk constituents, gradual changes in mammary secretion composition, and regression of mammary tissue. Near parturition, mammary glands again undergo marked transition characterized by intense growth, rapid differentiation of secretory epithelial cells, and synthesis and secretion of proteins, fat, and carbohydrates, which result in the accumulation of colostrum (21, 46, 67, 81, 109, 137). A unique feature of bovine colostrum is the high concentration of Ig, in particular IgG1, which serves as a source of passively acquired antibody for the newborn calf (66, 149).

Much of the early information on mammary involution was based on studies in laboratory species (41, 47, 48, 49, 50, 52, 76, 118, 119). In the rat, alterations in mammary secretory epithelial cells were observed within 24 h after milk cessation. Accumulation of milk in alveoli and ducts increased intramammary pressure, and caused degeneration of secretory cells and subsequent disruption of alveolar and lobular structures (48). Milk stasis resulted in the accumulation of fat droplets and secretory vesicles with a reduction in size of the rough endoplasmic reticulum (RER) (48, 49, 118). By 48 h after milk cessation, an increase in epithelial cell digestion by lysosomes within the epithelium was detected accompanied by leukocytic infiltration and notable reduction in cell volume (49, 50, 119). After 72 h of involution, macrophages were observed between epithelial cells and ingesting fragments of cellular debris (48, 49, 118, 119). Degenerative cells were shed into alveolar lumens by 48 to 72 h after weaning, leaving only basement membranes intact. Myoepithelial cells remained while secretory epithelial cells were eliminated. Myoepithelial cells appeared to play an important role in bridging gaps where necrotic epithelial cells had sloughed, thereby preventing total loss of organized structure (118, 119).

Similar events were thought to occur in bovine mammary glands after milk cessation. However, data from laboratory species are not easily extrapolated to the cow since conditions of mammary involution in dairy cows are generally quite distinct. For example, dairy cows are pregnant at the time of milk cessation, which is in marked contrast to most other species. In addition, mammary glands of cows at the time of milk cessation are still producing considerable quantities of milk, which again is in marked contrast to other species. Current gestation and active milk secretion are factors that immediately precede the nonlactating period and are likely to have a pronounced effect on the processes involved during mammary involution.

Recent studies have shown that ruminant mammary epithelial cells do not regress to the same extent as observed in rat mammary glands and appear to maintain some synthetic and secretory activity throughout the nonlactating period (53, 136, 137). Sloughing of mammary epithelial cells into alveolar lumina was not observed in caprine (140), ovine (66), or bovine mammary glands (53, 136, 137). Instead, alveoli exhibited small lumina that contained electron-dense proteinaceous material and undifferentiated epithelial cells (137). Following
the 1st wk of bovine mammary involution, secretory activity decreased, as evidenced by a marked reduction in alveolar luminal area with a concomitant increase in stromal area (53, 137). Once milk synthesis decreased and mammary fluid was resorbed, stromal areas increased proportionately to compensate for reduced alveolar luminal area. An increase in the prevalence of nonactive secretory epithelial cells was observed during the first 2 wk of involution (53, 137).

Involution of the bovine mammary gland results in mammary secretion that is distinct in composition compared with that of milk or colostrum (18, 54, 56, 62, 66, 157). However, alterations in mammary secretion composition do not occur instantaneously, but gradually over several days. Changes in the composition of mammary secretion during early involution reflect increased transfer of blood-derived factors and decreased synthesis and secretion of lacteal fluid. Changes in lacteal secretion reflect changes in mammary tissue ultrastructure.

The concentration of fat, casein, lactose, citrate, α-lactalbumin, and β-lactoglobulin, and the citrate to lactoferrin (Lf) molar ratio decrease markedly during the 1st wk of involution (4, 18, 54, 56, 127, 153). Conversely, the concentration of Lf, IgG, serum albumin, somatic cells, and the pH of mammary secretions increase dramatically during the early nonlactating period (18, 54, 57, 69, 132). Wheelock et al. (153) showed that changes in the composition of bovine mammary secretions during early involution were related to the reduction in yield of secretion.

The rate that mammary tissue regresses following milk cessation varies greatly by species (66). Based upon observed changes in bovine mammary tissue and mammary secretion composition, the process of active involution is most likely completed by 21 d after cessation of milking. The duration of steady state involution, or a time when mammary glands are fully involuted, varies and is influenced by length of the nonlactating interval (132). The morphology of bovine mammary tissue and the composition of mammary secretions remain relatively constant during steady state involution (90, 136), and further changes in mammary gland structure and function are not observed until parturition approaches (136, 137). The importance of steady state mammary involution has not been delineated. However, Smith and Todhunter (132) indicated that a minimal period of steady state involution may result in a less than optimal hormonally mediated lactogenic response. This could be related to suboptimal milk yield in the subsequent lactation in cows with nonlactating periods of less than 40 d.

Near parturition, mammary glands again undergo marked transition to a state of active milk synthesis. The dramatic changes that occur in mammary tissue and mammary secretion during the periparturient period are essentially the opposite of events that occur during active involution. Colostrogenesis is initiated hormonally near the end of pregnancy and occurs in two stages that can be characterized by both morphological and biochemical changes (40). The first stage comprises the prepartum period during which cytological and enzymatic differentiation of alveolar cells is accompanied by the appearance of precolostral fluid. Stage 2 begins immediately prior to parturition and is noted by the onset of copious colostrum secretion (40, 46).

The majority of cellular proliferation in ruminants occurs in nonlactating mammary glands during pregnancy (7, 8, 142). Mammary gland growth follows an exponential pattern during pregnancy in dairy heifers (142). Previous studies found that the greatest increase in mammary DNA content of goats (7), guinea pigs (8), and heifers (142) occurred in the last trimester of pregnancy. Cowie (27) observed limited changes in the structure of primigravid goat mammary glands during the first half of pregnancy. However, a period of advanced alveolar growth occurred between 60 and 120 d of gestation (27). Histological and cytological examination of prepartum goat mammary tissue exhibited characteristics indicative of copious milk synthesis and secretion (139, 140). Cytological examination of caprine mammary epithelium during the last 30 d of pregnancy indicated gradual differentiation with increased cytoplasmic to nuclear ratios, more cellular polarity, and abundant apically located secretory vesicles during the last trimester of pregnancy (140).

Morphogenesis of secretory capability in bovine mammary glands also became evident during the last few weeks of gestation (137). In-
creased synthetic and secretory activity of prepartum bovine mammary tissue was apparent by higher percentages of luminal area accompanied by lower percentages of stroma. As mammary fluid accumulated during colostrogenesis, luminal spaces became distended. Expansion of luminal areas resulted in a concomitant compression of surrounding stromal areas. Mammary secretory cells characterized as fully active also became more numerous as parturition approached while numbers of nonactive cells decreased. Ultrastructural analysis of bovine alveolar epithelium during the last week of gestation demonstrated enlarged cytoplasmic areas containing basally located nuclei and accumulated secretory vesicles at the apical region, which is typical of fully lactating cells (137). Percentages of total cytoplasmic area occupied by RER, Golgi apparatus, and mitochondria increased in mammary epithelium from periparturient glands when compared with cells obtained 2 to 4 wk earlier (136, 137).

Morphological alterations of mammary parenchymal tissue coincided with major changes in secretion composition during the periparturient period (136, 137, 138). Constituents found in mammary secretion during colostrogenesis result in part from shifts in the metabolic activity of alveolar epithelium and from changes in the integrity of the blood-milk barrier. In prelactating mammary glands, "leaky" tight junctions allow passive diffusion of components from blood to milk and vice versa (67, 109). Loss of cellular integrity is reflected in the relatively high concentrations of serum-derived immunoglobulins, sodium, chloride, and albumin (67, 109, 138). However, near parturition tight junctions become less permeable and inhibit paracellular movement of serum proteins and ions into milk. Instead, most transport is via the transcellular pathway (67). During the last 2 wk of gestation, a continuous increase in concentrations of milk constituents derived from de novo synthesis (fat, citrate, lactose, α-lactalbumin) was observed in caprine (139, 140) and bovine (138) mammary secretions, which indicated the onset of milk synthesis. Changes in mammary tissue and mammary secretion composition during involution are reviewed in several publications (55, 66, 132).

### Relationship of the Nonlactating Period with Susceptibility to New Intramammary Infection

Clinical and experimental data clearly support the concept that bovine mammary glands are markedly susceptible to new intramammary infection (IMI) during physiological transitions of the gland from lactation to involution and from involution to colostrogenesis (34, 35, 36, 78, 86, 93, 133). In contrast to transitional periods, the fully involuted mammary gland appears to be highly resistant to new IMI (35, 78, 87, 88, 133). Increased susceptibility to new IMI may be associated with the physiologic transition of the bovine mammary gland either from or to a state of active milk synthesis. However, reasons related to increased susceptibility during these times have not been fully delineated.

Many infections that originate during the nonlactating period persist into the subsequent lactation and are a major cause of clinical mastitis during early lactation (35, 36, 78, 133, 134). Intramammary infections contracted during the nonlactating period reduce milk yield after parturition, the decrease in production appears to be related to the duration of infection. Smith et al. (126) demonstrated that quarters infected throughout the nonlactating period produced 35% less milk during early lactation as compared to equivalent uninfected quarters. Quarters that were infected late in lactation and at parturition produced 48% less milk. If a quarter was infected at the time of milk cessation, but not at parturition, an 11% reduction was observed. These data suggest that IMI originating in the nonlactating period or persisting from late lactation have a significant effect on mammary function in the subsequent lactation.

After the last milking of lactation, at least three important changes occur that may affect susceptibility of mammary glands to new IMI: 1) the flushing effect of milking on bacteria colonizing the streak canal, teat cistern, or gland cistern is terminated; 2) mammary glands continue to synthesize and secrete milk during early involution, resulting in increased intramammary pressure that may cause leakage of milk from the mammary gland and facilitate bacterial penetration of the streak canal; and 3) mammary secretions during early involution contain low numbers of polymorphonuclear.
neutrophils, macrophages, and lymphocytes, and low concentrations of Lf and Ig, which have been implicated as factors of resistance to IMI, and high concentrations of fat, casein, lactose, and citrate, which can interfere with natural defense mechanisms as well as enhance bacterial growth (13, 16, 29, 56, 57, 117, 128).

Cessation of regular milking of dairy cows at the end of lactation results in increased intramammary pressure, which is thought to initiate the involutionary process. Smith and Schanbacher (131) indicated that the volume of mammary secretion increased 50 to 70 h after the last milking of lactation, and at 70 h bovine mammary glands accumulated 70 to 80% of their daily yield prior to cessation of milking. About 6 d were required before the volume of mammary secretion was significantly lower than that observed at 12 h after milk cessation. Similar results were reported by Wheelock et al. (153); however, marked variation among animals was observed. Cows producing higher quantities of milk during late lactation tended to retain larger volumes of mammary secretion longer during early involution than cows producing less milk at drying off. Near parturition, mammary glands again accumulate large quantities of colostrum, which often results in leakage of colostrum from teats. Cousins et al. (26) demonstrated that the streak canal was penetrated more readily by mastitis pathogens during early involution than during the mid- and late nonlactating period. The relative ease by which bacteria penetrate the streak canal may be associated with the accumulation of mammary secretion after cessation of milking (26, 86).

Increased susceptibility to new IMI during the early nonlactating and peripartum periods also may be a function of the relative capability of mammary gland defense systems. Protective factors associated with the mammary gland can be divided arbitrarily into at least three systems: 1) a phagocytic cellular component; 2) the specific immune system; and 3) bacteriostatic and bactericidal proteins. For a more comprehensive review of these systems refer to publications (29, 65, 66, 79, 117, 149).

Phagocytic cells present in mammary secretions are of two morphologically distinct types: polymorphonuclear neutrophils and macrophages. Studies (99, 101, 121) have shown that phagocytosis of mastitis pathogens by phagocytic cells is not an efficient process. This appears to be related to low leukocyte concentrations and increased metabolism of the phagocyte following indiscriminate ingestion of fat and casein. The process of phagocytosis is facilitated if bacteria have been opsonized. Specific IgG antibodies, complement components, and IgM antibodies in the presence of complement (29, 149) have opsonic activity. However, the concentration of all Ig isotypes and complement are very low during lactation and increase slowly as involution progresses (66, 113, 132, 150). Phagocytosis may be more efficient when mammary glands are fully involuted because of increased numbers of phagocytic cells and low concentrations of fat and casein in mammary secretions of the nonlactating gland.

Another natural defense system of bovine mammary secretions is the presence of the iron-binding protein Lf (123, 128, 131). Lactoferrin is thought to participate in the nonspecific defense of the involuting mammary gland by its ability to bind iron with great affinity. One molecule of Lf can bind two molecules of iron, and when not fully saturated with iron, Lf inhibits a variety of bacteria that require iron for their growth (13, 16, 85, 90, 128). In this regard, the ability of bacteria to acquire sufficient iron for growth in mammalian body fluids is a significant virulence factor (120). The interaction of citrate and Lf in bovine mammary secretions is of considerable importance since both compounds sequester iron (120, 132). Citrate binds iron in a form that can be obtained by bacteria via a mechanism that is activated by citrate. The concentration of Lf in milk is low, increases slowly during early involution reaching highest concentrations in mammary secretions of fully involuted glands, then decreases as parturition approaches. Citrate and Lf are reciprocally related and the high concentration of citrate in milk and colostrum (110) apparently prevents growth inhibition of bacteria by Lf (16, 128, 131). However, Lf in secretions from fully involuted mammary glands appears to be bacteriostatic since citrate concentrations are low, the concentration of Lf is high, and conditions for the iron binding activity of Lf are optimal (131, 132, 151).

Lactoferrin has several other functions in addition to iron binding. Studies in mice and
humans have shown that Lf interacts with receptors on the surface of macrophages, lymphocytes, and polymorphonuclear leukocytes. Lactoferrin may be associated with such diverse functions as influencing myelopoiesis by inhibiting the production of colony-stimulating factors through a series of complex interactions involving monocytes and certain T-lymphocyte populations (10, 12, 70), inhibiting antibody-dependent cell-mediated cytotoxicity (84), and blocking in vitro primary humoral immune response (32). In addition, Lf may play a regulatory role in the migration of mononuclear leukocytes during inflammation, and may direct or control influx of mononuclear cells into mammary tissue and influence or modulate their functional activity (31, 132).

Growth of Mastitis Pathogens in Bovine Mammary Secretions: Relationship with Experimental and Naturally Occurring Intramammary Infections During the Nonlactating Period

Changes in the composition of mammary secretion throughout involution appear to influence growth of mastitis pathogens differently. Several studies have shown that as involution progresses, mammary secretions become increasingly more inhibitory to growth of coliform mastitis pathogens (16, 33, 89, 90). However, Dutt et al. (33) showed that there were marked and nearly inverse changes in growth of *Escherichia coli* and *Streptococcus uberis* in mammary secretions obtained throughout the nonlactating period. *Escherichia coli* grew well in cell-free, fat-free mammary secretions obtained during lactation, but poorly or not at all in secretions from fully involuted mammary glands. Conversely, *Strep. uberis* grew well in mammary secretions from involuted glands and poorly in secretions obtained during lactation, which is in marked contrast with data reported by Breau and Oliver (16). Differences may be due to removal or alteration of components of mammary secretion by acid precipitation, since whey milks were used in the study by Breau and Oliver (16). *Staphylococcus aureus* grew well in mammary secretions throughout the nonlactating period; however, a slight reduction in growth was observed in mammary secretions obtained 7 d after drying off. Dutt et al. (33) suggested that the ability of mammary secretions to support or inhibit mastitis pathogen growth may influence susceptibility of mammary glands to infection.

Bovine mammary secretions obtained at 14 and 28 d of involution were poor media for growth of *Staphylococcus* species (92). Conversely, mammary secretions collected at cessation of milking, parturition, and during early lactation supported growth of all *Staphylococcus* species evaluated. *Staph. hyicus* and *Staph. chromogenes* growth was greatest followed by *Staph. epidermidis, Staph. xylosus* and *Staph. hominis*. However, all species evaluated followed similar patterns of growth. Growth of *Corynebacterium bovis* in bovine mammary secretions followed trends similar to *Staphylococcus* species (60). Thus, the ability of bovine mammary secretions to support or inhibit growth of minor mastitis pathogens appeared to be related to the stage of the nonlactating period.

The relationship between natural protective factors in bovine mammary secretion and in vitro growth of coliform mastitis pathogens correlates well with naturally occurring and experimental IMI by coliform organisms during the nonlactating period (16, 33, 89, 90). Studies (34, 35, 133, 134) have shown that the rate of new coliform IMI was highest during the first and last 25% of the nonlactating period. McDonald and Anderson (72) showed that during the first half of the nonlactating period, 32% of 34 mammary glands inoculated with *E. coli* became infected. However, all experimental IMI at this time were eliminated by the cow without therapy, and no signs of clinical mastitis were observed. In contrast, 88% of 42 inoculated mammary glands became infected during the prepartum period. Similar results were reported by Bramley (14) and Hill et al. (51) during the prepartum period. Thus, increased rates of new coliform IMI during early involution and near parturition may be attributable, in part, to changes in natural protective factors. Conversely, rates of new coliform IMI in the fully involuted mammary gland may be low because of high concentrations of antibacterial factors and conditions conducive for optimal antibacterial activity.

Changes in the growth of *Staphylococcus* species in bovine mammary secretions obtained throughout the nonlactating and peripartum
periods also correlates well with the pattern of IMI by these organisms during the nonlactating period. Oliver (87, 88) indicated that in the absence of antibiotic therapy at drying off, the number of quarters infected with coagulase-negative staphylococci increased markedly from the time of milk cessation to parturition. However, many quarters infected with coagulase-negative staphylococci during the early nonlactating period were not infected at parturition. Conversely, many new coagulase-negative staphylococcal IMI occurred during the peripartum period. Another study (94) showed that a high proportion of primigravid heifer mammary glands were also infected with coagulase-negative staphylococci near parturition. Harmon et al. (44) demonstrated that a high rate of spontaneous elimination of Staphylococcus species occurred during the nonlactating period. Thus, the ability of bovine mammary secretions to support or inhibit growth of Staphylococcus species may relate significantly to rates of new IMI that occur during the nonlactating period.

Growth of Staphylococcus species in bovine mammary secretions is also consistent with data on experimental infection of dairy cows with Staph. epidermidis. McDonald and Anderson (74) reported that 0 of 9 mammary glands at 7 d of involution, and 2 of 9 mammary glands at 14 d of involution became infected after intracisternal inoculation of Staph. epidermidis. However, 36 of 38 mammary glands became infected after inoculation of Staph. epidermidis during the last half of the dry period. Consequently, natural and experimental IMI by Staphylococcus species appear to be more prevalent at times when mammary secretions are better able to support the growth of these organisms. Previous studies (44, 87, 88) indicated that the rate of spontaneous elimination of C. bovis during the nonlactating period in the absence of antibiotic therapy ranged from 47.6 to 96%. Greatest inhibition of C. bovis growth occurred in mammary secretions obtained during involution (60). Growth of C. bovis in mammary secretions obtained at 14 d of involution was about 57% lower than that observed in milk obtained at drying off. Thus, one potential explanation for the high rate of spontaneous elimination of C. bovis during the nonlactating period is that nonlactating cow mammary secretions markedly inhibit their growth.

Changes in the growth of Strept. uberis in mammary secretions tend to correlate well with experimental infection of nonlactating bovine mammary glands but are inconsistent with the changing pattern of naturally occurring streptococcal infections during the nonlactating period. McDonald and Anderson (73) reported that mammary glands were resistant to intracisternal inoculation of Strept. uberis at drying off. However, an increasing proportion of quarters became infected following challenge as the nonlactating period progressed. Rates of new IMI by environmental streptococci, many of which are Strept. uberis, are highest during the first and last 2 wk of the nonlactating period and lowest when mammary glands are involuted (35, 87, 88, 133). However, growth of several Streptococcus species, including Strept. uberis, was extremely high in mammary secretions obtained from involuted mammary glands. Dutt et al. (33) speculated that factors other than protective components of mammary secretion are likely of greater importance in influencing the rate of new streptococcal infection. The degree of bacterial exposure of mammary glands to mastitis pathogens, anatomy of the teat canal, and cellular components of mammary secretion are most likely very important in the susceptibility or resistance of bovine mammary glands to new IMI during the nonlactating period (23, 37).

Manipulation of Bovine Mammary Involution

Current information suggests that natural defense systems of the bovine mammary gland during physiological transitions from lactation to involution and from involution to colostrogenesis are relatively ineffective when compared to their capacities in blood or other exocrine secretions. Mammary glands are highly susceptible to new IMI during these times. However, fully involuted mammary glands are highly resistant to new infection. Various components of natural defense such as Ig, Lf, lymphocytes, and phagocytic cells change dramatically during transitional periods. However, the rate of change of these components during early mammary involution apparently is not sufficient to prevent new IMI. It was hypothesized that augmentation or manipulation of the events that occurred during early involution
may be an effective means of increasing natural resistance of mammary glands to new IMI. Techniques that would hasten involution by reducing fluid volume and elevating natural protective factors could play an important role in the prevention and control of mastitis during the nonlactating period.

A series of experiments were conducted by Oliver and Smith (95, 96, 97) to evaluate this hypothesis. Colchicine, a plant alkaloid, and \textit{Escherichia coli} endotoxin were infused intramammary into both quarters of right udder halves at or near drying off (95, 96). Colchicine was chosen because it inhibits milk synthesis and secretion in lactating goats (102, 104, 108). Endotoxin was chosen because it caused an increase in the rate of mammary involution in mice and the involuted mouse mammary gland was more resistant to the establishment of new IMI (128).

Mammary secretions from quarters of cows infused intramammarily with colchicine or endotoxin had elevated concentrations of phagocytic cells, Lf, serum albumin, IgG, and a higher pH much earlier in the dry period when compared with uninfused control quarters (95, 96). The concentration of citrate and the citrate to Lf molar ratio, which are two excellent indicators of mammary involution (96, 132), were significantly lower in mammary secretions from colchicine or endotoxin infused mammary glands. Furthermore, fluid volume removed from mammary glands at 7 d of involution was 40% lower in quarters infused with colchicine or endotoxin compared to uninfused glands. These data demonstrated that intramammary infusion of colchicine or endotoxin near the time of milk cessation accelerated bovine mammary involution.

Mammary secretion whey obtained from quarters infused with colchicine or endotoxin during the 1st wk of involution was significantly more inhibitory to two strains of \textit{Klebsiella} species and two strains of \textit{E. coli} than whey from untreated quarters (128). As mammary involution progressed, wheys became progressively more inhibitory to the four strains of coliform bacteria. Changes in the concentrations of Lf, citrate, IgG, and the citrate to Lf molar ratio were correlated with changes in coliform growth inhibition. There was a strong positive relationship between coliform growth inhibition and concentrations of Lf and IgG, and a strong negative relationship between citrate and the citrate to Lf molar ratio and coliform growth inhibition.

Intramammary infusion of colchicine or endotoxin near drying off was associated also with a 50% reduction in the isolation of major mastitis pathogens during the first 4 wk of the nonlactating period (97). However, any advantage conferred to mammary quarters infused with colchicine or endotoxin at drying off appeared to have been confined to the first half of involution. The frequency of mastitis pathogen isolation and number of IMI during the periparturient period between colchicine-infused, endotoxin-infused, or uninfused control quarters was similar. These findings support the contention that mammary glands that involute more rapidly and have higher concentrations of natural protective factors such as phagocytic cells, Lf, and Ig appear to be more resistant to new IMI by certain mastitis pathogens.

Oliver and Hoffman (91) reported recently that intramammary infusion of lipopolysaccharide into bovine mammary glands after the last milking of lactation did not enhance the ability of mammary secretions obtained throughout the nonlactating period to inhibit growth of several species of \textit{Streptococci} or \textit{Staph. aureus}. Growth of all bacteria evaluated was similar in secretions from lipopolysaccharide and control mammary glands throughout most of the study. Marked differences between bacteria and among strains of a bacterial species were observed. \textit{Staphylococcus aureus} grew well in mammary secretions collected during early involution, but growth decreased at 7 and 28 d of involution, and increased again in secretion obtained near parturition. Growth of \textit{Strep. agalactiae} and \textit{Strep. uberis} in mammary secretions was related inversely to growth of \textit{Staph. aureus}. In general, \textit{Strep. agalactiae} and \textit{Strep. uberis} grew better in cell-free fat-free mammary secretions obtained during involution than in secretions at drying off and near parturition. Conversely, \textit{Strep. dysgalactiae} grew well in mammary secretions obtained during late lactation, early involution, midinvolution, and the periparturient period. These data indicate that intramammary infusion of lipopolysaccharide at drying off does not enhance in vitro growth inhibition of several gram-positive mastitis pathogens.
pathogens during the nonlactating period. These data demonstrate also the variability of mastitis pathogen growth in mammary secretions during transitions of the bovine udder, and that all strains of a bacterial species do not necessarily respond in similar fashion.

Todhunter et al. (144) reported that induced inflammation of bovine mammary glands at drying off or infusion of a nonlactating cow antibiotic preparation, novobiocin, had little effect on growth of *Strep. uberis*, *Strep. agalactiae*, *Strep. faecalis*, and *Strep. bovis* in mammary secretions obtained 7 d after treatment. In addition, they (144) showed that streptococcal growth was greatest in mammary secretions from fully involuted glands, and that apo-Lf, Ig, or both had little effect on streptococcal growth. Milk from lactating mammary glands inhibited growth of streptococcal species, and the inhibitory factor was identified provisionally as lactoperoxidase. Previous studies (17, 75, 98) have shown that the lactoperoxidase-thiocyanate-hydrogen peroxide system inhibited growth of Group B *Streptococci* and was bactericidal for certain Group A *Streptococci*.

Further attempts to manipulate bovine mammary involution utilized the plant lectins Concanavalin A (Con A) and phytohemagglutinin (PHA) (15, 16). Intramammary infusion of Con A into lactating cows or goats reduced milk yield by 12 h after infusion and yields remained lower for up to 96 h after treatment (42, 103, 105, 106, 107). Alterations in goat mammary secretion composition, such as decreased amounts of fat, casein, and lactose were detected by 12 to 24 h after infusion of Con A. Concanavalin A infusion into lactating bovine mammary glands resulted also in increased numbers of somatic cells, including polymorphonuclear leukocytes and lymphocytes, and increased concentrations of IgG1, IgG2, and IgA. Changes in milk yield and mammary secretion composition were still apparent 84 to 96 h after infusion (42).

Concanavalin A is thought to inhibit milk secretion by binding to mannosyl and glucosyl moieties of cell surface components and altering elements of the cytoskeleton associated with the plasma membrane (105, 106). Patton and Hubert (106) demonstrated that Con A binds to milk fat globule membranes, in particular to the membrane that enveloped the fat globule at the time of secretion. Concanavalin A has been reported also to increase membrane binding and uptake of calcium by cells (9, 106, 156). Most calcium in bovine mammary secretions is bound to casein and citrate (66). Changes in the electrolyte composition and pH disaggregate casein micelles into monomers (66), which may result in increased calcium flux into cells after Con A binding. High concentrations of intracellular calcium can inhibit microtubule formation, disrupt existing microtubules, and inhibit intracellular enzymes (6, 9, 105).

In addition to suppressing cellular secretion, Con A and PHA are T- and B-lymphocyte mitogens and are used widely in immunology research. Concanavalin A and PHA initiate polyclonal blastogenesis of lymphocytes with proliferation of antigenically committed and noncommitted T- and B-cells (24, 68, 112). However, the B-lymphocyte proliferative response apparently requires T-cell participation (68). Concanavalin A and PHA increase activity of cytotoxic T-lymphocytes and stimulate in vitro production of lymphokines and interferon (114, 154). Torre and Oliver (145, 146) and Concha et al. (24) demonstrated that lymphocytes obtained from bovine blood and dry secretion were stimulated by both Con A and PHA.

Intramammary infusion of Con A or PHA into bovine mammary glands near drying off (15) resulted in changes in mammary secretion composition similar to those observed following intramammary infusion of colchicine and endotoxin (96). However, effects of treatment were less dramatic and greater for Con A than for PHA. Infusion of Con A or PHA generally resulted in greater compositional changes earlier in the dry period compared with control glands. Concentrations of Lf, IgG, serum albumin, and somatic cells were higher, particularly during the first 2 to 3 d of involution, and citrate and the citrate to Lf molar ratio were lower in mammary secretions from treated quarters compared with control glands. Mammary glands infused with Con A or PHA had about 50% less fluid volume accumulation at 7 d of involution compared with controls. However, significant differences in milk yield or composition were not detected between Con A- or PHA-infused and control mammary glands.
at 7 and 14 d after parturition. These data suggest that intramammary infusion of these compounds near drying off does not adversely affect subsequent lactational performance.

Growth of *Klebsiella pneumoniae* in whey from lacteal secretions of quarters infused with Con A or PHA during the early nonlactating period was inhibited when compared with controls (16). At 7 d of involution, growth inhibition of *K. pneumoniae* was greater in mammary secretions from Con A- and PHA-treated glands than at drying off. However, growth inhibition in control secretions did not increase significantly during the 1st wk of involution. *Streptococcus uberis* growth inhibition increased by 3 d of involution after Con A treatment and by 7 d of involution after PHA treatment. Growth of *E. coli* in mammary secretions from control glands was similar to that of Con A- and PHA-treated glands. In vitro bacterial growth inhibition during early involution correlated positively with Lf, serum albumin, and IgG and correlated negatively with the citrate to Lf molar ratio (16).

Differences in growth inhibition of *Strep. uberis*, *K. pneumoniae*, and *E. coli* and mammary secretion composition were not observed in Con A- and PHA-treated glands and control glands during the peripartum period (16). In general, growth inhibition of all mastitis pathogens evaluated was greatest in mammary secretions obtained at 7 d prepartum, less at parturition, and least during early lactation. Bacterial growth inhibition during the peripartum period correlated positively with Lf, serum albumin, and IgG, and correlated negatively with citrate and the citrate to Lf molar ratio. Thus, intramammary infusion of Con A or PHA near drying off did not result in a carry-over effect in the prepartum period or in the early part of the subsequent lactation. These data agree with Oliver and Smith (97) who found that the acceleration of mammary involution decreased the frequency of major mastitis pathogen isolation during the 1st mo of the dry period only, and that any beneficial aspects of treatment were confined to the period of early involution.

A recent study by Bushe and Oliver (19) was conducted to determine if different methods of milk cessation influenced milk yield, composition, and in vitro growth of coliform mastitis pathogens. Cows producing about 13 kg of milk prior to experimentation were dried off by abrupt or intermittent milk cessation. An additional group was dried off by intermittent milk cessation and fed only hay during the last week of lactation. Cows milked intermittently produced 23% less milk during the last week of lactation than cows dried off by abrupt milk cessation. However, in spite of a significant reduction in milk yield for cows milked intermittently during late lactation, no differences in mammary secretion composition or in vitro growth inhibition of coliforms were detected when compared with mammary secretions from cows dried off by abrupt milk cessation. Mammary secretions from cows milked intermittently and fed only hay during late lactation contained higher concentrations of somatic cells, Lf, IgG, and serum albumin, a lower citrate to Lf molar ratio, and were more inhibitory to in vitro growth of *E. coli* and *K. pneumoniae* than mammary secretions from cows dried off by intermittent or abrupt milk cessation. Milk production in cows milked intermittently and fed only hay decreased 69.2% from 12.8 kg prior to experimentation to 4 kg during the last week of lactation, and significant differences in mammary secretion composition and growth inhibition of coliform mastitis pathogens occurred shortly after initiation of treatment. Increased concentrations of natural protective factors were not observed until milk volume was reduced below 9 kg.

The study by Bushe and Oliver (19) demonstrated that abrupt alteration of nutrient intake during late lactation significantly affected milk synthesis and secretion very rapidly. Changes in mammary secretion composition and fluid volume accumulation were similar, but more dramatic than following intramammary infusion of colchicine (96), endotoxin (96), PHA, or Con A (15). Intermittent milking of dairy cows with a simultaneous change in ration during late lactation seems to be a practical means of reducing milk yield at drying off and increasing natural protective properties of mammary secretions during early involution. Further studies are needed to determine if this approach influences the incidence of IMI during the nonlactating period.
Modulation of Mammary Cell Growth, Differentiation, and Milk Biosynthesis Near Parturition

Total mammary secretory cell populations and biosynthetic activity of these cells play a decisive role in determining milk yield. Mechanisms that regulate cellular differentiation and the onset of lactation need to be further defined. A greater understanding of these processes may provide new approaches to increase milk production in dairy cattle. The role of lactogenic hormones in ruminant mammary growth and differentiation during gestation have been reviewed extensively (1, 20, 27, 28, 30, 147) and will not be discussed in this paper. Instead, previous attempts to modulate the initiation of milk secretion through hormonal manipulation will be emphasized.

Considerable attention has focused on the influence of pituitary hormones, particularly prolactin and somatotropin, on mammary function during the periparturient period. The ergot alkaloid compound, 2-Br-α-ergocryptine-methane-sulphonate (CB154), was used in experiments to determine the importance of prolactin on mammary growth and lactogenesis in ruminants (1, 2, 3, 58, 61). Secretion of prolactin from the pituitary was inhibited specifically by CB154 (61). Karg and Schams (61) demonstrated that intramammary infusion of CB154 into the periparturient mammary gland reduced subsequent milk production by as much as 95%. However, administration of CB154 during lactation reduced milk production by 20%. When CB154 was administered to cows during the last 2 wk of gestation, reduced milk yields and lower α-lactalbumin concentrations in milk also were reported (58). The relationship between prolactin secretion and the cytological and biochemical differentiation of mammary secretory cells during the periparturient period was investigated further (2, 3). Mammary glands treated with CB154 from 10 d prepelum through 10 d postpartum produced 40% less milk when compared with either untreated glands or glands receiving CB154 and prolactin simultaneously. Metabolic measurements of mammary tissue slices from treated glands also indicated reduced rates of lactose, α-lactalbumin, and fatty acid biosynthesis in addition to lower acetyl coenzyme A carboxylase and fatty acid synthetase activities (2). Morphometric analysis demonstrated less synthetic and secretory ability of mammary parenchyma from CB154-treated cows as indicated by higher percentages of undifferentiated cells characterized by reduced cytoplasmic area occupied by RER and Golgi membranes (3). It was concluded that periparturient prolactin secretion was essential for optimal cellular biosynthesis and structural differentiation of alveolar epithelium during lactogenesis (2, 3, 61).

The plant alkaloid, colchicine, also has been used extensively to examine factors that regulate mammary gland development and milk production in several species. Colchicine is a microtubule-disrupting drug, which was shown to interfere with secretory processes in a variety of endocrine and exocrine glands (64, 71, 102, 116). Microtubules contain a high affinity binding site for colchicine. Subsequent binding of these sites prevents the protein interaction between tubulin dimers and blocks normal microtubule assembly (155).

Colchicine was used at different stages of the lactation cycle to clarify the role of microtubules in mammary secretory epithelium. Intramammary infusion of colchicine into lactating goats produced a reversible reduction in milk yield with concurrent inhibition of casein and fat globule secretion (104). Ultrastructural observations of lactating cells treated with colchicine demonstrated decreased secretory activity with extensive accumulation of secretory products throughout the cytoplasm (63, 82). Previous studies reported that colchicine interfered with secretion of lactose in guinea pigs (43) and rats (122), casein in rabbits (108), and milk fat globules in rats (105) and goats (135). Mode of colchicine action in the lactating gland is thought to be disruption of intracellular microtubular integrity necessary for exocytotic mechanisms (83, 115). It also was conjectured that colchicine interfered with the capability of secretory vesicles to fuse with the apical plasma membrane (63).

Effects of colchicine on mammary function appear to vary, depending on the physiological status of the gland. Although intramammary infusion of colchicine consistently decreased milk yield and altered normal composition in the lactating gland, these changes were transient. Patton (102) reported that the influence of colchicine on lactating mammary tissue was
reversed substantially by 72 to 96 h after treatment was discontinued. In contrast, limited information available in the periparturient mammary gland suggests that effects of colchicine on mammary function during lactogenesis are more permanent. Akers and Nickerson (5) found that repeated intramammary infusion of colchicine in periparturient heifers irreversibly reduced subsequent milk production compared with untreated mammary glands in the same animal. Prepartum colchicine treatment resulted in reduced rates of fatty acid synthesis, CO₂ production, and protein synthesis in tissue explants for up to 21 d following the last infusion (5). Cytological analysis demonstrated that prepartum infusion of colchicine into mammary quarters of heifers inhibited normal development of secretory tissue as indicated by less alveolar luminal area, more interalveolar stromal area, and the predominance of undifferentiated cells in the treated glands (80).

Similar results were observed in caprine mammary glands when a single intramammary infusion of colchicine was administered at parturition (139). During the 1st wk of lactation, milk composition from colchicine-treated udder halves had elevated somatic cell numbers, serum albumin concentration, and pH, whereas the concentration of citrate was lower than in uninfused glands. Milk yields from colchicine-treated udder halves also were 20% lower during the first 30 d of lactation compared with uninfused glands. Morphological examination of caprine mammary tissue showed that colchicine-treated udder halves consisted predominantly of undifferentiated mammary secretory cells, whereas uninfused udder halves appeared more cytologically differentiated (139).

Numerous studies have shown that the periparturient period is a critical time during which mammary secretory cells must respond to prolactin stimulation (1, 28, 38, 39). Microtubules are thought to play an important role in the response of mammalian cells to hormonal stimuli, and their disorganization may result in abnormal cellular activity (124). It was conjectured that temporary disruption of microtubule integrity with colchicine desensitized mammary epithelial cells to lactogenic hormone stimulation and inhibited irreversibly the biochemical and structural differentiation of secretory cells during lactogenesis (5, 80).

**Hormone-Induced Lactation**

Several researchers utilized available information concerning the hormonal control of lactogenesis and were successful in their attempts to induce lactation artificially in nonpregnant dairy cattle by administration of exogenous hormones (45, 77, 129, 130, 148). Smith and Schanbacher (129, 130) reported that a 7-d regimen of 17 β-estradiol and progesterone injections resulted in the initiation of lactation within a 3-wk period. Narendran et al. (77) analyzed mammary secretion composition and histologically examined mammary tissue from dairy animals induced to lactate with the same 17β-estradiol and progesterone therapy for 17 d. Milk from cows induced to lactate had similar percentages of fat, protein, and lactose when compared with milk obtained from postpartum animals during the first 21 d of lactation. However, milk yields from induced lactations were significantly lower than postpartum lactations. They also observed a large number of immature or developing alveoli and speculated that subsequent lactational performance was related to the degree of prepartum mammary growth in response to hormone treatment (77). Large variability in subsequent responses of animals to the estradiol-progesterone treatment (129, 130) also was explained in terms of time required for a majority of these immature alveoli to respond to treatment and reach maturity (77).

Modification of this procedure by others (39, 45, 59) indicated a positive relationship between subsequent lactational performance and lobulo-alveolar growth resulting from the synergism of both steroid and pituitary hormones (22, 100). It was hypothesized that high plasma insulin in conjunction with low prolactin and somatotropin may contribute to less than optimal milk production from induced lactations observed in some animals (59). Greater milk yields were reported for cows treated with reserpine when compared with cows treated with steroid hormones alone (11, 22). Reserpine stimulates the release of prolactin from the pituitary of dairy cows (11). It was conjectured that higher plasma prolactin was critical to the lactogenic hormonal complex in ruminants. The importance of the pituitary for mammary growth was demonstrated by Sykes and Wrenn.
(143). From histological observations, they observed normal mammary development in heifers treated with combinations of estradiol, progesterone, and pituitary extracts, but not in animals treated with steroids alone.

Procedures to induce lactation artificially in nonpregnant cows and heifers were associated with some distinct disadvantages. The success rate of inducing lactation was extremely variable and milk yields seldom reached those observed normally in postpartum cows. Increased estrous activity and relaxed pelvic ligaments following hormone injections resulted in physical injury to some treated animals (111). Addition of reserpine to the steroid regimen in heifers reportedly caused drowsiness, respiratory difficulties due to swelling of the nasal mucosa, and loss of appetite (125). In view of these undesirable side-effects, artificial induction of lactation in the bovine has limited practical value. However, it has proven to be a useful research tool for the study of bovine lactogenesis.

Summary

Marked changes occur in the bovine mammary gland and its secretion during involution. The early nonlactating period following cessation of milking is associated with transition from active milk synthesis to involution. This process is characterized by gradual changes in mammary secretion composition and regression of secretory tissue. The morphology of bovine mammary tissue and biochemical composition of mammary secretion remains relatively constant after mammary glands are fully involuted. Further alterations in mammary gland structure and function do not occur until colostragenesis. The dramatic changes that occur in mammary tissue and mammary secretion composition during the periparturient period are essentially the opposite of events that occur during early involution. Progression to a state of active milk synthesis is characterized by intense mammary growth, rapid differentiation of secretory epithelium, and increased synthesis and secretion of fat, protein, and carbohydrates that results in the accumulation of colostrum.

Bovine mammary glands are highly susceptible to new IMI during early involution and near parturition. Susceptibility to infection appears to be associated with transitions of the mammary gland either from or to a state of active milk synthesis. Techniques to hasten involution have resulted in reduced mammary secretion accumulation and elevated concentrations of natural protective components. More rapid changes in the composition of mammary secretion were associated with greater antimicrobial activity for some mastitis pathogens but had little effect on others. Additional studies are needed to determine if methods of decreasing milk production in high producing dairy cows during late lactation influence the incidence of IMI during the nonlactating period.

Studies that manipulated the periparturient mammary gland illustrated that the critical period for biochemical and structural redifferentiation was during the last few weeks of gestation. This is also a time of increased susceptibility to new IMI. Studies have shown that IMI during the nonlactating and periparturient periods suppressed irreversibly normal secretory cell development necessary for optimal milk production in the subsequent lactation (136, 137). A more thorough understanding of the events that occur during mammary involution, the relationship of involution with IMI, the ways in which mastitis affects mechanisms associated with cellular redevelopment, and factors related to mastitis resistance or susceptibility may lead to the development of improved methods of mastitis control.

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